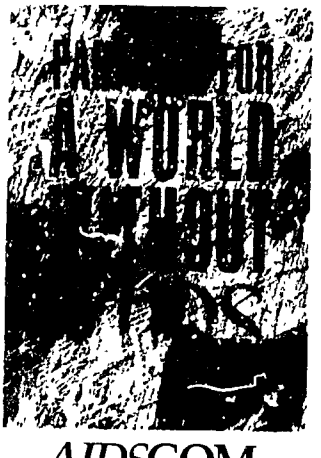
AIDS 90 Summary

A Practical Synopsis of the VI International Conference



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AIDS 90 Summary

A Practical Synopsis of the VI International Conference

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Publication

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TABLE OF CONTENTS

	EDITORIAL ADVISORY BOARD	iv
	PUBLISHER'S NOTE	v
CHAPTER 1	VIROLOGY	1
CHAPTER 2	PATHOGENESIS & IMMUNOLOGY	22
CHAPTER 3	EPIDEMIOLOGY	48
CHAPTER 4	DIAGNOSTIC TESTS FOR HIV	90
CHAPTER 5	VACCINE DEVELOPMENT	114
CHAPTER 6	ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS	138
CHAPTER 7	CLINICAL MANAGEMENT	168
CHAPTER 8	NEUROLOGICAL ASPECTS OF HIV INFECTION	230
CHAPTER 9	WORKPLACE ISSUES	264
CHAPTER 10	EDUCATION & COUNSELLING	286
	APPENDIX A: CDC DEFINITION OF AIDS/ARC CRITERIA	320
	APPENDIX B: CLASSIFICATION OF PEDIATRIC HIV INFECTION	321
	APPENDIX C: STAGING CLASSIFICATION SYSTEMS	322
	APPENDIX D: UNIVERSAL PRECAUTIONS	323
	INDEXES	327

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Publisher's Note

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Brigid M. Wallace
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Publishers

CHAPTER 1

Page 1 **BACKGROUND**
2 **VIRAL COMPONENTS**
2 Capsid and Envelope
3 RNA and DNA
3 **THE HIV LIFE CYCLE**
4 HIV Entry into Cells
6 Reverse Transcription
6 Circularization and Integration
7 Assembly, Budding, and Release
7 Maturation
7 Therapeutic Implications
8 **THE HIV GENOME**
10 Structural Genes
10 Regulatory Genes
12 The Long Terminal Repeat
14 **NATURAL HISTORY**
14 HIV-1
15 HIV-2
16 SIV
16 Evolutionary Divergence Among HIV-Related Viruses
18 **ANIMAL MODELS**
18 Nonhuman Primates
20 Cats
20 Mice

VIROLOGY

BACKGROUND

The virus that causes AIDS—human immunodeficiency virus, or HIV—while deceptively simple in structure has a highly complex life cycle. And this complexity may lead to its undoing by allowing scientists to exploit its vulnerabilities as precise targets. Virologists and molecular biologists have already discovered several apparently unique features of HIV, and these discoveries have led to the rational design of specific antiviral drugs now in various stages of development. Indeed, virology holds a central role in the multidisciplinary field of AIDS research and is crucial to exposing HIV's pathogenic mechanisms.

Identification and molecular characterization of discrete viral components, it is hoped, will help pinpoint HIV's vulnerabilities. Further understanding of individual events in the viral life cycle should also aid in addressing some global questions, such as:

- Is HIV a true lentivirus—a slowly replicating virus with a long incubation period? Or is it an evolved lentivirus that has developed a (genetic) switch with the potential to trigger rapid viral replication, thus speeding the onset of disease?
- What is the evolutionary relationship between HIV and other HIV-like animal viruses, such as simian immunodeficiency virus (SIV)? Will it be possible to establish the chronological evloution of these viruses?
- Has HIV been in the human population for a long time as an unrecognized nonpathogenic virus, and only recently (on an evolutionary scale) mutated into a pathogenic form?
- What controls HIV's well-recognized ability to rapidly and systematically mutate? Do such mutations affect infectivity and host-cell preference of the virus?
- What determines whether HIV will establish persistent (long-lasting) infection or cause acute infection leading to profound immunodeficiency? Might a genetic component of HIV be the determinant, or are cofactors required?
- How does HIV regulate its genes; how does it override normal host cell gene regulation?

Answers to these and other basic virology questions will figure heavily in the design of specific AIDS therapies and prophylaxes.

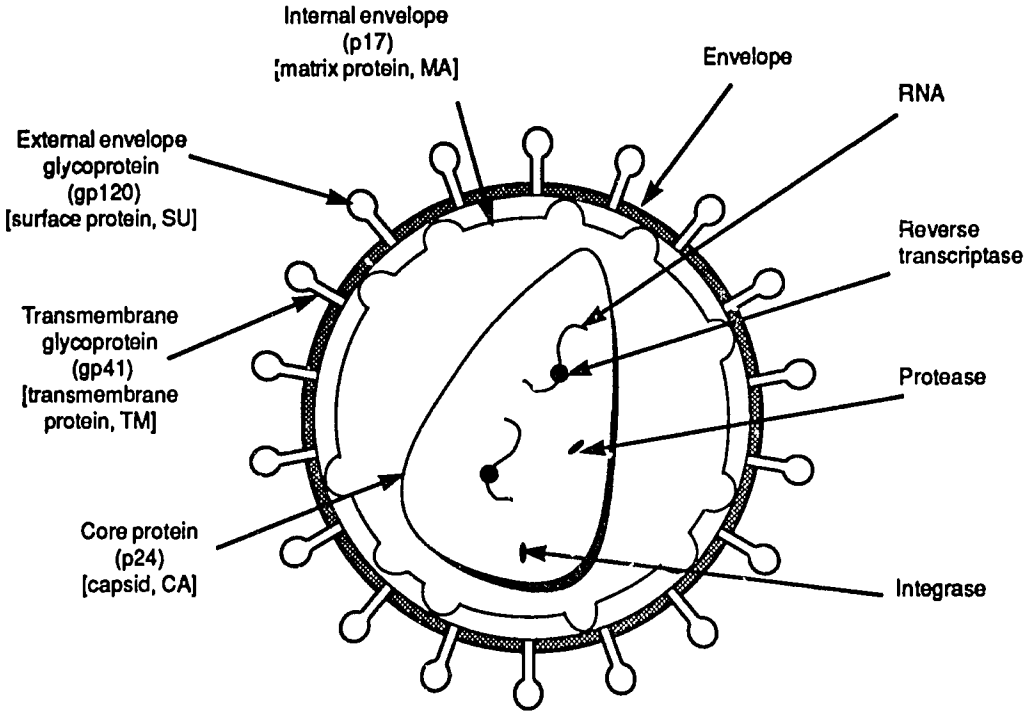


Figure 1.1. HIV (Human Immunodeficiency Virus)

This cross-sectional schematic of HIV reveals that the virus's genetic material (RNA) is protected by a protein coat (capsid protein, p24), which is itself surrounded and protected by a membrane-like envelope. The external glycoproteins, gp120, act as cell-docking agents for the virus. (Note that while the editors acknowledge the recommended nomenclature for retroviral proteins proposed in 1988 [shown in brackets], the "p" designations have persisted in the literature and therefore are included.)

VIRAL COMPONENTS

A brief overview of HIV's components, including its structural elements and known genes, offers a basis for understanding this exquisitely designed virus. Figure 1.1 provides a schematic representation of the HIV virion with all its components. (A virion is a single virus particle—a "cell-free" virus.)

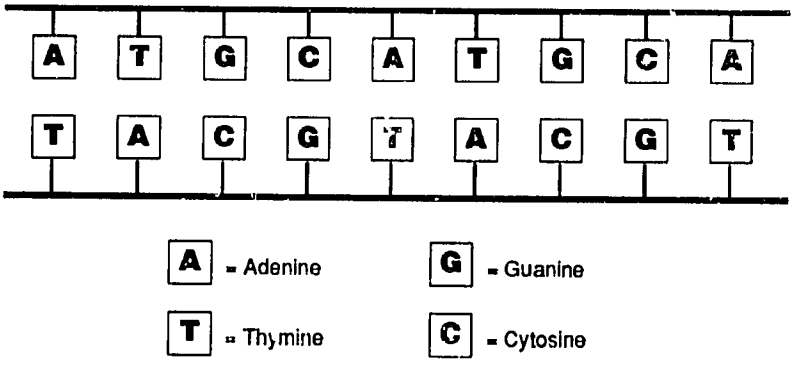
CAPSID AND ENVELOPE

From both a structural and biochemical standpoint, HIV is biologically very simple. It has been described as "a bag of proteins with some genetic material" with no other biomolecules and no means by which to propagate itself. Thus HIV, like all viruses, is a true intracellular parasite dependent on a host cell for replication.

The HIV capsid, or core protein, was originally defined by its molecular weight and was therefore designated p24. (Note: molecular weight determinations can differ slightly from laboratory to laboratory; in the literature both p25 and p24 are used, although they actually refer to the same protein.) The capsid encloses HIV's genetic information (in the form of two identical, single strands of RNA). Also housed within HIV's capsid are its three enzymes: reverse transcriptase, integrase (or endonuclease), and protease. (For a detailed discussion of HIV's enzymes, see "HIV Life Cycle" and "HIV Genome," below.)

Figure 1.2. Nucleic Acid Sequence of DNA and RNA Molecules

Shown here is a double-stranded DNA molecule. It is the sequence of the four nitrogen-containing compounds—adenine, thymine, guanine, and cytosine, which constitute nucleic acid molecules (either DNA or RNA)—that determines which proteins will be made. Note: in RNA molecules, thymine (T) is replaced by uracil (U). Determining the nucleic acid sequence is equivalent to "breaking the genetic code" for any given stretch of DNA or RNA.



HIV has an extra layer of protection: a lipid membrane, or "envelope," surrounding the capsid. Embedded within the HIV envelope are discrete glycoproteins (proteins with sugar molecules attached), which are described as "knobs" or "spikes" and give the viral particle its distinctive appearance. Each knob consists of an external glycoprotein (gp120), which is anchored to a transmembrane glycoprotein (gp41). An internal envelope, or matrix, protein (p17) lies between the capsid and envelope and is embedded in the envelope (on its interior aspect) at discrete points.

RNA AND DNA

HIV is classified as an RNA virus because its genetic information is encoded in ribonucleic acid (RNA) rather than deoxyribonucleic acid (DNA), which carries the genetic information for all known organisms and most viruses. (The central dogma of genetics, which originally stated that DNA was the universal genetic material, has been rewritten to include RNA.) DNA and RNA molecules are chains of *poly*nucleotides; each nucleotide includes a nitrogen-containing compound called a nitrogenous base, of which there are four: adenine (A), thymine (T), guanine (G), and cytosine (C). It is the precise sequence of these bases within the RNA or DNA molecule that specifies the genetic message and allows analysis of nucleic acid sequences in genetic studies (see Figure 1.2).

RNA viruses that convert their RNA to DNA once they enter a susceptible host cell are known as retroviruses. "Retro" refers to the one backward step in the viral life cycle, in which the normal pattern is reversed and the information contained in DNA is converted into RNA. In animal cells, the information contained in DNA is transcribed, or copied, into a specialized RNA molecule (mRNA, or messenger RNA) as a prerequisite to protein synthesis. Retroviruses, however, must first make a DNA version of their RNA in order to take advantage of the host cell's biochemistry; see "Reverse Transcription," below.

THE HIV LIFE CYCLE

While researchers continue to uncover specific features of the HIV life cycle, the general scenario of host cell invasion and assembly line viral replication remains unchanged. Figure 1.3 depicts the sequence of events in the HIV life cycle, which begins with free virus attaching to a receptor on the cell's surface.

HIV ENTRY INTO CELLS

HIV is an obligate intracellular parasite—that is, it cannot survive or propagate without access to a host cell's interior. Knowing this, researchers have been methodically dissecting the first step in the HIV life cycle—HIV's entry into a host cell—with the ultimate objective of identifying a site for therapeutic or prophylactic intervention.

Early laboratory experiments focusing on HIV-host cell interaction revealed a lock-and-key fit between the protruding "knobs" on the viral envelope and certain molecules (acting as receptors) displayed on the host cell's surface. These experiments identified the molecules to which HIV "docked" as CD4 receptors, now generally considered the major (if not the only) receptor that binds HIV. The tropism, or preference, HIV evidences for immune system cells bearing these receptors, notably the helper/inducer T lymphocytes, was thus explained.

The CD4 Receptor. A number of immune system cells—and some non-immune system cells, such as gastrointestinal cells—bear CD4 receptors. It has been reliably established that these receptors provide the major entry route for HIV into the helper/inducer T lymphocytes (also referred to as CD4 cells).

The glycoprotein knobs (gp 120) of the HIV envelope have been shown to dock directly to the CD4 receptor on the cell surface, and now researchers are attempting to pinpoint the precise region of the gp120 glycoprotein that interacts with and binds to CD4. Dr. Joseph Sodrosky (Dana-Farber Cancer Institute, Boston) and colleagues reported that by changing just three (discontinuous) amino acids of the gp120 glycoprotein, CD4 binding was reduced by more than 90%. The three amino acids were examined because they are common to all primate retroviruses and because, based on gp120's predicted molecular structure, they were likely to be on the exposed surface of gp120. One of the amino acids, the authors suggested, may not make direct contact with the CD4 receptor during binding but may be in close physical proximity to the binding site and necessary for optimal binding to CD4-bearing cells.

Other Receptors? Data appears to conflict regarding the possibility of other cellular receptors as entry vehicles for HIV infection of other cell types. Both *in vivo* and *in vitro* studies of several different cell types (macrophages, monocytes, and gastrointestinal cells, for example) show that these cells can be infected by HIV. However, it remains unclear whether the CD4 receptor is the entry route into these cell types. Although macrophages and monocytes are known to possess CD4 receptors, some investigators hypothesize that another receptor—possibly the Fc receptor—serves as the entry port for HIV. Dr. Thierry Jouault and colleagues (Pitié-Salpêtrière Medical School, Paris) presented data showing that HIV does indeed attach to monocytic cells by means of the Fc receptor, but that the CD4 receptor is still necessary for infection of these cells. (See Chapter 2: PATHOGENESIS & IMMUNOLOGY for further discussion.)

Figure 1.3. The HIV Life Cycle

Free virus enters a susceptible (host) cell via a membrane receptor. Once internalized, RNA is released from the capsid and immediately transcribed into DNA (reverse transcription). Double-stranded viral DNA is then integrated into the host cell's DNA. The integrated viral DNA, called proviral DNA, is transcribed along with cellular DNA into messenger RNA (mRNA) once the cell is activated. The mRNA "transcripts" are then translated into precursor polyproteins, which are later cleaved by viral and cellular protease into biologically active proteins. The polyproteins are assembled along with viral RNA into new viral particles, which bud from the cell surface, each acquiring an envelope as it exits the cell.

* Note: Protease activity is shown here for illustrative purposes only. It is now well established that polyprotein cleavage does not occur until after viral particles are released from the cell. Mature (infectious) viral particles can be distinguished from immature (noninfectious) viral particles by the presence of cleaved (active) or uncleaved polyproteins.

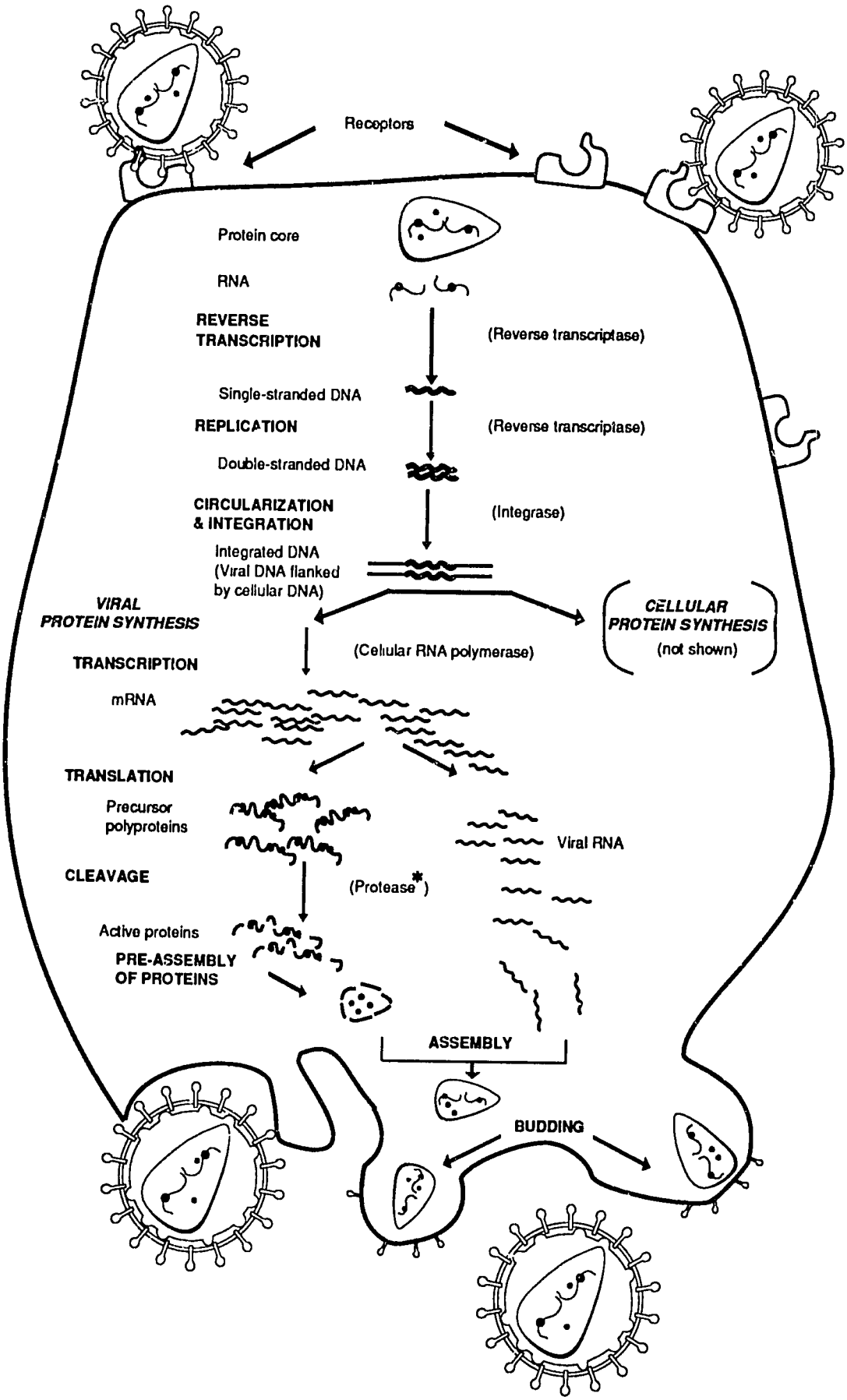
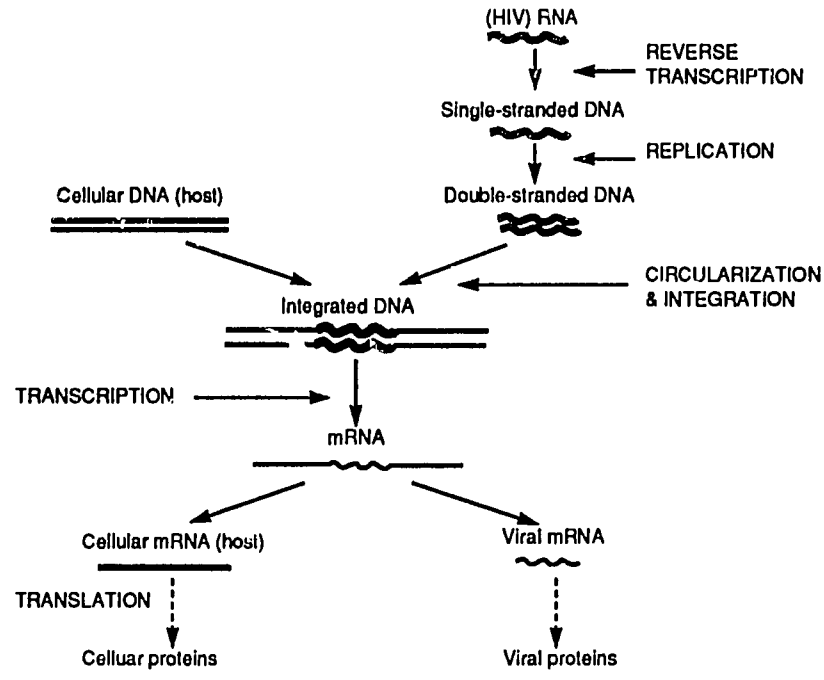

Receptors
Protein core
RNA
REVERSE TRANSCRIPTION
(Reverse transcriptase)
Single-stranded DNA
REPLICATION
(Reverse transcriptase)
Double-stranded DNA
CIRCULARIZATION & INTEGRATION
(Integrase)
Integrated DNA (Viral DNA flanked by cellular DNA)
VIRAL PROTEIN SYNTHESIS
CELLULAR PROTEIN SYNTHESIS (not shown)
TRANSCRIPTION
(Cellular RNA polymerase)
mRNA
TRANSLATION
Precursor polyproteins
Viral RNA
CLEAVAGE
(Protease*)
Active proteins
PRE-ASSEMBLY OF PROTEINS
ASSEMBLY
BUDDING

Figure 1.4. Protein Synthesis



Internalization of HIV. After the virus docks with a target cell, HIV is internalized by fusion of the viral envelope and cell membrane, possibly mediated by HIV's gp41 transmembrane protein. Some question remains about whether a cell receptor mediates fusion. Earlier models of endocytosis (whereby the virus is engulfed by the cell and pinched off into the cell's interior) have apparently been abandoned.

REVERSE TRANSCRIPTION

Once HIV is internalized the capsid (core protein) is shed and the viral RNA and viral enzymes immediately disentangle within the cell's interior. The single-stranded viral RNA is converted into single-stranded DNA, a process called reverse transcription. This essential conversion step requires HIV's reverse transcriptase enzyme, which is an RNA-dependent DNA polymerase. (Polymerases are a class of enzymes that catalyze the polymerization, or elongation, of certain molecules.) As described earlier, this process is the reverse of normal cellular transcription in which the information in cellular DNA is transcribed (copied) into RNA. Reverse transcriptase is also responsible for degrading the RNA molecule once the DNA molecule is synthesized and for replicating the newly synthesized single-stranded viral DNA into double-stranded viral DNA. Because reverse transcription is peculiar to the invading virus, it offers an attractive target for therapeutic intervention.

CIRCULARIZATION AND INTEGRATION

The double-stranded viral DNA migrates to the cell's nucleus, where the ends of some of the molecules join together to form a closed circle. This "circularized" DNA is then integrated into the host-cell DNA through the action of the HIV enzyme integrase, or endonuclease. (Note: Circularization may not be necessary for integration.) Mutation studies have demonstrated that integrase is critical for this event: Virus lacking integrase cannot replicate.

Several research teams are studying this pivotal step in the HIV life cycle, since preventing integration of viral DNA into the host cell DNA would necessarily avert virus production. In a study reported by Dr. James Fyfe and coworkers (Wellcome Research Laboratories, Research Triangle Park, North Carolina), HIV integrase was found to act as a "trimming" agent, cleaving HIV DNA sequences before insertion into cellular DNA occurs. Such characterization will aid in the rational design of anti-integrase drugs and antibodies to integrase.

Once viral DNA is integrated into the host cell's DNA, infection may become either productive (acute) or latent (dormant). Proviral DNA (the term for integrated viral DNA) can lie dormant within unactivated ("resting") cells as a silent infection until the cell becomes activated. Activated (proliferating or dividing) cells give rise to new virus production almost immediately. Approximately 90% of all T lymphocytes are resting.

After integration, proviral DNA is transcribed into many strands of messenger RNA (mRNA), triggered by an unknown activation signal (see "Regulatory Genes," below). As occurs during normal cellular protein synthesis, viral mRNA is translated into proteins. The cell manufactures viral proteins in a biologically inactive form as large precursor proteins (called *poly*proteins). Unless cleaved by the viral enzyme protease (see "Maturation" below), the precursor proteins remain inactive.

ASSEMBLY, BUDDING, AND RELEASE

Next, the polyproteins come together with viral RNA, assembling preparatory to budding and release. When assembled viral particles "bud" from the cell, they take along cell membrane components, forming an envelope.

New evidence presented by Dr. Heinrich Gottlinger and coworkers (Dana-Farber Cancer Institute, Boston) demonstrated by electron microscopy that, at a late stage in budding, viral particles can accumulate at the cell surface, remaining attached by a stalk. Prior to this finding, it was thought that budding from the cell surface and release were simultaneous events. They also presented biochemical data showing that efficient release of virus particles requires a capsid protein, p6.

MATURATION

Virus particles are released from a host cell as immature virions; conversion to mature, infectious virions requires the action of HIV protease (or proteinase). Like other naturally occurring protease enzymes, HIV protease is responsible for converting inactive, or precursor, forms of proteins into their biologically active form. Thus the HIV capsid proteins and HIV enzymes all depend on protease for a final processing step (see Figure 1.5). Indeed, genetic mutational experiments, in which the gene encoding for protease is altered or deleted, yield "immature" (noninfectious) virions. Although protease was previously thought to act prior to budding, studies in 1989 showed that processing by protease actually occurs after the virus has been released from the cell. (See also "Structural Genes," below.)

THERAPEUTIC IMPLICATIONS

As molecular details of the HIV life cycle are being worked out, discrete target sites for intervention are being identified. Examples of agents being designed to target major stages of the HIV life cycle are previewed below; Chapter 6: ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS reports these efforts in more detail.)

HIV Entry. Since identification of the CD4 receptor as entry portal for HIV into susceptible cells, much effort has been directed at developing agents to mimic this molecule as a means of interfering with viral attachment to the cell. Revamped versions of a soluble CD4 molecule (sCD4) that binds to HIV's envelope (thereby blocking attachment) are currently

in clinical trials. Other efforts aimed at HIV entry into susceptible cells include targeting the step immediately preceding internalization. Dr. John Bergmann and colleagues (Columbia University College of Physicians & Surgeons, New York) reported development of an assay that will enable testing of candidate agents for their ability to block HIV adsorption to and fusion with the cell's membrane.

Reverse Transcription. Interfering with the process of reverse transcription, and the HIV reverse transcriptase enzyme, remains a prime research target because production of virus from an infected cell can be halted if HIV's RNA cannot be made into DNA. AZT (zidovudine), the only drug approved by the U.S. Food & Drug Administration (FDA) for the treatment of AIDS, acts by interfering with reverse transcriptase.

At this year's Conference, novel approaches to interfering with reverse transcriptase activity were presented. Although the reports were only preliminary, an antibody (designated C2003) that recognizes and binds reverse transcriptase and inhibits its activity were described by Dr. Anthony DeVico and colleague (Advanced BioScience Laboratories, Kensington, Maryland).

A new class of drugs (called TIBO derivatives), believed to act specifically against HIV-1 reverse transcriptase, was described by Dr. Rudi Pauwels (Katholieke Universiteit Leuven, Belgium). Dr. Pauwels expressed hope that further characterization of these TIBO derivatives would help pinpoint their precise mechanism of action.

Integration. Much attention has recently turned toward HIV integrase and the integration phase of the HIV life cycle, with the goal of designing anti-HIV integrase agents. To aid development and testing of such agents, a speedy and efficient laboratory protocol for isolating and purifying the HIV integrase enzyme is necessary. Toward this end, Dr. Iain K. Pemberton and colleagues (The London Medical College) presented their methodology on cloning the gene and purifying its product. Dr. Viola Ellison (Stanford University, California) and colleagues, and independently, Christopher Farnet (Dana Farber Cancer Institute, Boston) reported on a newly developed cell-free system in which the mechanism of action of integrase can be studied.

Maturation. Perhaps because of past successes with other human protease enzymes, (notably, renin and pepsin), several research teams and pharmaceutical companies have made much progress in the design of specific anti-HIV protease. Data on one such compound now in development was presented at the Conference by scientists from Roche Products, Ltd., U.K. (see Chapter 6: ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS). Molecular characterization of this enzyme, including determination of its three-dimensional structure, and identification of its specific target sites (within HIV polyproteins) has greatly aided scientists in designing anti-protease drugs.

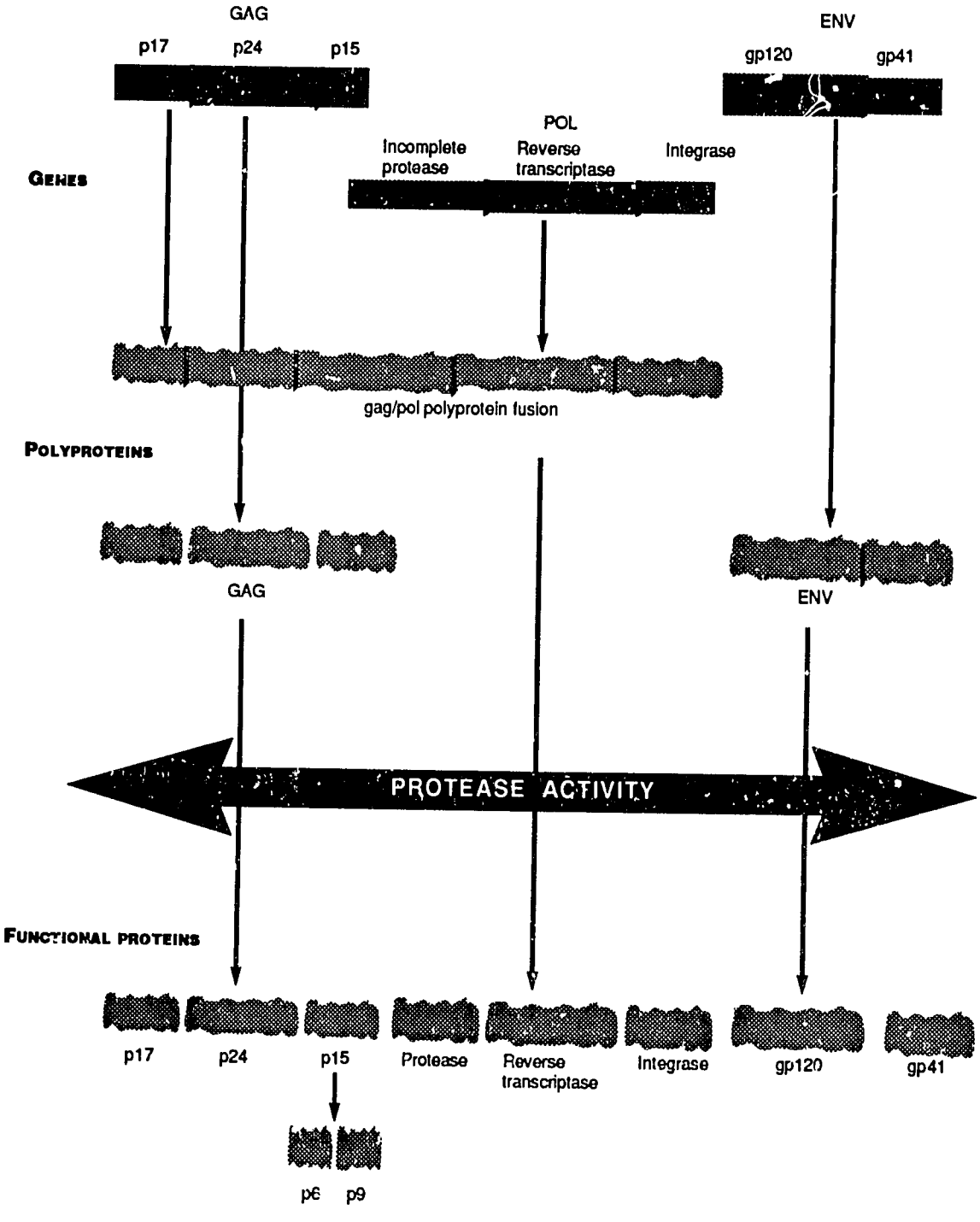
THE HIV GENOME

HIV's genome (its entire complement of genes) consists of three structural genes—which are common to all retroviruses—and six regulatory genes—several of which appear to be unique to HIV. (See Figure 1.6 for a "map" of the HIV genome.) Structural genes encode the genetic information necessary for the production of proteins, including enzymes; regulatory genes encode instructions for regulatory elements—proteins that control the synthesis and/or activity of other proteins. Regulatory gene products are also capable of self-regulation.

Given HIV's elusiveness within the human body, it is not surprising that scientists are uncovering a highly complex system of gene regulation. No other retrovirus appears to exhibit such multiple levels of control. Perhaps most intriguing is the finesse with which HIV's regulatory genes dictate the rate and timing of virus production. While HIV's regulatory mechanisms may at first appear ingenious, they may also prove to be its Achilles' heel, providing an ideal target for medical weaponry.

Figure 1.5. Protease Activity

The HIV protease enzyme cleaves the gene products (precursor polyproteins) of *gag and pol* into individual biologically active proteins. Protease must first undergo an autoprocessing event—that is, it must cleave itself from the gag-pol fusion polyprotein—before it can cleave the other polyproteins. (Note: The envelope polyprotein is cleaved by cellular protease.)



STRUCTURAL GENES

HIV possesses three structural genes whose products are essential to replication of the virus. Transcription and translation of these genes results in the generation of biologically inactive *poly*proteins. Processing by one of these structural gene products, the protease enzyme, is necessary for the "release" of individual structural proteins from their polyprotein precursors (see Figure1.5).

***Gag* (group-specific antigen).** The *gag* gene codes for (contains the genetic information for) the capsid, or core, protein designated p24 (or p25). Other *gag* gene products include

- the matrix protein p17 (also referred to as p18)
- p6, a protein recently shown to be necessary for the release of newly assembled viral particles from the cell surface
- p9, whose function is unknown

***Pol* (polymerase).** The *pol* gene codes for reverse transcriptase, a polymerase enzyme needed for the conversion of RNA to DNA. The *pol* gene also contains the blueprint for integrase (an enzyme essential for the integration of viral DNA into cellular DNA) and a partial blueprint for protease (the enzyme that cleaves large precursor polyproteins into smaller active proteins). Note that the complete "message" for protease is contained in a fused gene product, the *gag-pol* polyprotein. HIV must employ a special genetic maneuver—referred to by geneticists as a translational frameshift—to decode the information from both the *gag* and the *pol* genes in order to generate the protease enzyme.

***Env* (envelope).** The *env* gene encodes information for an external envelope protein, gp120 (which gives the virus its "knobby" appearance), and an envelope protein that is integrated directly into the envelope, the transmembrane protein gp41. Both of these gene products are believed to be significant determinants of HIV's pathogenicity.

REGULATORY GENES

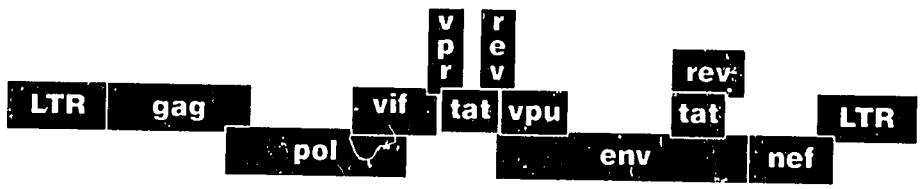
The rate of protein synthesis can be promoted or inhibited by genes referred to as positive or negative regulators. Investigations into HIV's regulatory genes seek to define and characterize not only these genes and their products, but also the interactive elements that enable them to act as positive or negative regulators of gene activity. Although HIV's regulators were previously categorized as either positive or negative, data from interim studies have blurred such clear-cut distinctions (except for *tat*, see below). It is conceivable that a given regulatory gene may act in a positive manner in a particular circumstance (of environment or time), but in a negative manner in another.

Regulatory genes can interact with short nucleotide sequences (building blocks of the nucleic acid molecules) located within either DNA or RNA molecules. These short sequences are known as "responsive" elements. Numerous reports have shown that cellular elements also play a role in the mechanism of action of regulatory genes. New findings on the mechanism(s) by which HIV regulates its own production are slowly revealing the truly intricate nature of HIV's regulatory pathways.

***Nef* (negative regulatory factor).** Much controversy surrounds the precise function of the *nef* gene product. Not only has it been postulated that the *nef* protein negatively regulates HIV transcription (through interaction with a specific region within the long terminal repeat—the negative regulatory element, NRE), but also that *nef* plays an indirect role in the establishment of latency, or at least "slow infection." Some research groups, however, contend that no such activity can be attributed to the *nef* gene. And some are now interested in whether the discrepant observations can be attributed to strain variations or to differences in its activity in different cell types.

Figure 1.6. The HIV-1 Genome

The entire complement of HIV genes are represented below: The LTR = Long terminal repeat. gag = group-specific antigen; pol = polymerase; env = envelope; vif = virion infectivity factor; rev = regulator of virion protein expression; nef = negative regulatory factor; vpu = virion productivity factor "u"; tat = trans-activator of transcription; and vpr = viral protein r.



The conflicting findings on *nef* function may be explained, at least in part, by new evidence on *nef*-defective HIV isolates presented by two independent research teams. Dr. Ernest Terwilliger and colleagues (Dana-Farber Cancer Institute, Boston) reported that an HIV isolate maintained in stable T cell cultures (as opposed to fresh primary T cell cultures) lacked an intact *nef* gene. In a parallel study using biochemical analyses, Dr. Anne Laurent (Institut Pasteur, Paris) and colleagues found the *nef* protein of an HIV isolate—also raised in a stable T cell culture—to be nonfunctional. Both sets of data may shed some light on previous conflicting reports regarding the role of *nef* in HIV replication. More importantly, this new evidence raises concern about the possibility of *in vitro* artifacts, bringing into question the reliability of data obtained using HIV isolates from such cell culture systems and its applicability to *in vivo* behavior of HIV.

***Rev* (regulator of virion protein expression).** The *rev* gene product is now known to control the shuttling of structural messenger RNA molecules (which carry the message for structural proteins) from the nucleus, where they are generated, to the cell cytoplasm, where they are translated into proteins. The mechanism by which *rev* is able to exert such control is now under intense investigation. It is known that *rev* must bind to a specific RNA sequence, called RRE—for *rev* responsive element. Several independent teams are working on identification and characterization of the RNA that contains this sequence in an effort to obviate normal *rev* function. Dr. James Rusche (Repligen Corporation, Cambridge, Massachusetts) and colleagues presented data that implicated cellular factors in the *rev*/RRE interaction.

***Tat* (transactivator of transcription).** The *tat* gene has been heavily scrutinized since its identification as a gene capable of increasing HIV transcription—and therefore ultimately controlling replication. *Tat* is considered a potent positive regulator because it has been shown to dramatically speed the rate of transcription of viral genes. Since the general function of *tat* has been defined, researchers are now anxious to find out how *tat* exerts its control. Once this process and its kinetics are laid bare, efforts can begin to target its disruption.

The *tat* gene product (transactivation, or tat, protein) is described as a *trans*activator because it activates transcription by interacting with *noncontiguous* target nucleic acid sequences located within the genome. These sequences, situated at the ends of the genome in the long terminal repeat (see below) and also in newly synthesized RNA, are called transactivation responsive elements (or TAR sequences).

Through the efforts of several independent research teams, a clearer picture of the complex interaction of the *tat* protein with TAR sequences is coming into focus. As pointed out by

Dr. Flossie Wong-Staal (University of California, Davis), it is now apparent that *tat* protein acts at multiple levels:

- increasing the efficiency of initiation of viral gene transcription
- enhancing elongation of growing mRNA molecules
- increasing the speed and efficiency of translation of mRNA into proteins by increasing the level of newly synthesized RNA in the nucleus

It is hoped that these findings, together with data from ongoing studies, will enable scientists to dismantle the critical "interaction site" within the transactivation process.

***Vif* (virion infectivity factor).** Two separate pieces of data presented at the Conference implicate the *vif* gene in controlling infection kinetics and cytopathicity (ability to induce pathological effects on a cell). Dr. Kazuyoshi Ikuta (Hokkaido University, Sapporo, Japan) and associates reported their results on a series of deletion mutation experiments in which an HIV strain lacking *vif* was able to infect susceptible cells but could not kill them. These results were not obtained, however, with natural HIV or deletion mutants defective in the *nef*, *vpu*, and *vpr* genes. In an independent study conducted by Dr. Koji Sakai (St. Luke's/Roosevelt Hospital Center, New York) and colleagues, cytopathic and noncytopathic isolates of HIV were examined in an effort to identify differences between the two at the molecular level. The researchers found that deletion of the *vif* gene (producing either truncated *vif* protein or no *vif* protein) gave rise to a noncytopathic isolate.

***Vpr* (viral protein r).** Dr. Eric Cohen and coworkers (Dana-Farber Cancer Institute, Boston) presented new data regarding the *vpr* gene product, which until this time has only been characterized as a nonessential gene. The researchers found, rather surprisingly, that the *vpr* protein is in cell-free viral particles in multiple copies. Such a finding implies that the *vpr* protein is necessary to (or at least facilitates) the early stages of the viral life cycle.

***Vpu* (virion productivity factor "u").** Independent studies reported at the Conference confirmed earlier work showing that the *vpu* gene product is associated with (intra)cellular structures and acts in the later stages of the viral life cycle by facilitating assembly, budding, and release of new viral particles.

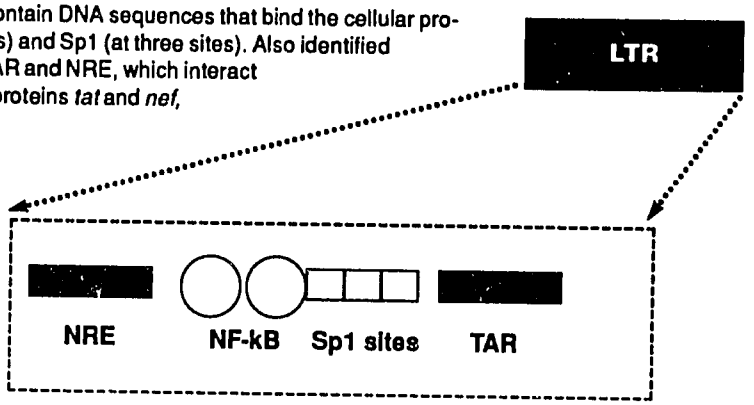
Therapeutic Implications. As outlined out by Dr. John Coffin (Tufts University, Boston), the interrelationships among HIV's regulatory proteins have proved to be much more complex than those of any other retrovirus studied. Nevertheless, the potential for gene therapy is significant. Several sessions on the molecular biology of HIV discussed the prospects of designing gene fragments that, once within a cell, can compete with HIV genes and gene products—and thereby negatively regulate HIV replication. Dr. Remi Cheynier and colleagues (Institut Pasteur, Paris) compared the ability of defective *tat* gene products to interfere with the activity of normal *tat* gene products. *In vitro* studies showed that when a defective *tat* gene is introduced with normal proviral HIV DNA, replication of HIV is diminished. When added in excess quantities, the defective *tat* gene product (*tat* protein) could outcompete normal *tat* protein and effectively inhibit replication by as much as 90%. Many researchers are excited about the prospects of transforming the molecular details of HIV gene regulation into a realizable clinical rationale.

THE LONG TERMINAL REPEAT

As in all retroviruses, proviral DNA is physically flanked at both ends by identical segments called long terminal repeats (LTRs). Each of the LTRs contains many regulatory signals. Sequences in the left (or 5') LTR and right (or 3') LTR of either DNA strand serve as initiation sites for the transcription of viral genes. Turning on, or activating, the LTR, then, means turning on transcription, the beginning of viral protein synthesis.

Figure 1.7. The Long Terminal Repeat

HIV's LTR is known to contain DNA sequences that bind the cellular proteins NF-kB (at two sites) and Sp1 (at three sites). Also identified are sequences called TAR and NRE, which interact with the HIV regulatory proteins *tat* and *nef*, respectively.



Transactivation. HIV LTR is activated when binding occurs between sequences within the LTR (responsive elements) and regulatory proteins. Such activation by noncontiguous elements is more accurately called *trans*activation. Interaction between *tat* and TAR (the tat-responsive element), for example, results in an increased rate of transcription.

Cellular proteins have been shown to bind to—and thereby activate—the HIV LTR. It is more than curious that the HIV LTR contains sequences resembling cellular DNA sequences. This similarity enables the binding of cellular proteins that normally interact with cellular DNA to speed transcription of cellular genes. In the normal environment of an activated cell, proteins such as nuclear factor kappa B (NF-kB) bind to DNA sequences and thereby speed transcription. Unactivated, or "resting," cells do not appear to have NF-kB available for binding to these sequences.

Stretches of DNA in the HIV LTR that bind NF-kB (at two sites) and another cellular factor, called Sp1 (at three sites), are being exhaustively tested (see Figure 1.7). Several groups of investigators are trying to establish the precise role these sites play in controlling the virus's capacity to replicate. In summarizing the deletion and reconstruction experiments being conducted in his laboratory, Dr. Malcolm Martin (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) reported that the NF-kB and Sp1 sites in the HIV LTR are critical for HIV infection of CD4-bearing T cells. Dr. Martin presented data demonstrating that HIV cannot replicate if all of these sites are eliminated from the LTR (a so-called "empty LTR"). And when the HIV LTR contains only a single NF-kB site and none of the Sp1 sites, viral replication is significantly delayed (occurring several weeks rather than hours after the DNA is introduced into susceptible cells) and virus production is significantly depressed compared with normal LTR constructs.

Transactivation of HIV LTR lacking NF-kB *and* Sp1 binding sites apparently *can* occur in the presence of coinfecting viruses. Dr. David Margolis and colleagues (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) found that the herpes simplex virus (HSV-1) was able to transactivate an LTR lacking all NF-kB and Sp1 sites in cultured (Jurkat) T cells, but not in cultured kidney cells (Vero cells).

The ability of other viruses, including human T-lymphotropic virus, type 1 (HTLV-1), Epstein Barr virus (EBV), cytomegalovirus (CMV), and JC virus, to transactivate the HIV LTR continues to be studied. Several authors cautioned that although non-HIV viral gene products can be shown to transactivate the HIV LTR *in vitro*, such stimulation may not actually be occurring *in vivo*. Furthermore, as illustrated by work presented by Dr. Kei Amemiya (National Institute of Neurological Disorders and Stroke, Bethesda, Maryland),

HIV may replicate more efficiently in the absence of transactivating proteins from coinfecting viruses. Dr. Ameiniya's group found that a JC virus protein previously identified as transactivating could not stimulate LTR-driven HIV replication to the levels obtained without the protein.

Dr. Vincent Emery (Royal Free Hospital School of Medicine, London) and colleagues presented new data on the NRE situated in the HIV LTR, which they showed contains two previously unidentified binding sites for cellular proteins. Upon binding, transcription is either activated or repressed depending on certain molecular characteristics of the protein-DNA complex. This finding raises the possibility that NRE is able to act as a switch from latency to productive infection. If such a mechanism does exist, intervention at this site may well permit HIV infection to be kept in check.

Therapeutic Implications. Perhaps one of the most compelling reasons to dissect the HIV LTR dates back to experiments with a pathogenic murine retrovirus, which was rendered nonpathogenic following experimental substitution of certain sequences in its LTR. Many factors, both cellular and viral in origin, have been shown to activate the HIV LTR in *in vitro* studies. Such substances that bind to DNA sequences in the LTR can conceivably regulate HIV replication through this interaction. Alternatively, Dr. Kuan-Teh Jeang (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) discussed the design of special RNA molecules, called ribozymes, which possess scissor-like cleaving capabilities and can act as homicidal agents within the cell. Ribozymes that specifically recognize LTR sequences could be used as a therapeutic tool for interfering with transactivation of the HIV LTR and ultimately the production of viable virus.

NATURAL HISTORY

Although many molecular details of HIV have been sorted out, a clear picture of its natural history—delineation of its origins and immediate relations—has yet to emerge. A second human retrovirus associated with AIDS-like symptoms (HIV-2) was isolated from persons in West Africa 2 years after the AIDS virus (HIV-1) was identified. Identification, that same year, of a simian retrovirus in laboratory-raised rhesus macaques exhibiting immunosuppression, prompted researchers to examine the evolutionary relatedness of the primate retroviruses. Molecular and immunological analyses of human and simian immunodeficiency viruses (SIV), in combination with surveys determining the prevalence of HIV and SIV infection, will it is hoped eventually allow researchers to piece together the ancestry of these retroviruses.

HIV-1

Human immunodeficiency virus, type 1 (HIV-1), was identified in 1983 as the causative agent of AIDS, just 2 years after the first reports of an immunodeficiency syndrome appeared in the medical literature. Determination of the DNA sequence of the entire HIV genome and characterization of its organization (in 1985) led to the finding that individual HIV isolates showed marked strain variation from patient to patient and among different tissue types. Such variability was consistently reported by independent laboratories and is now recognized as a hallmark of this virus. (Chapter 2: PATHOGENESIS & IMMUNOLOGY discusses the pathologic consequences of HIV strain variation.)

HIV-1 isolates from diverse geographic areas—North America, Europe, and Africa, for example—exhibit characteristic differences in specific regions of their DNA sequence. Such differences are apparent in immunological profiles of infected individuals: antigen-antibody reactivities of samples taken from persons in distinct geographic regions yield slightly different patterns.

Constructing an evolutionary tree of the HIV and SIV isolates, then, must take into account the strain variation that exists within HIV-1 and, as is discussed below, within HIV-2, and the SIV subtypes. Comparative genetic analyses of each viral isolate permits calculation of the degree to which the strains are related, based on variables such as nucleic acid sequences. Establishing evolutionary relatedness, it is hoped, will facilitate "backtracking" studies seeking to determine the origin of HIV-1.

HIV-2

Human immunodeficiency virus, type 2 (HIV-2) was isolated in 1985 from a Portuguese man living in the Cape Verde Islands who had symptoms resembling those seen in AIDS patients (specifically, immune suppression and lymphadenopathy). Recent regional and worldwide epidemiological surveys show that HIV-2 is endemic to West Africa, and that, with few exceptions, HIV-2 infected persons have some connection with West Africa, such as having

- lived or travelled in West Africa
- had sexual contact with persons in or from West Africa
- received transfused blood/blood products from a West African donor

HIV-2 was identified through diagnostic screening for HIV-1 when an antibody profile was seen that had definite similarities to HIV-1—but which also showed distinct differences. Genetic characterization of the virus and comparison with HIV-1 revealed differences in DNA sequence that explained the discrepancies in antibody banding patterns. Comparative genetic analyses were also able to show that the *env* gene was significantly distinct from that of HIV-1. Differences between the *gag* and *pol* genes of HIV-1 and the virus that would be designated HIV-2 are now also well documented. Perhaps the most important distinction—and one that may provide the link between HIV-1 and HIV-2—is the absence of the *vpu* regulatory gene and the presence of a previously uncharacterized regulatory gene, *vpx*, in HIV-2.

Until recently, the variability among individual HIV-2 isolates has been reported to be rather low (87–90% identical). Dr. Ursula Dietrich (Georg-Speyer-Haus, Frankfurt, FRG) reported her team's work with a "new" HIV-2 strain (HIV-2 ALT) that is only 76.2–76.8% identical to the HIV-2 prototype and to other characterized isolates. Dr. Michael Tristem and colleagues (University of Cambridge, U.K.) also noted substantial variation between their isolate (from an infected person in Guinea Bissau) and the HIV-2 prototype (from Cape Verde Islands). Data presented by Dr. Tristem showed that the lowest homologies (similarities) were in the *env* and *tat* amino acid sequences. The authors suggest that HIV-2 strains do indeed exhibit considerable variability—perhaps to a greater extent than that seen with HIV-1 strains.

Clinical correlates of HIV-2 infection show similar, but not identical, features to HIV-1 infection. Dr. Wanda Canas-Ferreira (New University of Lisbon, Portugal) and colleagues observed that although lymphadenopathies, ARC, and AIDS are associated with HIV-2 infection, the clinical evolution of infection is slower and less aggressive than that seen in HIV-1 infection. Indeed, naturally occurring HIV-2 isolates with markedly reduced cytopathicity (ability to induce cell killing or cell fusion) have been reported.

In vitro study of HIV-2 strains that differ in their ability to cause cell damage may lead to the identification of specific molecular determinants involved in *in vivo* pathogenicity. Dr. James Hoxie (University of Pennsylvania, Philadelphia) presented his group's data on a highly cytopathic HIV-2 variant derived from a noncytopathic, replication-competent, isolate. The cytopathic HIV-2 isolate was found to differ from the noncytopathic parental isolate in its affinity for the CD4 receptor, level of "down-modulation" of CD4, size of the external envelope glycoprotein, and ability to interfere with co-infecting cytopathic viruses. It is hoped that continued *in vitro* comparisons, together with further characterization (at the DNA and amino acid level) of the two variant strains, will provide insight into the mechanism(s) controlling pathogenicity.

SIV

The simian immunodeficiency virus (SIV) was first identified in laboratory-raised macaques exhibiting immunosuppression. Examination of data from many studies since then has revealed that, while SIV is found in animals in the wild, it is nonpathogenic to these natural hosts—several species and subspecies of African Old World monkeys, including African green monkeys, sooty mangabeys, mandrills, and patas. Extensive serological testing of Old World monkeys native to Africa indicates that SIV infection is quite widespread among this species in the wild. However, monkeys native to Asia, while not infected in the wild, have thus far been the only species found to develop a fatal immunodeficiency condition (called simian AIDS or SAIDS) when experimentally infected with certain SIV strains. (The rhesus macaque from which SIV was first isolated was of the Asian type and was probably infected by a sooty mangabey while they were housed together in the laboratory.)

These findings have led to speculations that SIV crossed species, infected humans, and eventually evolved into the highly pathogenic HIV. Numerous research teams are looking at this hypothesis and are comparing the genetic sequences of individual isolates to approximate evolutionary divergence (see below).

The genetic organization of most SIV strains reveals that, like HIV-2, the SIV genome contains a *vpx* gene and lacks a *vpu* gene. Dr. Thierry Huet (Institut Pasteur, Paris) and colleagues, however, presented new data on an SIV strain isolated from two chimpanzees (SIVcpz) captured in Gabon: the genome of SIVcpz contains the exact same complement of genes as HIV-1, including the *vpu* gene. (Note: the study's authors recommended using the acronym "CIV," for chimpanzee immunodeficiency virus, rather than SIV.) See below for a discussion of the evolutionary implications of this finding.

Dr. Martine Peeters (Institute of Tropical Medicine, Antwerp, Belgium) and colleagues, who originally identified SIVcpz from two chimpanzees in Gabon in 1989, are now studying the prevalence of natural infection of SIVcpz in chimpanzees. It remains to be seen whether SIVcpz infection is widespread throughout the species in the wild or is regional, as seen in the African Old World monkeys.

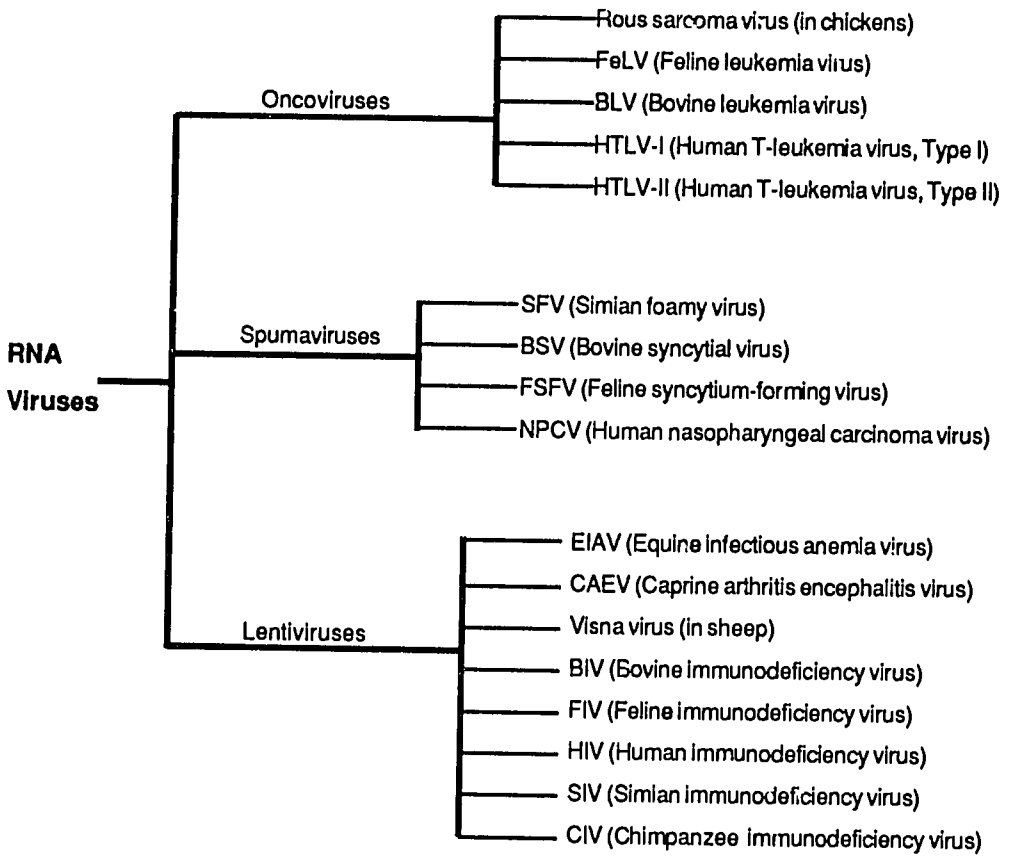
EVOLUTIONARY DIVERGENCE AMONG HIV-RELATED VIRUSES

Researchers attempting to define the evolutionary relationships between the various SIV subtypes and HIV-1 and HIV-2 use three basic lines of evidence—genomic organization, nucleic acid homology, and amino acid homology. Dr. Etsuko Moriyama (National Institute of Genetics, Mishiwa, Japan) and colleagues pointed out that different relationships emerge depending on which genes are being examined and compared. For example, when the *gag* and *env* genes are examined, SIVagm (SIV found in African green monkeys) is observed to be closest to HIV-2, but when *pol* and *nef* are examined, SIVagm is closer to HIV-1. The authors suggest that these differences can be reconciled in a phylogenetic tree where HIV-1 and HIV-2 are shown to evolve independently from SIV, and where HIV-1 and HIV-2 represent recombinant viruses derived from SIV and other related viruses.

Most theories for the emergence of HIV-1 and HIV-2 are based on the premise that HIV has been in nature "all along" and has (relatively) recently crossed species barriers. Perhaps the strongest evidence for this cross-species transmission theory comes from recent work showing considerable nucleic acid and amino acid homology between SIV from sooty mangabeys (SIVsmm) and HIV-2. The degree of homology at the amino acid level argues that SIVsmm and HIV-2 may be as closely related as the individual SIVsmm isolates are to each other. Furthermore, the natural habitat of sooty mangabeys is West Africa, where HIV-2 infection is known to be endemic. Together these data support the theory that

Figure 1.8. RNA Viruses

Animal RNA viruses are classified into three families; no evolutionary relatedness is implied in this illustration. Oncoviruses, or transforming viruses, cause cancer; spumaviruses are called "foamy" viruses because of the distinct appearance they induce in cells they infect; and lentiviruses, or slow viruses, are so named because of their lengthy incubation period.



SIVsmm could have crossed the species barrier, infected humans, and in changing hosts, mutated to HIV-2. These findings do not, however, explain the origin(s) of HIV-1.

A theory for the origin of HIV-1 does emerge from new findings on an isolate recovered from chimpanzees (SIVcpz), reported by Dr. Thierry Huet (Institut Pasteur) and colleagues; see "SIV" above). Previously, SIV strains have been shown to be closer to HIV-2 than to HIV-1 with respect to genomic organization and sequence homology, suggesting that SIV may have crossed species lines and infected humans, eventually giving rise to HIV-2.

However, Dr. Huet's report gives evidence for one of the missing links between SIVs and HIV-1. The genomic data, as well as the amino acid comparison data, place the chimpanzee isolate SIVcpz closer to HIV-1 than to HIV-2 or any of the other SIV strains. Although SIVcpz may be closer to HIV-1 than any other primate retrovirus studied thus far, it is nevertheless sufficiently divergent from HIV-1 to warrant classification as a distinct primate retrovirus: Sequence analysis revealed divergence greater than 50% (in nucleic acid sequence) between SIVcpz and HIV-1 for several regions, including the *gag* gene. This represents a greater divergence than any seen for all the HIV-1 sequences characterized to date. SIVsmm and SIVmac, while distinct from HIV-2, do not show the same high degree of divergence, suggesting that a strain, or strains, more closely related to HIV-1 will likely be found.

ANIMAL MODELS

Research into the disease-causing mechanisms of HIV cannot rely on test tube or other artificial environment experiments exclusively. Data from *in vivo* studies (both in man and in relevant animal systems) is essential to the identification of underlying causes of disease manifestations, as well as to the development of therapeutic and prophylactic intervention strategies. Furthermore, other aspects of HIV infection—such as the transmissibility of the virus and clinical disease course—necessitate the use (and in some cases, the development) of animal models. Chapter 5: VACCINE DEVELOPMENT discusses animal models used in the testing of candidate HIV vaccines.

HIV and AIDS have some predecessors in the animal kingdom—specifically, lentiviral infections in horses, sheep, cows, goats, cats, monkeys, and perhaps apes (see Figure 1.8). Lentiviruses, the family of retroviruses to which HIV belongs, exhibit a peculiar "slowness": The onset of overt disease symptoms takes a relatively long time from the moment of infection. Other common features of lentiviruses include cell tropism, cytopathic consequences—cell death and cell fusion—the ability to establish persistent infection, and the ability to mutate at a horrendously rapid rate.

Dr. Bill Narayan (Johns Hopkins University School of Medicine, Baltimore, Maryland) discussed the many similarities and important differences of lentiviral infections among various animals. An overview of lentiviral infections reveals that all animals show a loss in their helper/inducer T lymphocyte population, but apparently in ungulates (horses, sheep, cows, and goats) the loss is not as detrimental as in human and nonhuman primates and in cats.

Infection in ungulates is accompanied by inflammatory responses (the result of overstimulation of the immune system) with a relatively low degree of immunosuppression, whereas infection in primates and cats results in severe immunodeficiency. Molecular examination of individual animal lentiviruses shows strong similarities in life cycles and in the complexity of their genetic regulation. Ongoing investigations into viral gene activation by cellular factors in each model system may provide some clue to the observation that ungulates are generally able to survive persistent (lifelong) infection.

One intriguing common thread being studied by Dr. Narayan's group and others is cell tropism by lentiviruses. The primary target of lentiviral infection appears to be macrophages in ungulates but T lymphocytes in primates and cats. These host preferences may ultimately explain the differences in pathogenesis of the individual animal lentiviruses.

NONHUMAN PRIMATES

Because of their close evolutionary relationship to man, simians and great apes (chimpanzees) are considered to be potentially the most informative animal model for the study of AIDS pathogenesis. To date, only experimentally infected Asian macaques develop an AIDS-like disease; naturally infected simians (African green monkeys, for example) do not seem to manifest any disease symptoms. However, it is hoped that the study of nonpathogenic and pathogenic lentiviruses, individually and comparatively, will help pinpoint pathogenic determinants of HIV infection in man. Studies of simians and chimpanzees artificially infected with HIV-1 and HIV-2 seek to define host responses, which may lead to new approaches for the development of antiviral drugs or protective vaccines.

Simians. Macaques experimentally infected with SIV typically develop disease symptoms approximately 2 to 12 months and die within 6 to 24 months after infection. Disease manifestations are quite similar to those seen in humans: lymphadenopathy, dramatic declines in T lymphocytes (CD4 cells), numerous opportunistic infections, and, in some cases, encephalopathy (degenerative brain disease) and lymphoma. Parallel immune system changes have been observed as well: Dr. Archie Shum (University of California, Los Angeles,

School of Medicine) and colleagues reported that two markers of immune activation (neopterin and beta-2-microglobulin) were found to change over time and relative to the immunodeficiency state of the animal. Elevated levels of these immune activation markers—and of cellular markers (B cells and killer cells)—correlated with disease progression.

Several other investigators are attempting to systematically characterize host responses. Professor Reinhard Kurth and coworkers (Paul Ehrlich Institute, Langen/Frankfurt, FRG), for example, reported findings suggesting that host defense mechanisms may account for the lack of pathogenic effect in SIVagm-infected African green monkeys. Cellular immune responses and antibody responses were noted to be less vigorous in these monkeys than responses seen in HIV-infected humans. The authors pointed out that several factors could account for this observation, notably limited variation within a strain of SIVagm (as shown by comparing sequential isolates with the originally injected isolate) combined with low virus production, perhaps reflecting a high degree of intracellular suppression of viral replication.

In an overview of simians as animal models, Dr. Murray Gardner (University of California, Davis) pointed out that many simian experiments are based on the use of clones of the prototype SIVmac strain (isolated from immunosuppressed rhesus macaques). Infection of macaques with this prototype strain results in a highly aggressive clinical course in animals that fails to muster a strong antibody response: Death usually occurs within 7 months. Dr. Gardner remarked that such infection more closely resembles acute pediatric AIDS cases, with the much shorter incubation period.

Dr. Patricia Fultz (Emory University, Atlanta) discussed her group's interesting findings: An extremely virulent SIV strain was isolated from a pig-tailed macaque 14 months after experimental infection with a less virulent SIV strain (from sooty mangabey monkeys). The "parental" isolate (designated SIVsmm) was shown to possess very different cytopathic properties from the derived isolate (designated SIVsmmpbj) when examined *in vitro*. Most impressive, however, were the *in vivo* findings: The parent SIVsmm strain induces progressive immunodeficiency, whereas SIVsmmpbj causes acute disease with death resulting within 1-2 weeks.

Dr. Fultz stressed that these findings mean that we now have an animal system in which the *in vitro* biologic and cytopathogenic properties of these two viral isolates can be correlated with their *in vivo* pathogenesis. Preliminary *in vitro* data from Dr. Fultz's group and from Dr. Deborah Birx and coworkers (Walter Reed Army Institute of Research, Rockville, Maryland) show evidence for SIVsmmpbj-induced cytokine production—a potentially important finding that may ultimately prove to be related to the observed acute *in vivo* pathogenesis. (Cytokines are soluble proteins, produced and released by various immune cells, that act as chemical messengers in an elaborate cell-cell communication network; see Chapter 2: PATHOGENESIS & IMMUNOLOGY for a detailed discussion of cytokines.)

Although early studies showed that rhesus macaques are not sensitive to HIV-1 infection, Dr. Nicholas Lerche (California Primate Research Center, Davis) and coworkers presented data at the Conference demonstrating that these monkeys are sensitive to HIV-2 infection: seroconversion (the development of a specific antibody response) was noted 4 weeks after experimental inoculation. However, immunological and clinical findings revealed normal CD4 cell (helper/inducer T lymphocyte) counts, and no clinical symptoms have been observed (the oldest monkey has been infected for 1 year). Since the preliminary data indicate that HIV-2 is nonpathogenic in these infected macaques, the authors are looking for clues to the pathogenic determinants of this virus. Sequential isolates have not thus far differed much from the original inoculated isolates.

Chimpanzees. Much interest has been generated by the recent identification of an HIV-related isolate in two naturally infected but healthy chimpanzees in Gabon, reported by Dr.

Martine Peeters (Institute of Tropical Medicine, Antwerp, Belgium) and colleagues in 1989. Since chimpanzees are man's closest relatives, they would likely be excellent animal models. As discussed above, this isolate (SIVcpz) is very close in genomic content to HIV-1, another finding that makes the chimpanzee attractive as a model of HIV-1 infection.

Previous chimpanzee studies have shown that they can be infected by HIV-1 through inoculation (seroconversion is seen after 6 weeks), but they do not manifest disease symptoms, although some HIV-1-infected chimpanzees have developed transient immunosuppression. The longest-infected chimpanzee has been infected now for a little more than 7 years, which makes the chimpanzee unsuitable as a model system for HIV infection. (Note: chimpanzees are not susceptible to SIV infection.)

CATS

Two retroviruses are known to cause immunodeficiency states in domestic cats and both are apparently natural infections, prevalent worldwide. Feline leukemia virus (FeLV), first identified in 1963, is an oncovirus (tumor-causing virus). Feline immunodeficiency virus (FIV), a lentivirus first described in 1986, shows remarkable similarities to HIV in its virologic, immunological, and clinical aspects of infection. At the molecular level, the FIV genome exhibits gene regulation akin to that seen in HIV, particularly with respect to the LTR. The FIV LTR can be transactivated by cellular activation signals and by genes of certain co-infecting viruses, as shown by data presented by Dr. Ellen Sparger (University of California, Davis).

Cats infected with FIV manifest overt disease symptoms comparable to those seen in AIDS patients (lymphadenopathy, opportunistic infections, and neurological symptoms, for example) approximately 2 years after infection. The immune response pattern in FIV-infected cats also correlates with the immune response to HIV in humans. Dr. Takuo Ishida and coworkers (Nippon Veterinary & Zootechnical College, Tokyo) compared the immunological status of cats in various stages of infection (asymptomatic, ARC, and AIDS) with that of healthy control animals. The degree of cellular dysfunction, such as decreased responsiveness to mitogen stimulation, was noted to parallel the disease state. Abnormalities in antibody response and cytokine production were also observed.

Dr. Michael Tortem (University of California, Davis) presented his team's recent findings that implicate FeLV as a viral cofactor in disease progression of FIV-infected cats. Cats infected with either virus typically have a relatively long latency period, remaining healthy for about 2 years. Molecular analysis of dually infected cats showed a 10-fold increase in the amount of FIV RNA and DNA in lymphocytes and lymph nodes when compared to cats infected with FIV alone. Furthermore, FIV DNA was detected in co-infected cats, in tissues not normally targeted by FIV (kidneys, for example).

Ongoing studies will seek to define, if possible, the mechanism(s) by which disease progression is enhanced. Several potential mechanisms are already being discussed:

- the possibility of transactivation of FIV by FeLV
- immunosuppression by FeLV leading to enhanced FIV replication
- *in vivo* evolution of a chimeric isolate between FIV and FeLV

Other viral cofactors are also being investigated (including herpesviruses, infectious peritonitis virus, and foamy virus).

MICE

For several reasons, the laboratory mouse is a convenient animal model for studying human diseases and disorders. Unlike the other animal models of HIV infection discussed above, mice apparently are not naturally infected by a lentivirus and are not sensitive to

HIV infection. However, as past studies have shown, mice strains can be manipulated to provide a choice animal model system for HIV infection.

SCID Mice. Laboratory mice with severe combined immunodeficiency, or SCID (a mutation that can be created in inbred mouse strains), have no T or B lymphocytes and therefore cannot mount an immune response. Reconstitution of the immune system with human cells has proved successful: Introduction of adult human blood lymphocytes into SCID mice apparently does not lead to tissue rejection when these cells are injected intraperitoneally (but not intravenously). This model was pioneered by Dr. Donald Mosier and colleagues at Medical Biology Institute, La Jolla, California, in 1988, and is now being used by numerous research groups in the development of HIV vaccines.

SCID-hu Mice. Alternatively, SCID mice can receive implants of fetal human lymphoid tissue; now referred to as SCID-hu mice, this model was developed by Dr. Joseph McCune (SyStemix, Inc., Palo Alto, California). In both types of SCID mice, human lymphoid and myeloid (bone marrow) cells are identified and both can be readily infected by HIV.

Dr. Linda Rabin (SyStemix, Inc.) reported her team's finding that SCID-hu mice are not susceptible to infection with laboratory-adapted strains of HIV. In contrast, when fresh isolates from HIV-infected patients are injected (intravenously), new virus production can be detected, signaling the establishment of infection. Virus is localized in the engrafted lymph nodes; detection of virus in the blood, however, is a time- and dose-dependent phenomenon. Dr. Hideto Kaneshima and coworkers (SyStemix, Inc.) reported data demonstrating active infection 10–14 days following inoculation and lasting for as long as 12 weeks.

Because the SCID-hu mouse has circulating human lymphoid cells susceptible to HIV infection, it can be used to evaluate HIV-specific drugs. Dr. McCune discussed antiviral drug testing using this model: When HIV-infected SCID-hu mice were treated with the reverse transcriptase inhibitors AZT (azidothymidine or zidovudine) and ddI (dideoxyinosine), viral activity was suppressed. This effect was observed to be dose-dependent, and removal of the drug resulted in subsequent reappearance of virus.

Transgenic Mice. Mice can also be manipulated at the genetic level: the complete or partial HIV proviral genome can be inserted into chromosomes of "founder" mice, which because they now carry specific transgenes (genes of another species), are called transgenic mice. To establish strains of mice harboring all or part of the HIV genome, founder transgenic mice are bred to nontransgenic mice and progeny mice are examined for the presence of the transgene(s). Reported at the Conference were data from ongoing studies on transgenic mice that carry HIV *tat*, *nef*, and LTR and also those which carry noninfectious proviral DNA (deleted *gag-pol* sequences). Although such studies are in the early stages, it appears that transgenic mice that carry even a single HIV gene develop some kind of abnormality. Dr. Peter Dickie (now at National Institute of Allergy & Infectious Diseases, Bethesda) reported that the litters of founder transgenic mice carrying the *nef* transgene are much smaller in number than those of control mice, and a significant percentage of surviving progeny mice carrying the transgene(s) show persistent dermatosis. It is hoped that careful study of individual gene products *in vivo* will shed light on the function and full range of properties of these gene products.

22

CHAPTER 2

Page 23 **Background**
23 **An Overview of the Immune System**
24 Cellular Components
26 Cytokines and Soluble Components
27 Normal Immune Response
28 **Immune System Under Attack**
28 HIV Heterogeneity
29 Preferential Infection
31 HIV Entry: CD4-Receptor Mediated
33 HIV Entry: Non–CD4-Receptor Mediated
34 Direct and Indirect Immune Cell Destruction
36 **Natural Defenses**
36 Neutralizing Antibodies
37 Cytotoxic T Lymphocytes (CTLs)
38 Antibody-Dependent Cellular Cytotoxicity (ADCC)
38 **Disease-Causing Mechanisms**
39 B-Cell Activation
39 Autoimmunity
39 Monocyte/Macrophage Inefficiency
40 Immature-Cell Depletion
40 Cytokine Induction
42 Enhancing Antibody Induction
43 HIV-Associated Malignancies
44 **Suggested Cofactors**
44 Mycoplasma
45 Other Cofactors
46 **Factors Affecting Disease Progression**
46 Viral Properties
46 Immune Response Factors

PATHOGENESIS & IMMUNOLOGY

BACKGROUND

The insidious disease process triggered by HIV infection lays its foundation by undermining the body's intricate defense system—the immune system. This sophisticated network is formed by many cell types that act in concert to combat the innumerable pathogens, or disease-causing agents, that bombard the body daily.

HIV infection provides a chilling example of how the entire immune system can be crippled by compromising several of its key cellular components. An efficient immune response relies on the ability of multiple cell types to coordinate their attack; any disruption of this cellular cooperation can wreak havoc. By incapacitating certain cells, HIV insulates itself from immune attack so it can continue to replicate and infect and kill other host cells. Of even more significance is the compromising of potential immune responses against future infections. Thus, persons with established HIV infection frequently succumb to "opportunistic" infections that would normally be eliminated by a healthy immune system.

AN OVERVIEW OF THE IMMUNE SYSTEM

The uncompromised immune system functions through a series of defense mechanisms led by the skin and the cilia lining the trachea and the intestines, which usually provide effective physical barriers against the many invading microorganisms. Pathogens that penetrate these barriers encounter the complex array of immune system cells, each of which performs a precise function in the recognition of foreign microorganisms as "non-self" and the subsequent elimination of them. Some immune system cells eliminate pathogens such as bacteria and viruses; others are key to surveillance and removal of cancerous cells or important in neutralizing toxins produced by certain microorganisms. Still other of these cells have crucial regulatory roles, integral to initiation or curtailment of immune responses.

CELLULAR COMPONENTS

The primary immune system cells are lymphocytes and macrophages. (See Figure 2.1). Macrophages are extremely versatile cells, which can be found in virtually every tissue of the body; their major functions include scavenging (phagocytosis) and antigen presentation (see "Monocytes and Macrophages," below). Lymphocytes—white blood cells distributed throughout the body—can be further subdivided by function. These cells recognize antigens (substances that trigger an immune response) and mount a specific response against them. Lymphocytes can be divided into two main classes:

- B cells—responsible for antibody production (referred to as humoral immunity)
- T cells—responsible for a variety of antigen-specific "priming" and killing functions as well as regulatory functions (cellular immunity).

B and T cells, as well as macrophages and other immune system cells, arise from "unprogrammed" cells called stem cells, which can either develop into specialized (differentiated) cells or self-replicate, replenishing the stock of generic stem cells.

B cells. Stem cells must undergo a series of molecular and cell-surface changes before they become mature B cells, which bear antibody molecules (immunoglobulins) on the cell surface. These surface molecules enable the cell to recognize, and bind to, a specific antigen, such as a viral protein.

When a mature B cell encounters the antigen to which its antibody molecules can bind, the cell is triggered to clonally expand, generating many copies of the sensitized cell. Some of these clonally expanded cells become *plasma cells*, which secrete large quantities of antigen-specific antibody. High levels of circulating antibodies maximize the binding and removal, or clearance, of the offending pathogen from the system. (Antibody response to an antigen can be diagnostically monitored by measuring the titer, or concentration, of antigen-specific antibodies in the blood. Thus exposure to HIV elicits an antibody response that can be detected by screening a blood sample for the presence and titer of antibodies that specifically recognize HIV. See Chapter 4: DIAGNOSTIC TESTS FOR HIV for details.) On exposure to antigen, not all of the clonally expanded B cells actively secrete antibody—some become *memory cells*. These serve as an expanded reservoir of antigen-specific cells, primed to initiate an accelerated secondary response should the antigen reappear months, or even years, later.

T Cells. Circulating stem cells that reach the thymus gland are stimulated to undergo the molecular and cell-surface changes that will transform them into mature T cells. Prior to their entry into the thymus, stem cells lack two cell-surface proteins, CD4 and CD8; for this reason, these cells have been dubbed "double negative." Within the thymus, immature "double-positive" ($CD4^+CD8^+$) cells, possessing both cell-surface proteins, develop from these precursor cells. A small proportion of these cells are thought to further differentiate into mature, functional $CD4^+CD8^-$ or $CD4^-CD8^+$ cells, although these mature subsets may also differentiate directly from double-negative ($CD4^-CD8^-$) precursors. These proteins together with another cell surface protein, the T cell antigen receptor (TCR), enable mature T cells to effectively bind to target antigens and carry out their "effector" functions—such as cell killing or cell regulation.

Mature T cells leave the thymus and enter the circulation, traveling throughout the body and concentrating in the spleen and lymph nodes where they are likely to encounter circulating antigens. T cells, like B cells, also have the capacity to reside for many years as memory cells after an initial encounter with antigen. T cells are subdivided into four main categories:

- **Cytotoxic T lymphocytes (CTLs)** destroy foreign cells, virally infected cells, and other infected or chemically altered host cells. Most CTLs bear the CD8 surface protein and lack CD4.

Figure 2.1. Major Cellular Components of the Immune System

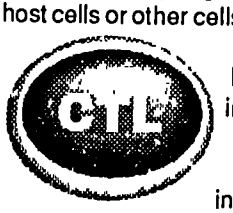
B cells
Lymphocytes responsible for the humoral (antibody) arm of the immune response. On recognition of foreign antigen, B cells expand in number; mature B cells, or plasma cells, secrete large quantities of antibody (immunoglobulin molecules).

Suppressor T cells
Lymphocytes that bear the CD8 surface receptor and inhibit the response of other immune cell types, probably by means of cytokines.

CD4 (helper/inducer) cells
This class of T lymphocytes, which bears the CD4 receptor, can be further distinguished according to function as either helper cells or inducer cells. On recognition of foreign antigens, CD4 helper cells bolster the response of other immune cell types, whereas CD4 inducer cells promote the development of immature lymphocytes. Both "helping" and "inducing" functions are carried out by cytokines (chemical messengers) released from activated CD4 cells.

NK cells
Lymphocytes that function in the destruction of virally infected and damaged host cells. NK cells are believed to be one of the effector cell types of antibody-dependent cellular cytotoxicity (ADCC). Cells coated with antibodies are recognized by NK cells, which may release pore-forming proteins to solubilize the cell's membrane.

Cytotoxic T cells
Lymphocytes that bear the CD8 surface receptor and are capable of lysing, or killing, virally infected host cells or other cells "seen" as nonself. It is thought that cytotoxic T lymphocytes (CTLs) kill infected cells by making irreversible lesions in the membrane of the infected cells on contact; these lesions may or may not be caused by pore-forming proteins.



Macrophages
Highly mobile immune cells that act as scavengers, engulfing and destroying foreign antigens and damaged host cells, including virally infected host cells. (Monocytes, which are restricted to bone marrow and the circulatory system, are the precursor cells of tissue macrophages.) Macrophages also play a primary role in presenting antigens to CD4 cells and other cell types, "gearing up" the immune response. Macrophages produce and release a number of cytokines, including tumor necrosis factor and gamma-interferon.

- **Suppressor T cells** inhibit B and T cell function, "turning off" an immune response that is no longer needed. Suppressor cells bear CD8 cell-surface proteins and lack CD4 molecules.
- **Helper T cells** secrete proteins called lymphokines that regulate the activity of B cells, other T cells, macrophages and other accessory cells. Helper cells possess the CD4 cell-surface protein and are therefore referred to as $CD4^+$.
- **Inducer T cells** support the maturation of immature T and B cells. These cells, like helper cells, are $CD4^+$. (Helper and inducer cells are functionally distinct populations; however, they are not separable in routine laboratory assays and are therefore collectively referred to as helper/inducer cells)

  Note: while many cell types (other than T cells) carry the CD4 protein on their surface, cells designated as CD4 cells—in the context of HIV research—actually refer to T helper/inducer lymphocytes.

Although both B and T cells are equipped to specifically recognize antigen, there is an important functional difference between the two cell types. Whereas the B cell receptor (an antibody molecule) is secreted and circulates in the bloodstream to bind soluble ("free") antigen, the T cell antigen receptor (TCR) is affixed to the T cell surface and can only bind to antigen that is appropriately displayed on the surface of so-called "antigen-presenting cells" such as macrophages, dendritic cells, and activated B cells. This added complexity of antigen recognition ensures that T cells will be correctly targeted to the appropriate antigen. Thus a CTL that recognizes a viral envelope protein will recognize its antigen on the surface of a virally infected cell, which it will then effectively destroy. Circulating (non-cell associated) virus, which would only distract the T cell from its proper function, are invisible to the T cell despite the presence of the "correct" viral protein antigen.

Natural Killer Cells. Natural killer (NK) cells are lymphocytes that are not targeted to specific antigens; thus NK cells are referred to, along with macrophages, as the "nonspecific" arm of the immune system. These highly potent cell killers destroy infected or malignant cells by poking holes in the target cell membrane with pore-forming proteins.

Monocytes and Macrophages. The precursor stem cells, which give rise to T and B cells, also differentiate into monocytes. While similar in function to their mature "fixed-tissue" counterpart (the macrophage), monocytes circulate in the blood. Differentiation of monocytes into macrophages occurs when the circulating monocyte penetrates into tissue.

Macrophages are highly mobile units of the immune system, patrolling body tissues in search of foreign substances. One of the primary functions of macrophages is phagocytosis—the engulfment and degradation of invading pathogens and cellular debris. Before this can occur, macrophages must first bind to the antigen on the pathogen's surface. Degraded antigens (antigenic peptides) are presented on the macrophage's surface and are then recognized by T cells; it is this function to which "antigen presenting cell" refers. Thus macrophages serve both as a surveillance cell and as a primer for the T lymphocyte.

CYTOKINES AND SOLUBLE COMPONENTS

Cytokines are soluble proteins released by various cells to act as chemical messengers, giving specific signals to target cells (those bearing appropriate receptors). Cytokines secreted by lymphocytes are termed *lymphokines*. Some cytokines promote the maturation of specific target cells, while others are important in augmenting or suppressing a variety of immune responses. Many of these cytokines have multiple functions, which vary according to the type of cell targeted by their signal. Relevant cytokines, the cells they target, and their functions are summarized in Table 2.1.

Table 2.1. Cytokines: Targets and Functions

Cytokine	Target Cell	Functions
IL-1	All immune cells	Co-stimulator of growth and differentiation
IL-2	T cells	Growth factor
	NK cells	Growth factor; activator
IL-3	Stem cells	Induce macrophage, granulocyte and erythroid differentiation
IL-4	B cells	Activate B cells
	T cells	T cell growth factor
IL-5	B cells	Growth factor
IL-6	B cells	Stimulate B cell differentiation and antibody secretion; growth factor
	T cells	Activation; differentiation factor
	Thymocytes	Proliferative signal
	Myeloid cells	Proliferative signal
TNFs	Macrophages	Recruit and activate macrophages; increase their cytotoxic potential
	T cells	Co-stimulator in antigen-induced T cell proliferation
IFNs	Antigen-presenting cells	Induce expression of MHC antigens; increase NK cell activity
CSFs	Stem cells	Induce macrophage and; or granulocyte differentiation

Increasingly clear is that many of the cytokines interact to mediate their effects. Thus, a proper balance of these soluble messengers is critical for a coordinated and effective immune response; a balance that in many HIV-infected individuals is disrupted (see "Cytokine Induction," below).

NORMAL IMMUNE RESPONSE

The normal immune response against an invading pathogen is characterized by several key features:

- recognition of specific foreign antigens
- correct cellular signaling between different arms of the immune system
- establishment of memory cells "primed" to respond to the antigen, should it reappear

Antigen Recognition. The ability to discriminate between "self" and "non-self" (foreign) molecules is integral to an efficient immune system. Interestingly, this capability is not built into the genetic program of an individual—it is "learned" during fetal life, when the immune system is first developing. Occasionally, this state of "self-tolerance" breaks down, and the immune system mounts an inappropriate attack against the body's own tissues. Diseases such as rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis are examples of autoimmune disorders; others such as juvenile-onset diabetes (Type II diabetes) and multiple sclerosis are thought to have an autoimmune component that has not as yet been identified.

Proper Cellular Signaling. Once an invading pathogen has been identified as foreign, an attack is triggered, setting off a complex interactive cascade of events mediated by multiple cellular and soluble immune components. For example, viral invasion first signals macrophages and NK cells to carry out their respective functions: NK cells destroy virally infected cells, while macrophages ingest the viral particles and display the viral antigens on their membranes, thereby alerting T cells to the antigenic challenge. Only T cells bearing antigen receptors specific for those viral antigens will be stimulated to proliferate; the activated T cells (primarily the helper T cell subset) "turn on" other immune cells, including virus-specific CTLs, which can rapidly clear the virus by destroying virally infected cells. Similarly, B cells bearing cell-surface antibody molecules specific for the virus are stimulated to clonally expand and secrete large quantities of antibodies that aid in the clearance of remaining free virus. As the threat diminishes, suppressor T cells are stimulated to terminate the response, probably by secreting soluble suppressor factors.

Laying Down Memory. Antigen-specific T and B cells are clonally expanded as a consequence of their activation by antigen. Once the immune response has subsided, these cells become quiescent and return to the circulation and body tissues as an expanded reservoir of antigen-specific "memory" cells. Should the same virus invade the host again (months, or even years later) these cells will be primed to respond more rapidly and vigorously in a secondary immune response. Antigen-specific memory is the basis for the success of vaccines in the prevention of many viral diseases: Immunization with a modified (non-disease-causing) form of the virus elicits a primary immune response that establishes virus-specific memory cells capable of clearing the "real" virus in a future (secondary) response, should the host become infected.

IMMUNE SYSTEM UNDER ATTACK

The AIDS virus has proved to be a very elusive pathogen for many reasons. First of all, HIV has adopted a strategy by which it can move about virtually freely within the immune system, dodging destruction as a foreigner by various immune system cells. HIV may be able to evade recognition by a clever, although not unique mechanism, whereby its surface antigens—primarily the envelope glycoproteins—change. Therefore, memory immune system cells primed to detect a specific HIV antigen will not recognize the altered antigen. Also, HIV can hide within host cells in a so-called latent state. In essence, HIV outwits the system.

HIV proceeds to attack the immune system by choosing as its host the very cells that are designed to protect the body from disease. As discussed below, HIV preferentially infects the T helper/inducer cells, which serve as the primary coordinators of the immune system. Depletion of these cells through direct cell killing is complemented by an oblique attack on various immune system cells: HIV infection can indirectly deplete reserves of immune system cells by inducing formation of abnormal giant cell masses, called syncytia.

HIV HETEROGENEITY

Many researchers have speculated that HIV's heterogeneity—its ability to vary its antigens—seems to be the key to this virus's ability to cause disease. It was shortly after the discovery of HIV that subtle differences were observed among the individual HIV strains that had been isolated through that date: the French isolate (LAV), the Washington, D.C., isolate (HTLV-III), and the San Francisco isolate (ARV) shared many essential molecular and immunological characteristics but were clearly (although only slightly) different from one another. Since those early observations, it has become evident that HIV has the capacity to mutate very quickly.

Not only have diverse "geographical" strains exhibited degrees of variability, but also strains isolated from an infected individual: HIV isolated at different times from the same

person have been found to vary. As pointed out by Dr. Jay Levy (University of California, San Francisco), different HIV strains have distinct properties that determine

- the types of cells they are able to infect
- the extent and efficiency to which they replicate in these cells
- the amount of cell killing that results from productive infection

Indeed, the replication pattern of a particular HIV strain is thought to influence the rate of disease progression in certain individuals.

Even strains of HIV that are only mildly cytopathic (inducing only a relatively small degree of cell damage), such as those isolated from asymptomatic individuals, may become more virulent with time. Dr. Levy's research team has found that viruses isolated from these patients after onset of AIDS display a wider cellular host range, spread more rapidly to infect new cells, and are highly cytopathic. Furthermore, strains isolated from one individual may exhibit a preference for certain tissues, such that viruses from the brain or gastrointestinal tract can often be distinguished from those residing in other tissues.

Dr. Levy summarized recent work in his laboratory that seeks to pinpoint portions of the viral genome responsible for these differences in virulence and tissue distribution. A small change in a tiny portion of the gene that contains the blueprint for the HIV gp120 envelope protein seems to be sufficient for increasing the virulent properties of the virus (see "Factors affecting Disease Progression," below). Locating such areas in the viral genome will allow scientists to target these regions for specific antiviral therapies.

PREFERENTIAL INFECTION

HIV mortally wounds the immune system by preferentially infecting and destroying the CD4, or helper/inducer, T cell. (In the context of HIV research, the term CD4 cell—previously T4 cell—refers only to the helper/inducer T cell subset, even though some non-T cells also bear the CD4 molecule on their surfaces.) Other cell types bearing the CD4 molecule also appear to be infectible by HIV, but further study is needed to ascertain whether this is an *in vitro* artifact. T cells lacking the CD4 molecule (CTL and suppressor T cells, for example) do not appear to be infectible by HIV. Monocytes and macrophages, which are now known to bear at least small quantities of CD4 on their surfaces, have been shown to be infected both *in vitro* and *in vivo*. Indeed macrophages have turned out to be a reservoir for HIV and have been implicated in spreading HIV infection throughout the body. Research reported by Tatsuo Shioda and coworkers (UCSF) suggests that the HIV's differential tropisms—for macrophages and CD4 cells—can be programmed by a region within HIV's gp120.

CD4 (T helper/inducer) Cells. Disease progression in HIV-infected individuals has been consistently observed in association with the depletion of CD4 cells (see "Markers of Disease Progression" in Chapter 7: CLINICAL MANAGEMENT). Because CD4 cells act as the crucial regulatory arm of the immune system, their destruction renders the remaining cells of the immune system functionally impotent. CD4 cells stimulate and coordinate the functions of B cells, NK cells, monocytes/macrophages, and other classes of T cells (see Figure 2.1). They secrete cytokines (or lymphokines) that stimulate their own proliferation and that ensure the proper functioning of many of the other immune system cells. However, in HIV-infected CD4 cells, cytokines such as interleukin-2 (IL-2) and gamma-interferon are not properly synthesized, or are generated in insufficient amounts. Consequently, the functions of other cells that depend on these cytokines are adversely affected.

Dr. Steven Schnittman and colleagues (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) presented data indicating that HIV preferentially infects memory CD4 cells on the basis of a cell marker, designated UCHL1. This marker has been found on memory CD4 cells but not on other CD4 cells. And, in fact, of all CD4 cells obtained from infected individuals, these memory CD4 cells were found to serve as the principal reservoir

for HIV. *In vitro* studies show that memory CD4 cells (with the marker UCHL1) are infected 4 to10 times as efficiently as cells lacking this marker. Preferential destruction of memory cells translates to dire consequences for the host, since these cells would be unavailable to respond to re-exposure to, or activation of, the vast number of infectious agents to which the person has already been exposed over a lifetime. Indeed, many of the opportunistic infections that prove life-threatening for AIDS patients are thought to have been lying dormant within the body (rather than newly infectious), formerly kept in check via efficient immune responses initiated by memory cells.

Macrophages. Because their role endows them with the capability of leaving the lymph circulation and penetrating tissues in any organ of the body to reach sites of infection, macrophages may actually serve as a Trojan horse for HIV. Originally macrophages were thought to play only a minor role in HIV-related disease, since few infected cells could be found. However, increasing evidence suggests that this cell type is a major reservoir for virus in infected individuals; more importantly, these cells are uniquely equipped to disseminate virus throughout the body, and (although controversial) may even transport HIV into the brain.

HIV infection in T cells differs notably from that in macrophages. Unlike the dramatic depletion of CD4 cells that is the hallmark of progressive disease, changes in number and overall characteristics of macrophages are relatively few, even in late disease stages, although some changes in function are observed, such as excess production of the cytokine gamma interferon. (Whereas all CD4 cells appear to be targets of HIV infection, not all macrophages are readily infected. Infected macrophages are found with high frequency in the brain, lymph nodes, and lung, but not in the liver (although the liver contains the greatest percentage of tissue macrophages).

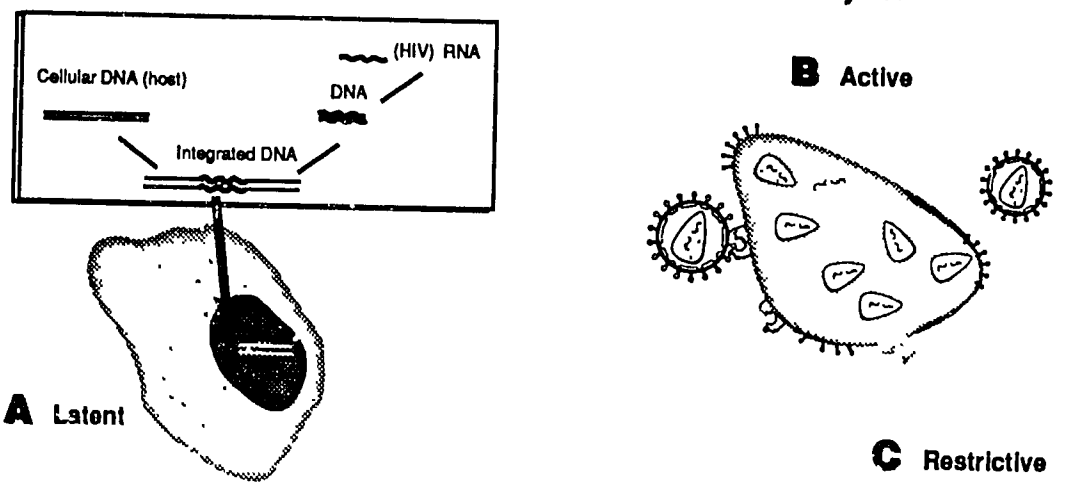
Some experimental models suggest that the ability of HIV to infect tissue macrophages depends on cellular differentiation or activation. While many cell types differentiate following cell division, macrophages are different—they replicate little, if at all, and instead become activated or differentiate on exposure to a variety of signals from the external environment. Activation of tissue macrophages during normal immune responses may render these cells susceptible to viral infection, and permit them to readily infect many other immune cells that have been attracted to the site of the immune response. In this way, HIV may have cleverly exploited the normal immune response to its own advantage.

While infection of CD4 cells results in copious viral replication and large numbers of new viral particles that bud from the infected host cell, HIV infection of macrophages is restricted—apparently very few virions are actually released from the cell surface. However, electron microscopy of infected macrophages reveals enormous numbers of viral particles accumulating within vacuoles, or cavities, within the cells (see Figure 2.2). Macrophages thus represent efficient viral factories, sequestering actively replicating virus with little apparent negative effect on the host cell. Release of this stockpiled virus during an active immune response would allow its dissemination and infection of other susceptible cells.

Other Cells. In addition to CD4 cells, monocytes and macrophages, a number of other immune and non-immune cell types are infectible by HIV, and may serve as potential reservoirs of replicating or latent virus. Diverse cell types are infectible with HIV *in vitro;* (their infectivity *in vivo* remains unclear) and include B cells, NK cells, glial cells in the brain, epithelial cells lining the colon and gastrointestinal tract, and fibroblasts.

A report from Dr. Georgi Kojouharoff and coworkers (Georg-Speyer-Haus, Frankfurt, FRG) indicates that immature stem cells from the bone marrow, which give rise to all of the white blood cells, are infectible by HIV *in vitro*. The destruction of such cells could thwart attempts by the immune system to bolster its ranks of mature cells depleted by the virus, thereby exacerbating the disease process. However, these cells do not appear to house a large viral reservoir in infected individuals, according to results from Dr. Dorothee von Laer

Figure 2.2. Latent, Active, and Restrictive Infection by HIV



A In latent infection, HIV can enter a susceptible cell, process its genetic material through the stage of integration with cellular DNA, at which point it lies dormant until the cell is stimulated. The precise triggering mechanism(s) for stimulation is unknown.

B In cells that have been stimulated, HIV viral proteins—and ultimately new virions—are produced and released; active production of HIV may or may not result in the death of the infected cell.

C Restrictively infected cells are those which produce viral proteins and new virions, but the new virions bud into intracellular sacs rather than budding from the cell surface. It is possible that the virus has adopted this mechanism so that a greater number of virions can be produced using the cell's machinery before the cell is killed. Alternatively, the cell mav inhibit viral release, because the same HIV isolate replicates actively in T cells.

and colleagues (Bernhard-Nocht-Institute of Tropical Medicine, Hamburg, FRG) and Dr. Brian Davis and associates (Stanford University Medical School, Palo Alto, California), who estimate the infection of bone marrow precursor cells at less than 1 in 1,000. Whether infection of these progenitor cells contributes to the suppression of hematopoiesis (differentiation of blood cells) observed in AIDS patients remains to be determined.

Similarly, reports from Dr. Rosemarie Pereira and coworkers (Institute of Medical and Veterinary Science, Adelaide, Australia) indicate that both immature double-negative ($CD4^-CD8^-$) and double-positive ($CD4^+CD8^+$) thymocytes (T lymphocytes residing in the thymus) are readily infected with HIV *in vitro*, although evidence for infection of these cells in HIV-positive patients was not presented by these or other investigators. Immature eosinophils (a small subset of white blood cells) are also highly susceptible to HIV infection *in vitro*, according to reports from Dr. Pierre Cornuet and colleagues (INSERM, Hôpital Paul-Brousse, Villejuif, France).

HIV ENTRY: CD4-RECEPTOR-MEDIATED

HIV utilizes a specific cell surface molecule to gain access to the interior of host cells; without access to host cells' biochemical machinery, HIV could not survive. The CD4 receptor, whose normal function is related to antigen recognition in coordination with histocompatibility proteins, was identified as HIV's portal of entry into susceptible host cells in early *in vitro* studies. (Histocompatibility proteins, found on all cells of the body, interact with the

CD4 receptor present on certain immune cells in the recognition of foreign, or non-self, antigens.) Apparently HIV's surface proteins—the protruding, knobby envelope glycoproteins—"fit" with the CD4 receptor to allow binding to take place.

T Cells. HIV clearly demonstrates a distinct preference for certain cell types, most notably, the CD4 cell (helper/inducer T cell). To appreciate the mechanisms by which HIV may gain entry into CD4 cells, it is necessary to examine how these T cells normally function. CD4 cells only recognize antigen presented on the surface of antigen-presenting cells (APCs) such as macrophages, B cells, and dendritic cells (immune cells residing in the lymph nodes and spleen). Antigen, complexed with "self" histocompatibility proteins (called major histocompatibility, or MHC, antigens), is displayed on the surface of antigen presenting cells. Displayed in this manner, specific antigen receptors on the T cell can recognize the foreign antigen, but it is the CD4 molecule that makes this bond even tighter by binding to a portion of the MHC protein. In this way, the CD4 molecule enhances the ability of the T cell to recognize antigen through its specific antigen receptor (see Figure 2.3).

The HIV gp120 envelope protein has an extremely high affinity for the CD4 protein. Once bound to the cell surface, the virus is able to enter the interior of the cell by fusion of the viral and cellular membranes. For this reason, the CD4 molecule is commonly referred to as the viral "receptor," perverting its primary role in recognition of self and nonself antigens.

Considerable effort is being made to identify specific amino acid stretches (peptides) on both CD4 and gp120 that are required for binding of HIV and subsequent infection of the host cell. Professor Ronald Penny (University of New South Wales, Sydney, Australia) explained that although HIV gp120 binds at a site distinct from the portion of CD4 that interacts with MHC antigens, HIV gp120 nevertheless interferes with the ability of CD4 to associate with MHC (Figure 2.3). Binding also interferes with the association between adhesion molecules on the T cell and others on the antigen presenting cell—these adhesion molecules act like glue to keep the cells in contact until antigen presentation is complete.

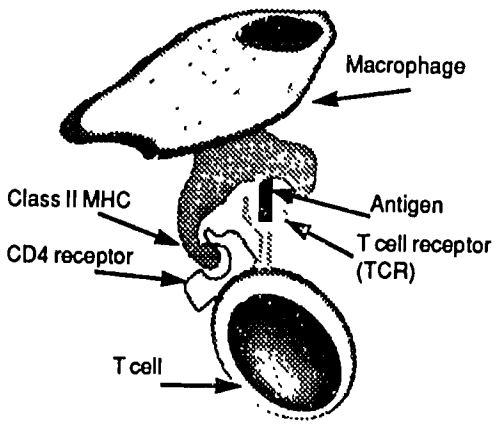
The end result of gp120 binding is that CD4 cell function is severely impaired. A major research goal is to identify the binding site, and prevent infection by blocking these sites with tailored peptides that will not interfere with the primary function of the T cell—that of antigen recognition.

Non-T Cells. Monocytes and macrophages also express (display) CD4 molecules on the cell surface, but in far fewer numbers than those on T cells. The function of this molecule on macrophages is not clear. However, according to some researchers, even a few CD4 proteins are sufficient to render cells susceptible to HIV infection. Data presented by Dr. Ronald Collman and coworkers (University of Pennsylvania Medical Center, Philadelphia) and by Dr. Thierry Jouault and associates (Pitie-Salpêtrière Medical School, Paris) indicate that CD4 functions as an obligate receptor in macrophages, even when virus initially binds to the cell via other molecules (see "Fc receptor," below).

In addition to T cells, monocytes, and macrophages, other cells in the body have been found to express CD4, seemingly providing HIV ready access to the cell's inner machinery. These include microglial cells in the brain and, possibly, epithelial cells of the bowel and dendritic cells in lymph nodes. Fibroblastic cells—previously thought to be lacking CD4—may actually bear a few CD4 receptors: In some cases infection can be blocked by adding antibodies to CD4, thereby essentially "hiding" the receptor from HIV.

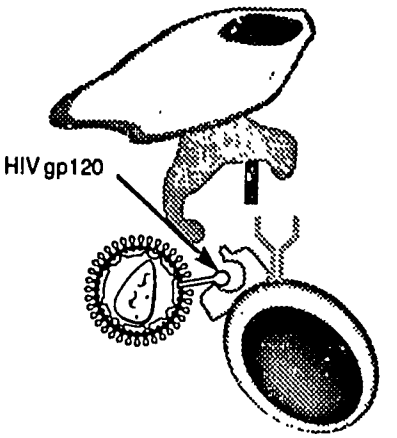
Dr. Pierre Cornuet and colleagues (Hôpital Paul-Brousse, Villejuif, France) reported that cell lines made from immature eosinophils (white blood cells involved in inflammatory responses) bear CD4 molecules and are highly susceptible to HIV infection *in vitro*, with little impact on the cell's survival. Similarly, Dr. Mamoru Sakaguchi and coworkers (New England Deaconess Hospital, Harvard Medical School, Boston) demonstrated the *in vitro* infectivity of CD4-bearing human megakaryocytic cells (the progenitors of circulating platelets).

Figure 2.3. Antigen Recognition by T Cells



Normal Antigen Presentation

T helper/inducer cells can only recognize (and respond to) antigens that are presented with surface proteins called class II major histocompatibility proteins (MHC proteins). Depicted here is normal presentation of antigens by a macrophage. Once T cells "see" antigens in this context, they begin secreting substances (cytokines) to mobilize other immune cells, such as B cells, CTLs, and other T helper/inducer cells.



HIV Interference with Antigen Presentation

When HIV gp120 binds to the CD4 receptor on T cells, normal antigen presentation cannot take place. Interaction of the antigen/MHC protein complex with the CD4 and TCR (T cell receptor) is physically prevented. (Note that it is not clear exactly how gp120 binding to CD4 prevents MHC and CD4 binding, because gp120 does not bind precisely at the MHC binding site on CD4.)

As vital as *in vitro* studies are to HIV research, numerous investigators are careful to point out that their results must be interpreted with caution. Cells cultured for any period of time in the laboratory can undergo subtle changes that can result in new surface proteins, or increased susceptibility to infection from many viruses, likely including HIV. Thus the presence of CD4 on many of these cell types, and their susceptibility to infection by HIV, must be confirmed by *in vivo* studies.

HIV ENTRY: NON-CD4-RECEPTOR-MEDIATED

Many studies suggest that CD4 is not the only mechanism by which HIV gains access to the interior of target cells. Based on several Conference presentations and studies reported in the scientific literature, supporting evidence for a second viral portal can be summarized as follows:

- HIV can infect a variety of human cells lacking CD4.
- Some human cells, despite high levels of CD4, cannot be infected with HIV.
- Some animal cells on whose surface the CD4 molecule has been experimentally placed still do not permit viral entry, although the virus successfully binds to the CD4.

A great deal of effort is being devoted to identifying non-CD4 receptors used by HIV, with therapeutic intervention the ultimate goal. Typically, such experiments involve trying to block viral infection by the known CD4 route: either by adding antibodies to CD4 (which would occupy the potential binding sites for HIV), or by adding soluble CD4 molecules (which can effectively bind free virus, thereby preventing its attachment to the cell-surface CD4 receptor). Presumably, if the CD4 receptors are occupied and HIV infection of the cell still occurs, the presence of another receptor is implicated.

Work presented by Dr. Janet Harouse and coworkers (University of Pennsylvania School of Medicine, Philadelphia) using these techniques indicates that glial cells (supportive cells in the brain) lacking the CD4 molecule are efficiently infected with HIV *in vitro* by a mechanism other than CD4 binding. Dr. Louise Evans and associates (University of New South

Wales, Sydney, Australia) used a similar strategy to demonstrate that CD4 is not involved in HIV infection of epithelial cells from the colon. Implicated thus far are the Fc and the TM (transmembrane) receptors.

Fc Receptor. Macrophages have structures on the cell surface that act as a receptor for antibodies: during a normal immune response, binding of antibodies to this Fc receptor activates the macrophages, and triggers them to increase their cytotoxic and phagocytic functions. The Fc receptor may inadvertently serve to "focus" antibody-coated viral particles to the macrophage cell surface until they can successfully infect the cell via CD4 (see "Enhancing Antibody Induction," below). In certain cells, neither CD4 nor Fc receptor binding appears to be involved in HIV infection. Dr. Jihed Chehimi and coworkers (Children's Hospital of Philadelphia) presented data indicating that HIV infection proceeds in the absence of CD4 or Fc receptor binding in purified NK cells cultured briefly *in vitro*.

TM (Transmembrane) Receptor. Dr. Lee Henderson (Tulane Medical School, New Orleans) presented data from his laboratory identifying a second cellular receptor that binds to a different HIV envelope protein, gp41. This newly identified receptor (designated TM, for transmembrane) allows HIV-cell fusion and productive infection. Cells lacking CD4 (in these experiments, neuroblastoma cells) have the TM receptor, suggesting that this cellular receptor may permit HIV entry on diverse cell types via a non-CD4 mechanism, by directly fusing with the cell membrane. Cells bearing CD4 (T cells and monocytes) also possess the TM receptor, and therefore may be more readily infected.

DIRECT AND INDIRECT IMMUNE CELL DESTRUCTION

Cell destruction and cell dysfunction characterize the pathological consequences of HIV infection; the means by which these events occur vary.

CD4 Cell Depletion. The loss of CD4 cells appears to result primarily from their destruction following infection. HIV may directly kill the host lymphocyte as a consequence of its own reproduction: when large numbers of newly assembled virions bud from the surface of the cell, the integrity of the cell is disrupted and the cell dies. In late-stage disease, the loss of CD4 cells is acute, and those which remain are defective—they cannot respond normally to HIV or other antigens and do not appear to secrete the appropriate levels of cytokines when stimulated. Abnormal CD4 function, especially insufficient cytokine production, would in turn affect the functional capabilities of other cell types such as B cells, macrophages, and natural killer (NK) cells.

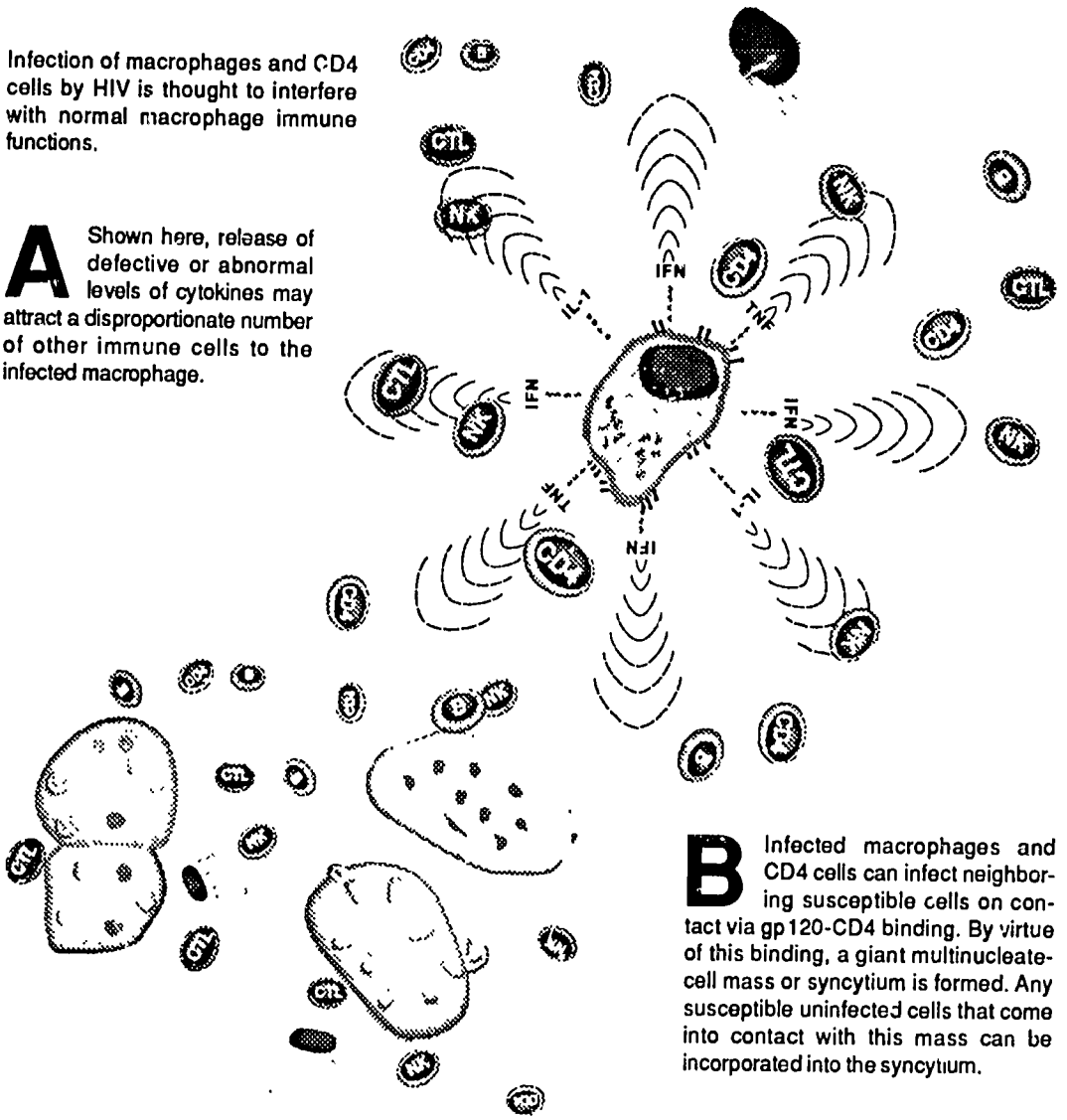
CTL/NK Cell-Mediated Killing. Infected cells may be killed not directly by HIV, but by virus-specific CTLs (cytotoxic T lymphocytes) or by NK cells. Dr. David Paul and coworkers (Rush-Presbyterian-St. Luke's Medical Center, Chicago) and George Fust (National Institute of Hematology and Blood Transfusions, Heidelberg, FRG) and associates presented evidence that HIV infection of lymphocytes *in vitro*, as well as *in vivo*, results in coating of the infected cells with complement proteins—proteins that signal scavenger macrophages to phagocytose the infected cells. Dr. Fust's team suggests that this coating contributes to the destruction and shortened survival of lymphocytes in HIV-infected patients. (See Figure 2.5.)

NK cells, although not depleted in AIDS patients, do not function properly; they cannot carry out their cell-killing function to completion. It is not clear whether these deficiencies are due to direct or indirect effects of HIV. Data presented by Dr. Jihed Chehimi and colleagues (Children's Hospital of Philadelphia) indicate that while HIV can infect NK cells *in vitro*, infection does not kill the cells, and they retain full cytotoxic capabilities. No direct evidence for NK cell infection *in vivo* has been demonstrated to date, supporting the idea that the NK cell defects in AIDS patients may result from an indirect mechanism. Since production of IL-2 (a cytokine made by CD4 cells) is low in AIDS patients, and NK cells are

Figure 2.4. Syncytia Formation

Infection of macrophages and CD4 cells by HIV is thought to interfere with normal macrophage immune functions.

A Shown here, release of defective or abnormal levels of cytokines may attract a disproportionate number of other immune cells to the infected macrophage.



B Infected macrophages and CD4 cells can infect neighboring susceptible cells on contact via gp120-CD4 binding. By virtue of this binding, a giant multinucleate-cell mass or syncytium is formed. Any susceptible uninfected cells that come into contact with this mass can be incorporated into the syncytium.

IL-2-dependent, this fact offers a potential mechanism for diminished NK cell function *in vivo*. Evidence for a direct effect of HIV on NK cells was introduced by Sirianni and coworkers (University of Rome), who suggest that interaction of the viral envelope protein (gp120) with a surface protein on NK cells may perturb NK function in the absence of infection.

Syncytia Formation. Indirect cell killing can also occur by the recruitment of uninfected cells into giant multinucleated cell masses called syncytia. A syncytium is thought to form when a single infected cell fuses with other cells via gp120-CD4 binding as they come into contact with each other. Macrophages appear to be the primary cell type involved in syncytia formation, although T lymphocytes and other cells have also been identified in these cell masses. Although syncytia are a hallmark of HIV infection, little is actually known regarding the how and why of induction of syncytia formation. One plausible theory holds that HIV-infected immune cells secrete cytokines inappropriately, calling in other immune cells, which are then in position to come into physical contact with the infected cell and become part of a cell mass.

Macrophage Dysfunction. Whereas HIV infection of CD4 cells results in their destruction, infection of macrophages is not usually cytolytic; that is, HIV does not appear to kill the host macrophage. Abnormal production and secretion of cytokines as well as altered antigen presentation capabilities are thought to be the primary pathological consequences of HIV infection of macrophages. By disabling—without killing—these cells, HIV can travel virtually unnoticed throughout tissues. Infection of macrophages also contributes indirectly to the pathologic effects, tissue injury, and cell destruction observed in other immune system cells and possibly within the nervous system. For example, macrophages release many secretory products that influence nerve growth and tissue repair following injury. Inappropriate secretion of these cytokines in brain tissues may cause localized tissue injury, which may contribute to some of the neurological symptoms of advanced disease (see Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION). To compound this potential problem, macrophages can also respond to signals from injured nerve cells that instruct them to secrete even more toxic substances—in this way, a cyclic pattern of cellular damage may be initiated.

NATURAL DEFENSES

In the early stages of HIV infection, the body elicits a vigorous immune attack against the infiltrating virus (see Figure 2.5). Dr. Andrew McMichael (John Radcliffe Hospital, Oxford University, U.K.) explained that all of the cellular participants in a normal immune response appear to function as they would upon any other viral attack:

- Antigen-presenting cells engulf the virus and initiate the immune response.
- CD4 cells become activated through their antigen receptors (TCR) and release cytokines that stimulate the immune response.
- B cells stimulated by these cytokines secrete virus-specific antibodies.
- Cytotoxic T lymphocytes (CTLs) release interferons and kill virally infected cells.

Yet despite this staunch attack, the immune system does not oust the invader—the virus is not cleared from the body. A fuller characterization of the natural defenses, and how they go awry, should provide the basis for future vaccine design and immunomodulatory therapies.

NEUTRALIZING ANTIBODIES

During an immune response triggered by HIV infection, thousands of B cells respond by secreting large numbers of antibodies. Each B cell makes a single type of antibody that can bind to a specific part of the virus, usually a small portion of an "exposed" viral protein. However, since many B cells are involved, each producing its own type of antibody, the result is a varied antibody response against diverse viral proteins. Certain of these antibodies are particularly efficient at binding to and aiding in the removal of the virus from the system, and are therefore considered to be "neutralizing antibodies." The most efficient neutralizing antibody response is one that is generated early in the host immune response—at this time the viral burden is low, fewer cells are infected, and the pathogen can be more easily eliminated. Neutralizing antibodies can accomplish this by various mechanisms:

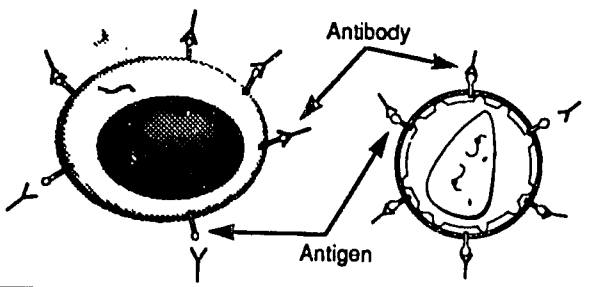
- by binding to free virus or virally infected cells, targeting them for destruction by other immune cells
- by binding to viral proteins that are important to fusion with the host cell membrane and entry into host cells, thereby preventing productive infection

This second mechanism is the subject of a great deal of research: Pinpointing the precise *portions* of the viral antigens that stimulate efficient neutralizing antibodies is crucial to designing appropriate vaccines. A major candidate for the "prime" neutralizing target on the virus is the so-called "V3 loop" of the gp120 protein, which has been described by Dr. Scott

Figure 2.5. Triple Defense

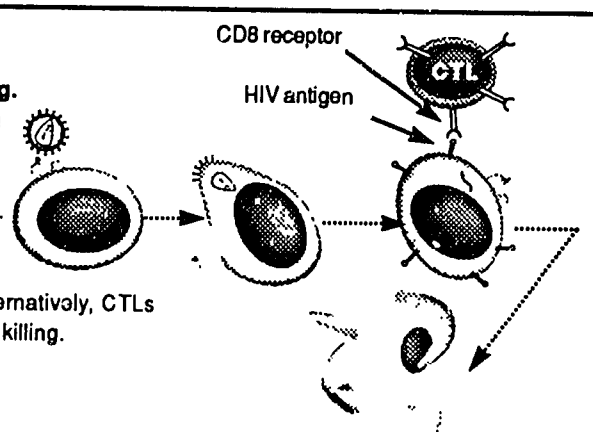
Three natural defense mechanisms have been demonstrated to act specifically against HIV.

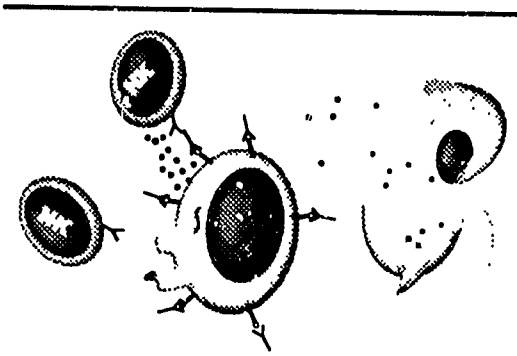
Antigen/antibody complexes



A **Neutralizing Antibodies.** Antibodies that recognize and bind to either free HIV or HIV-infected cells are called neutralizing antibodies. The resulting antigen-antibody complexes serve as targets for destruction by other immune components, and block the virion from binding to and entering susceptible cells.

B **Cytotoxic T Lymphocyte Killing.** A class of CD8-receptor–bearing T lymphocytes (CTLs) can recognize and destroy HIV-infected cells that display viral antigens on their surface. It is thought that when these cells come into contact, an irreversible lesion may be made in the infected cell's membrane. Alternatively, CTLs may suppress viral replication without cell killing.





C **Antibody-Dependent Cellular Cytotoxicity (ADCC).** Natural killer (NK) cells recognize and destroy antibody-coated HIV-infected cells. Cell killing is thought to occur through release of pore-forming proteins, which destroy the cell's membrane.

Putney and researchers at Repligen Corporation (Cambridge, Massachusetts). Dr. Mary White-Scharf and associates and Dr. Charles Scott and coworkers (Repligen Corporation) have mapped the V3 loop even further. To gauge the universality of the high level of reactivity to the V3 loop, Dr. Emily Carrow and coworkers (FDA, Bethesda, Maryland) examined sera from HIV-infected individuals from the United States, Haiti, Brazil, and Zaire. Their results indicate that the V3 loop is indeed the major neutralizing antibody target of all the isolates represented in the study. (See also Chapter 5: VACCINE DEVELOPMENT for a discussion of the V3 loop in the design of candidate vaccines.)

CYTOTOXIC T LYMPHOCYTES (CTLs)

In addition to the antibody response to HIV infection, considerable evidence testifies to a vigorous CTL response early in infection. On appropriate cues from helper/inducer T lymphocytes, CTLs are primed to seek and destroy HIV-infected cells—but they are unable to

"see" free circulating virus. The levels of circulating CTLs activated to kill HIV-infected cells can be monitored by simple *in vitro* tests. Guido Vanham and associates (Institute of Tropical Medicine, Antwerp) presented data indicating that the level of CTL activity increases during the early clinical stages of disease. However, by the time infected individuals progress to clinical AIDS, CTL activity has dropped precipitously.

Most cell types known to be infectible by HIV *in vivo* also seem to be good targets for CTL lysis (cell killing); Dr. Fernando Plata (Institute Pasteur, Paris) and associates confirmed that the HIV-specific CTL response is effective in destroying a number of infected cells of diverse origins, including infected lung fibroblasts, lung macrophages, and CD4 cells. Similarly, Dr. David Torrey and coworkers (University of Pittsburgh) described their observations that macrophages displaying HIV antigens were readily lysed by circulating CTLs obtained from the peripheral blood of seropositive individuals. Despite this apparent efficiency in killing infected cells, potent CTL activity alone does not result in the complete clearance of the virus. Using the polymerase chain reaction (PCR)—an exquisitely sensitive method for detecting small amounts of the HIV DNA sequences in infected cells—Dr. Andreas Meyerhans (Institute Pasteur, Paris) and coworkers demonstrated that although vigorous anti-HIV CTL responses could be observed *in vitro*, resting (non-activated) lymphocytes from seropositive individuals were nevertheless found to harbor proviral DNA.

In addition to lysing virally infected cells, CTLs in some HIV carriers also inhibit the replication of the virus in other white blood cells. Mari Kannagi and colleagues (Kumamoto University Medical School, Japan), Carl Mackewicz and coworkers (University of California School of Medicine, San Francisco), and Christopher Walker (Chiron Research Laboratories, Emeryville, California) and colleagues independently noted that asymptomatic HIV-infected individuals possessed CTLs that drastically inhibited HIV replication within their CD4 cells. The infected cells were not killed, but were prevented from replicating HIV. This protective effect, however, was lost with progression to AIDS. Although the mechanisms at work are not clear, it is possible that such CTLs are involved in maintaining disease latency via cytokines in asymptomatic individuals.

ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC)

Virally infected cells displaying viral antigens on their surface, to which antibodies have bound, are targeted for lysis by NK cells. Unlike CTLs, NK cells are not antigen-specific; they recognize all "antibody-coated" cells, bind to them, and then secrete pore-forming proteins, which destroy the cell by permeabilizing the membrane. Because of the role antibodies play in this process, such cell killing is referred to as antibody-dependent cellular cytotoxicity (ADCC).

Previous studies have shown that circulating NK cells from sera of HIV-positive individuals possess ADCC capabilities. At the Conference, Dr. Lauren Wood (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) reported that her team identified specific peptide sequences within gp41 that elicit ADCC activity. Although many researchers have argued that a strong ADCC response probably occurs early in HIV infection and wanes during disease progression, several groups (including Dr. Wood's group) have failed to correlate the level of ADCC with clinical status.

DISEASE-CAUSING MECHANISMS

It is becoming increasingly evident that the progression of disease in HIV-infected individuals is intimately intertwined with the normal functioning of the immune response. A multifaceted, volatile relationship forms between HIV and the immune system, and the complexity of this interaction is daunting. How does this virus adversely affect so many different cell types and so thoroughly disrupt the body's immunological network? Each year more details of HIV's pathogenic mechanisms are revealed.

B-CELL ACTIVATION

A striking feature of progressing HIV infection is the enormous numbers of activated B cells in these patients. Although many of these B cells are churning out antibodies that recognize the virus, they do not account for all the activated cells observed. Early in the course of infection, virtually every type of antibody is produced in large amounts. This condition (called hyperimmunoglobulinemia) in itself would not necessarily be detrimental to the host; however, in HIV-infected individuals, some of these antibodies are directed against "self" rather than foreign antigens. These so-called auto-antibodies can devastate the body by attacking such essential components as red blood cells, platelets, and connective tissue (see "Autoimmunity," below).

B cells do not appear to be infected *in vivo*, leading to a search for the source of their activation in HIV-infected individuals. Cytokines such as IL-5 and IL-6 can activate B cells or induce them to secrete antibody. Elevated levels of IL-6 in particular have been observed in individuals infected with HIV and may contribute to B cell activation in these patients. Dr. Peter Rieckmann and associates (National Institutes of Allergy & Infectious Diseases, Bethesda, Maryland) have shown that activated B cells not only respond to cytokines such as TNF-alpha and IL-6, but actually secrete them as well. The increased local levels of these cytokines could help maintain the activated state of these cells. To make matters worse, both of these cytokines also stimulate HIV activity in cells that have already been infected with the virus (see "Cytokines," below). Thus a cyclic pattern of activation of B cells, cytokine release, and HIV activation of other cells can emerge.

AUTOIMMUNITY

In the frenetic response to HIV infection, the massive overproduction of antibodies seems to result in a breakdown in the immune system's normal ability to distinguish between "self" and "non-self." Dr. Daniel Volker and coworkers (University of Heidelburg, FRG) presented confirming evidence that HIV-infected individuals produce a variety of antibodies that recognize CD4 cells, as well as the CD4 receptor itself. The clinical relevance of these antibodies is still unclear, although it is possible that these antibodies play a role in CD4 cell depletion. Other autoantibodies (as these self-directed antibodies are called) have been described that recognize and attack host tissues such as collagen (a structural protein), platelets (blood components crucial for clotting), neutrophils (a class of white blood cells) and lymphocytes, muscle, nerves, and even other antibodies.

This pattern of autoantibody production may lead to a variety of clinical disorders, among which may be the myopathy, or muscle dysfunction, and peripheral neuropathy, or nervous dysfunction seen in many patients in the later stages of disease (see Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION). In addition, evidence has been found implicating cellular autoimmunity as well; in cellular autoimmunity, immune cells rather than antibodies are responsible for "self" tissue damage. Dr. Ahvie Herskowitz and associates (Johns Hopkins Hospital, Baltimore, Maryland) presented evidence for HIV-associated cardiomyelopathy (breakdown of cardiac muscle) that appears to be the result of direct destruction by CTLs attacking the heart tissue. Thus numerous autoimmune disorders may be (directly or indirectly) triggered by and contribute to the general pathogenesis of HIV disease.

MONOCYTE/MACROPHAGE INEFFICIENCY

Macrophages constitute another cellular pool whose function is perturbed by HIV infection. Like B cells, monocytes and macrophages obtained from HIV-infected individuals have certain characteristics of activated cells. In addition, according to data presented by Dr. Guiseppe Gianni (Johannes Gutenberg Universitat, Mainz, FRG) and coworkers,

macrophages from HIV-infected patients are unable to kill appropriate target cells as efficiently as do those from uninfected individuals. As disease progresses, the cells become less and less potent in their ability to lyse target cells, and by the development of full-blown AIDS, this capacity seems to be entirely lost. Yet the macrophages are not compromised completely; they still retain their ability to phagocytose foreign particles and secrete cytokines such as TNF-alpha. Thus the macrophage defect is not a general one, but appears to be linked to the lytic function of these cells.

A potential mechanism for this macrophage defect was suggested by studies presented by Dr. Nejat Duzgunes and associates (University of California, San Francisco). These researchers obtained normal peripheral blood monocytes, activated them *in vitro*, and examined their ability to kill target cells under various conditions. Normal lytic function was reduced or completely abolished if they infected the cells *in vitro* with HIV. Lysis could also be blocked in *uninfected* cells if antibodies recognizing the monocyte-borne CD4 molecule were included in the experiment. (Both the virus and the antibody bind to CD4 molecules on the monocyte surface.) These experiments suggest a novel mechanism of immunosuppression by HIV: Once bound to the CD4 molecule, the viral protein blocks the receptor and renders the cell useless in its normal function—the lysis of target cells. Since destruction of virally infected cells by monocytes and macrophages is crucial to the effective clearance of virus early in infection, incapacitating this function bears severe consequences.

IMMATURE-CELL DEPLETION

In most HIV-infected individuals, a vigorous immune response is mounted against the virus. Nevertheless, natural replacement of CD4 cells does not keep pace with their HIV-induced destruction. Even in patients receiving antiviral drugs, despite suppression of viral replication, the levels of CD4 T cells never return to normal, although slight or transient increases in the cell counts are sometimes seen. This puzzling observation suggests that HIV infection may also be affecting the immature precursor cells that would normally repopulate the diminished cell community by giving rise to new, mature CD4 cells.

Dr. Anthony Fauci (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) remarked that in HIV-infected individuals, the thymus is virtually destroyed. Furthermore, very primitive precursor cells isolated from the thymus *in vitro* are readily infected with HIV. These cells are generally considered to be $CD4^-$ (so-called "triple-negative" $CD4^-CD8^-TCR^-$ cells), however, recent studies suggest that, in fact, they do have tiny amounts of CD4 on their surface. Indeed, Dr. Rosemarie Periera (Institute of Medical and Veterinary Science, Adelaide, Australia) and associates presented work indicating that in the thymus, both "double-positive" ($CD4^+CD8^+$) and "triple-negative" ($CD4^-CD8^-TCR^-$) immature cells are readily infected *in vitro* with HIV, suggesting the presence of CD4.

In addition to immature T cells, there is evidence that other types of immature white blood cells are easily infected with HIV. Dr. Andrea Sperduto and colleagues (University of California, Los Angeles, School of Medicine) presented evidence suggesting that HIV infects macrophages obtained from newborn babies 3 to 5 times more efficiently than it infects macrophages obtained from adults. These authors are currently trying to determine why a newborn's cells are so much more susceptible to viral infection, and why the disease course is so much shorter and more aggressive in children than in adults.

CYTOKINE INDUCTION

Nearly all immune system cells secrete cytokines, the soluble mediators that allow cells to communicate with each other and carry out their various functions (see "Cytokines" in "Background" section above). Cytokines can orchestrate various immune functions in the following ways:

- Expanded populations of $CD4^+$, $CD8^+$, and NK cells require cytokines such as IL-2, IL-4 (interleukins) and TNF (tumor necrosis factor) to proliferate and mediate efficient cytotoxic responses against virally infected target cells.
- B cells mature and produce antibody in response to lymphokines produced by CD4 cells (IL-2, IL-4,-5, -6 and -7).
- Inflammatory responses at the site of infection are enhanced by interferons (IFNs), TNF, IL-1,-6,-8 and the colony stimulating factors (CSFs).
- Hematopoietic responses to infection (those involved in the differentiation of blood cells) are influenced by IL-1,-3,-5, -9, and the CSFs.

Cytokines can have their own pathogenic consequences, independent of their direct effects on immune function. During the course of disease progression in HIV-infected patients, the cytokine balance becomes severely disturbed, and these pathogenic effects can become quite severe.

Tumor Necrosis Factor (TNF). One cytokine in particular, tumor necrosis factor (TNF), has recently received a great deal of interest for its possible role in HIV-associated pathogenesis. (The name tumor necrosis factor stems from its original discovery as a potent anti-cancer agent.) TNF is normally produced by monocyte/macrophages in response to inflammation. The levels of TNF are generally elevated in HIV-infected persons, although the mechanisms by which this occurs are not completely understood. In laboratory animals, chronically elevated TNF levels have been implicated in the pathogenesis of cachexia, a clinical condition characterized by rapid wasting and the suppressed production of new blood cells. Cachexia and cytopenia (a reduction in overall blood cell number) are often observed in AIDS patients, although a definitive causative role for TNF in these disorders has yet to be established.

Considerable evidence suggests that the elevated TNF levels found in HIV-infected individuals could be a direct effect of the virus, rather than the consequence of perturbation of another immune component. Dr. Annapurna Vyakarnam and colleagues (Middlesex School of Medicine, London) presented results indicating that HIV entry into CD4 cells directly causes the cells to secrete large amounts of TNF, as well as another cytokine, gamma-interferon (gamma-IFN). Interestingly, the elevated cytokine levels enhanced HIV's ability to replicate in the CD4 cells and cause syncytia formation, providing evidence for a feedback loop between HIV-induced cytokine production and enhanced viral replication.

TNF can also enhance HIV replication in monocytes and macrophages. Since this cytokine normally acts to recruit new macrophages to a site of infection, the pathological consequences of this cytokine are two-fold: not only does the increased level of TNF recruit a steady supply of uninfected cells to the virus, but it also increases the ability of the virus to replicate in these new target cells.

Interleukin-6 (IL-6). Other cytokines also may be able to stimulate HIV replication; data presented by Dr. Guido Poli (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) and coworkers indicate that IL-6 can cooperate with other cytokines, such as TNF and GM-CSF (granulocyte-mononuclear colony stimulating factor), to enhance viral replication *in vitro*. The levels of serum IL-6 are high in HIV-positive asymptomatic individuals, according to results from Mitsuo Honda and colleagues (National Institute of Health, Tokyo), and the detected levels of this cytokine appear to reflect the degree of HIV disease progression.

Cytokine Feedback Loop. Still other data presented by J. S. Justement and colleagues (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) indicate that at low levels, glucocorticoids (substances normally produced by the body in response to stress) cooperate with various cytokines such as IL-6, TNF and gamma-IFN to increase HIV replication. These results suggest a vicious cycle of HIV infection and escalated viral activity in HIV-infected individuals:

- First, HIV infection leads to increased production of TNF, IL-6, gamma-IFN, or all three cytokines.
- Increased TNF levels, in particular, recruit more uninfected cells to the site.
- Once cells become HIV-infected, these high cytokine levels result in enhanced HIV replication and viral pathogenesis.
- Increased production of HIV induces further cytokine production.

This positive feedback loop can have devastating pathogenic consequences (see Figure 2.6).

How do these cytokines lead to elevated HIV production? It appears that the mechanisms are again intimately coupled with normal immune function. During a normal immune response T cells become activated, which is a prerequisite for their full immune function. To become activated, cellular proteins (within the T cell) bind to specific (enhancer) regions of the cell's own DNA; these regions have been designated as NF-kB.

Interestingly, NF-kB sequences are also present in the HIV genome, and it is this similarity that gives HIV such a destructive advantage. Dr. Anthony Fauci (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) reported that his team examined the effects of cytokines on this portion of the HIV genome. They found that infected cells were induced by TNF to produce a protein that bound to the NF-kB site within the HIV genome. Once bound, the cellular protein appeared to stimulate the production of new virus. Thus the viral genome may remain in an inactive (latent) state in an infected T cell, and be activated to replicate when the host cell itself becomes activated through its interaction with cytokines. Several speakers at the Conference pointed out that by using such a common regulatory mechanism, the virus can undermine the immune system and cause its collapse. Further understanding of this mechanism will help direct the development of new therapeutic strategies aimed at interrupting this destructive cycle of viral induction and cell death.

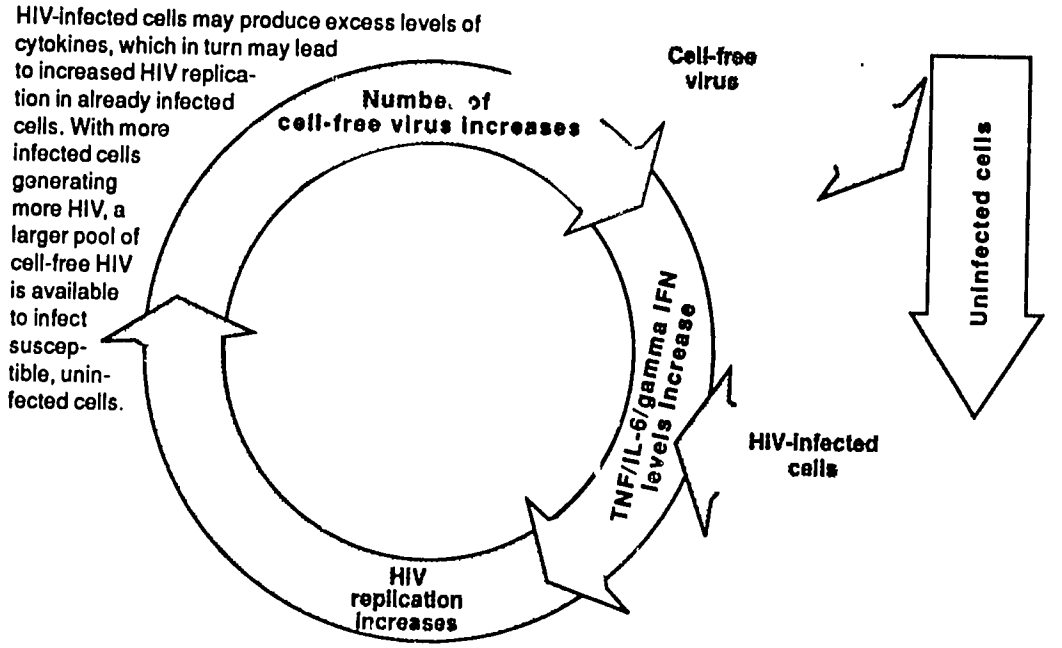
ENHANCING ANTIBODY INDUCTION

Although the immune response to HIV early in infection produces antibodies capable of neutralizing the virus (see "Neutralizing Antibodies," above), this same immune response also produces antibodies that do exactly the opposite—that is, they enhance the ability of the virus to infect cells. Several investigators theorize that this may be yet another example of how HIV has exploited the immune system to its own advantage: immune responses to the virus generate antiviral antibodies that recognize and attach themselves to HIV. The other end of the antibody molecule is free to bind to the macrophage Fc receptor, inducing the macrophage to phagocytose the virus, which then infects the cell.

Dr. Andrew McMichael (Oxford University, U.K.) pointed out that antibodies to nonneutralizing regions of the virus bind to HIV; the antibody-coated virus then becomes a target for cells such as monocytes and B cells bearing Fc receptors that can attach to the antibody. Alternatively, enhancing antibodies can activate the complement system; complement proteins bind to HIV's envelope glycoproteins, which then bind to cells bearing complement receptors and CD4 molecules.

Dr. Akira Takeda and associates (University of Massachusetts, Worcester) presented data indicating that Fc receptor–mediated enhancement probably requires the presence of the CD4 molecule for infection of target monocytic cells; that the CD4 molecule is necessary to viral entry was demonstrated by addition of soluble CD4, which blocked enhancement. The Fc receptor may serve instead to focus the viral gp120 proteins onto surface CD4 molecules, according to data presented by Carlo-Federico Perno and coworkers (National Cancer Institute, Bethesda, Maryland). However, Dr. Jay Levy and colleagues (UCSF) and others have shown that the Fc receptor is sufficient for enhancement of infection of macrophages; the presence of CD4 may serve as a facilitator of viral entry. In some cases, viral entry is achieved when the target cell phagocytoses the antibody-coated virus.

Figure 2.6. Cytokine Feedback Loop



Although some researchers find the evidence for Fc receptor involvement in HIV infection compelling, others are not yet convinced, and this question merits additional inquiry. If enhancing antibodies are a factor in HIV-related disease, a great deal of caution must be exercised in designing vaccine strategies so that vaccinated individuals produce neutralizing, and not enhancing, antibodies.

HIV-ASSOCIATED MALIGNANCIES

In addition to the opportunistic infections that pose a fatal threat to persons infected with HIV, many of these individuals also succumb to rare malignant conditions such as Kaposi's sarcoma and lymphomas (see Chapter 7: CLINICAL MANAGEMENT). Early in the AIDS epidemic, infectious disease specialists and epidemiologists noted that these two types of cancers, in particular, were commonly associated with HIV infection. The connection between HIV infection and lymphoma (cancer of the lymphoid tissue) can conceivably, at least in part, be explained by HIV's insidious infiltration—and thorough disruption—of the lymph tissue and the lymphatic system. A link with Kaposi's sarcoma—a connective tissue cancer, affecting endothelial cells that line the blood vessels—was somewhat puzzling, however. (Prior to the AIDS epidemic, Kaposi's sarcoma was primarily restricted to pockets in Africa, Mediterranean countries, and, in the United States, to older men of Jewish or Mediterranean descent. By the end of 1981, however, 96 cases of Kaposi's sarcoma had been reported to the CDC—the great majority of which were in homosexual men who were later found to be HIV infected.) Researchers in basic science have been looking for a specific etiologic agent in Kaposi's sarcoma, as well as possible mechanisms by which HIV could induce a cancerous state in endothelial cells.

Dr. Oto Martinex-Maza (University of California, Los Angeles, School of Medicine) discussed his team's recent work on cytokine involvement in HIV-related Kaposi's sarcoma. A number of Kaposi's sarcoma cell lines obtained from HIV-positive individuals were examined and

found to produce copious quantities of IL-6. Interestingly, these cells also bear specific receptors on their surface that bind to IL-6, suggesting that Kaposi's sarcoma cells (cancerous endothelial cells) may respond to this cytokine, which acts as a growth factor. Infection by HIV, then, may encourage the uncontrolled expansion of Kaposi's sarcoma cells through the overproduction of IL-6.

Another group reported their finding of a specific HIV product which stimulates the growth of Kaposi's sarcoma cells. Dr. Barbara Ensoli and colleagues (National Cancer Institute, Bethesda, Maryland) presented data implicating an HIV regulatory gene product—the *tat* protein—as the growth stimulant for Kaposi's sarcoma cells. *In vitro* experiments demonstrated that cells derived from Kaposi's sarcoma lesions of AIDS patients respond to and grow rapidly in the presence of cell culture fluid containing HIV *tat* (that is, exogenous *tat*). To test the specificity of this proliferative effect, other HIV proteins, namely gp160 and *nef* protein, were tested, but no stimulation was observed. Furthermore, when antibodies against *tat* were added (anti-*tat* antibodies), growth of Kaposi's sarcoma cells was inhibited. The authors suggest that *tat*, released by HIV-infected cells, may be responsible for either the induction or progression of HIV-related Kaposi's sarcoma.

Several groups have hypothesized and subsequently tested the involvement of various agents, including viruses, in HIV-related Kaposi's sarcoma. Dr. Jacques Corbeil (St. Vincent's Hospital, Sydney, Australia) reported that his team's PCR studies revealed no HIV, EBV, HSV-1, HSV-2, human papilloma virus (HPV), or CMV DNA in Kaposi's sarcoma cells. Perhaps what is most perplexing—and frustrating—is the knowledge that Kaposi's sarcoma was recognized as a cancer in the 1800s, obviously unrelated to HIV. Sidestepping the question of the precise agent or substance responsible, Dr. Ronald Penny (University of New South Wales, Australia) proposed a working model for the evolution of Kaposi's sarcoma in the context of HIV infection (see Figure 2.7).

SUGGESTED COFACTORS

One of the most controversial issues in the scenario of HIV pathogenesis is whether HIV alone can cause all of the associated pathological consequences, or whether other cofactors contribute to or are required for the establishment of disease.

MYCOPLASMA

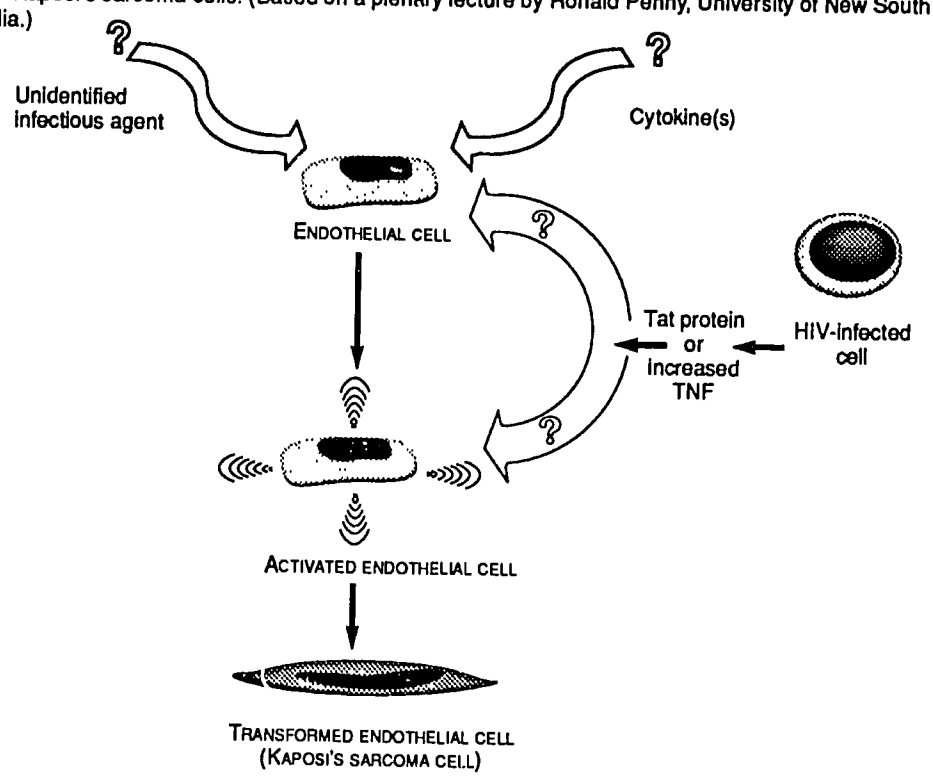
Recent studies have suggested that a small microorganism called mycoplasma may act as a cofactor. Dr. Luc Montagnier (Institut Pasteur, Paris) discussed various aspects of mycoplasma, a well-characterized parasitic organism similar to tiny bacteria, that is found in the mucosa of many individuals. Mycoplasma are also a confounding contaminant in many cell line cultures. Dr. Montagnier reported a rather surprising finding—that in almost 40% of HIV-positive individuals, mycoplasma could be detected from the blood, from tissue biopsies, and in several patients, from brain biopsies. Some HIV-infected individuals showed evidence of several distinct types of mycoplasma.

The crucial question is whether mycoplasma are simply another opportunistic infection associated with the acute immunosuppression observed in these individuals, or whether the microorganism is in fact contributory to the disease state. Interestingly, an independent report implicating mycoplasma in immune suppression appeared in the medical literature in 1986. Although originally reported as a "virus-like agent," Dr. Shyh-Ching Lo (Armed Forces Institute of Pathology, Washington, D.C.) later identified the microorganism that was isolated from patients with AIDS and other immunosuppressed patients as mycoplasma.

Dr. Montagnier's observations that some of the cytopathic effects of HIV infection, including syncytia formation and cell lysis, are inhibited or controlled *in vitro* with antibiotics specific

Figure 2.7. Working Model for HIV-Related Kaposi's Sarcoma

Numerous investigators have postulated that either a transmissible infectious agent (as yet unidentified) or soluble factors (such as excess cytokines or HIV proteins) may induce the transformation of healthy endothelial cells into cancerous Kaposi's sarcoma cells. (Based on a plenary lecture by Ronald Penny, University of New South Wales, Australia.)



for mycoplasma (such as fluoroquinolines), suggest that this microorganism may play a role in the disease process. His team also tested 21 patients before and after treatment with specific antibiotics; while some showed marked improvement in their clinical status, others showed no significant change. However, it is possible that the antibiotic regimens used were simply not sufficient to eliminate all of the mycoplasma, since the microorganism could still be detected in some patients despite treatment.

Dr. Montagnier and his team have postulated that mycoplasma can exacerbate the cytopathic effects of HIV by influencing the rate of viral replication in infected cells. *In vitro* experiments suggest that co-infection of cells with HIV and mycoplasma results in an increase in viral replication and viral antigen production that is inhibited with anti-mycoplasma drugs, such as quinolone. These researchers are quick to add that these results do not mean that HIV, in the absence of mycoplasma, is harmless—rather, they suggest that perhaps mycoplasma infections simply increase the viral load such that it is not as easily controlled or cleared by the immune system.

While these observations are not conclusive and require confirmation, they are provocative, and will likely accelerate the design and testing of new combination drug therapies aimed at eliminating both mycoplasma and HIV.

OTHER COFACTORS

Since HIV can remain in a latent state for long periods before being activated to replicate, any environmental change that might activate the host cell also has the potential to activate HIV's genome. T cells and macrophages are principal reservoirs for the latent virus; thus

their activation by any antigenic challenge may induce viral activation. This is especially true of challenge by another virus, which can stimulate both T and macrophage cell types to become activated (see "Normal Immune Response," above).

Viruses such as cytomegalovirus (CMV), Epstein-Barr (EBV), hepatitis B (HBV), herpes simplex (HSV) and human herpes virus-6 (HHV-6) have all been implicated as possible cofactors in HIV-related disease. These viruses may activate HIV indirectly through the cellular secretion of cytokines (see "Cytokines," above), or exert their modulatory effects by other mechanisms, as yet undefined.

FACTORS AFFECTING DISEASE PROGRESSION

Why do some HIV-infected individuals develop rapidly progressive disease, while others remain relatively free of any disease symptoms for as long as 10 years after seroconversion? This question is being addressed by numerous research teams. Factors such as the virulence of the particular strain of HIV involved, the individual's immune response, the status of the individual's immune system at the time of exposure, and genetic differences among infected persons may all contribute to the pathogenic course of HIV infection.

VIRAL PROPERTIES

The recent work described by Drs. Jay Levy and Cecilia Cheng-Mayer (University of California, San Francisco) highlights the potential role for small changes in the viral genome—in the envelope region—as influencing disease progression. Virus isolated from an asymptomatic individual was compared with viral samples isolated from the same patient after development of severe disease symptoms (5 months later). Although they could pinpoint only minor changes in the viral DNA (less than 3% change in the entire viral genome), these changes were sufficient to render the virus more cytopathic and cytotoxic. The later viral isolate was also able to infect a greater variety of cell types, and could multiply faster in the infected cells. These results imply that even "minor" changes in the viral genome can lead to significant differences in the properties of the virus.

IMMUNE RESPONSE FACTORS

While virtually all HIV-infected individuals mount an immune response against HIV, the potency or duration of the response may differ, allowing some people to "control" the virus for longer periods of time. A provocative study presented by Dr. Jonathan Weber and coworkers (Royal Postgraduate Medical School, London) suggests a correlation between the type of antibody response to HIV and the clinical outcome at 84 months after seroconversion. High antibody responses specific to the p24 protein were associated with prolonged survival, whereas high responses to the gp120 protein were associated with rapid progression to AIDS.

Similarly, Dr. Homayoon Farzadegan and associates (Johns Hopkins University, Baltimore, Maryland) reported data indicating that a high CD4 cell count was maintained in individuals with high titres of antibodies to the p24 protein; low antibody titres to this protein were correlated with low CD4 counts. However, similar studies of Ugandan HIV patients reported by Pontiano Kaleebu and colleagues (Uganda Virus Research Institute, Entebbe) indicated that 93% of these individuals maintained circulating anti-p24 antibodies irrespective of their clinical outcome, although the levels of these antibodies were lower in patients with progressive disease.

The genetic makeup of an individual may also influence the immune response to HIV and, in turn, disease progression. Dr. Leslie Louie and coworkers (University of California, Berkeley) studied a group of individuals already infected with HIV, and tried

to correlate their disease progression with differences in certain genes that regulate immune responses. These genes encode the major histocompatibility antigens (in humans, called HLA antigens,) that, together with the antigen, are presented to T cells to initiate an immune response (see "T cells," above). This group determined that individuals who remained asymptomatic for at least 5 years had a particular form of the HLA DR beta protein, although the mechanism by which this protein could influence disease progression is not yet known.

The clinical aspects of disease progression and the effects of early treatment with antiviral or immunotherapeutic agents are discussed in Chapter 7: CLINICAL MANAGEMENT.

CHAPTER 3

Page 49 **Background**
50 **Global Trends**
52 Americas
56 Europe
58 Africa
60 Asia
60 Australia
60 **High-Risk Groups**
60 Homosexual/Bisexual Men
62 Intravenous Drug Users
67 Sexually Active Heterosexuals
69 Hemophiliacs and Other Transfusion Recipients
70 **Other Groups Under Study**
70 STD Clinic Attendees
71 Prostitutes
73 Prisoners
74 Women
75 Adolescents/Young Adults
77 Children
77 The Homeless
77 U.S. Military Personnel
78 **Perinatal Transmission**
80 **Blood Supply Safety**
81 **Incubation Time**
81 **HIV-Associated Mortality**
83 **Other Retroviruses**
83 HIV-2
84 HTLV-I and HTLV-II
86 **Epidemiological Modelling**
86 Incubation Time
87 New Approaches to Modelling
87 AIDS Projections in the United States
88 Epidemiologic Modelling in Europe
88 Models in Pattern II Countries

EPIDEMIOLOGY

BACKGROUND

The number of diagnosed AIDS cases worldwide, as reported by Dr. Michael Merson (World Health Organization, Geneva), totaled more than 250,000 by mid-1990—almost 100,000 more than the cumulative cases in June of 1989. However, considering underreporting, incomplete or delayed reporting, and under-diagnosis, the actual figure is believed to be closer to 700,000. Current estimates (revised in July 1990 by WHO and considered conservative) suggest that between 8 and 10 million people are infected with HIV worldwide. These statistics provide an overall sense of the magnitude of the pandemic (global epidemic), but it is the detailed analyses—those which reveal the patterns and how they shift—that serve as the keystone for public health planning.

Extensive worldwide collaborative efforts by scientists in many disciplines led to identification of the etiologic (causative) agent of AIDS and the means by which this disease was being spread. Epidemiologists served as global "detectives," using demographic information as clues to identify transmission modes—ways in which the then unidentified infectious agent was being spread from person to person. Based on the early case reportings (in 1981 and 1982) of what would later be called AIDS, it was suspected that a transmissible agent was involved and that it was spread by blood-to-blood contact or via semen. After a great deal of study and scientific debate, transmission of HIV is now known to occur via blood, semen, and breast milk.

Other body fluids continue to be evaluated as potential routes of transmission. Although HIV has been detected in saliva, most researchers think it an unlikely vehicle because the harsh enzymatic environment of the oral cavity and upper gastrointestinal tract is believed to preclude survival of the virus. No evidence has been found for aerosol (airborne) transmission, and no cases have been reported of HIV infection acquired through casual contact (such as hugging, shaking hands, or sharing utensils) with an infected person.

Differences in transmission patterns can be distinguished around the globe, and epidemiologists have attributed this largely to cultural distinctions. For example, although needle sharing accounts for many AIDS cases in western countries, little if any, IV drug abuse

Table 3.1. Groups Identified as At-Risk Worldwide

- Male homosexuals/bisexuals
- Intravenous (IV) drug users
- Persons born in, residing in, or traveling to countries where heterosexual transmission is the primary mode of transmission (Pattern II countries, such as sub-Saharan Africa and the Caribbean)
- Sexual contacts of HIV-infected or at-risk individuals (such as sexual contacts of IV drug users)
- Hemophiliacs
- Blood transfusion recipients
- Children born to seropositive mothers
- Occupationally exposed personnel

occurs in parts of rural Africa, where the prevalence of HIV infection in the general population is relatively high. In North America, Western Europe, and Australia, homosexual/bisexual men and IV drug users (IVDUs) constitute the largest at-risk populations, whereas in Africa and the Caribbean, prostitutes, promiscuous heterosexuals, and persons with a history of sexually transmitted diseases have been identified as the groups at highest risk for acquiring HIV infection.

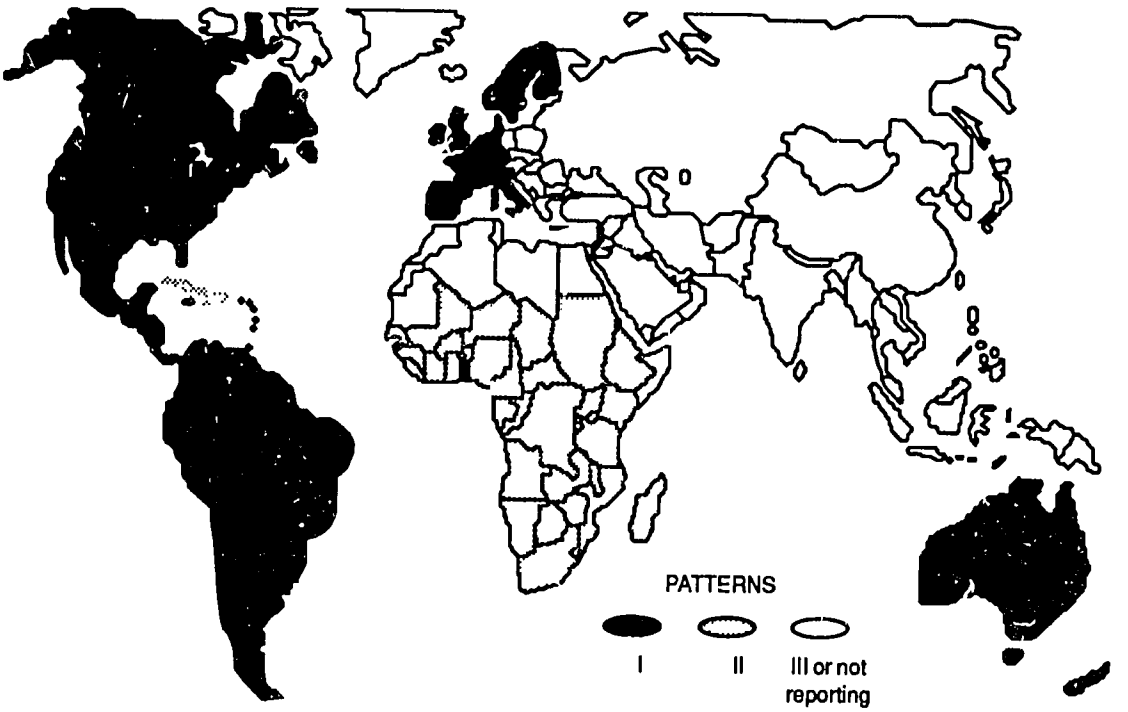
Numerous epidemiological studies are being conducted in an effort to ascertain the extent of HIV infection in discrete populations, either defined geographically or by risk category. One of the best ways to collect such information is by determining the serostatus of the population—that is, establishing the incidence or seroprevalence of HIV infection among the population by screening blood samples for the presence of antibodies to HIV. Public health officials use past and current epidemiological data for discrete populations, together with relevant demographic information, as the structural basis for their ultimate goal: the design and implementation of AIDS prevention programs.

GLOBAL TRENDS

Analysis of AIDS incidence data from across the world has led to the recognition of three distinct geographic patterns of HIV transmission. Several speakers at this year's Conference, however, stated outright that such a division may be an oversimplification of the epidemic—primarily because the epidemic has not stabilized in populations in different areas of the world. Nevertheless, these patterns can serve as a basis for evaluating the current status and emerging trends of the HIV epidemic in various parts of the world.

- Pattern I is characterized by disproportionately high numbers of AIDS cases among homosexual/bisexual males and IV drug users: roughly 80% to 90% of all cases occur within these two risk categories. Pattern I countries—including North, Central, and South America, Western Europe, Scandinavia, Australia, and New Zealand—currently show a relatively small percentage of heterosexual transmission cases; the male to female ratios typically range from 10:1 to 15:1.
- Pattern II has heterosexual sexual contact as its primary mode of transmission; the number of HIV-infected females and males is approximately equal. HIV transmission in Pattern II countries occurs almost exclusively by heterosexual contact, transfusions with blood or blood products, and perinatal transmission. Pattern II countries include sub-Saharan Africa and the Caribbean.
- Pattern III is generally restricted to those countries in which HIV has recently been introduced—and therefore relatively few AIDS cases have been reported. The first reported AIDS cases in Pattern III countries were in general linked to imported (contaminated) blood or blood products from Pattern I countries. Sexual contact and/or needle sharing with persons from Pattern I or II countries now also appear responsible for the spread of HIV into Pattern III countries. North Africa, Eastern Europe, the Middle East, and Asia are considered to be in the Pattern III category.

Figure 3.1. Global Transmission Patterns



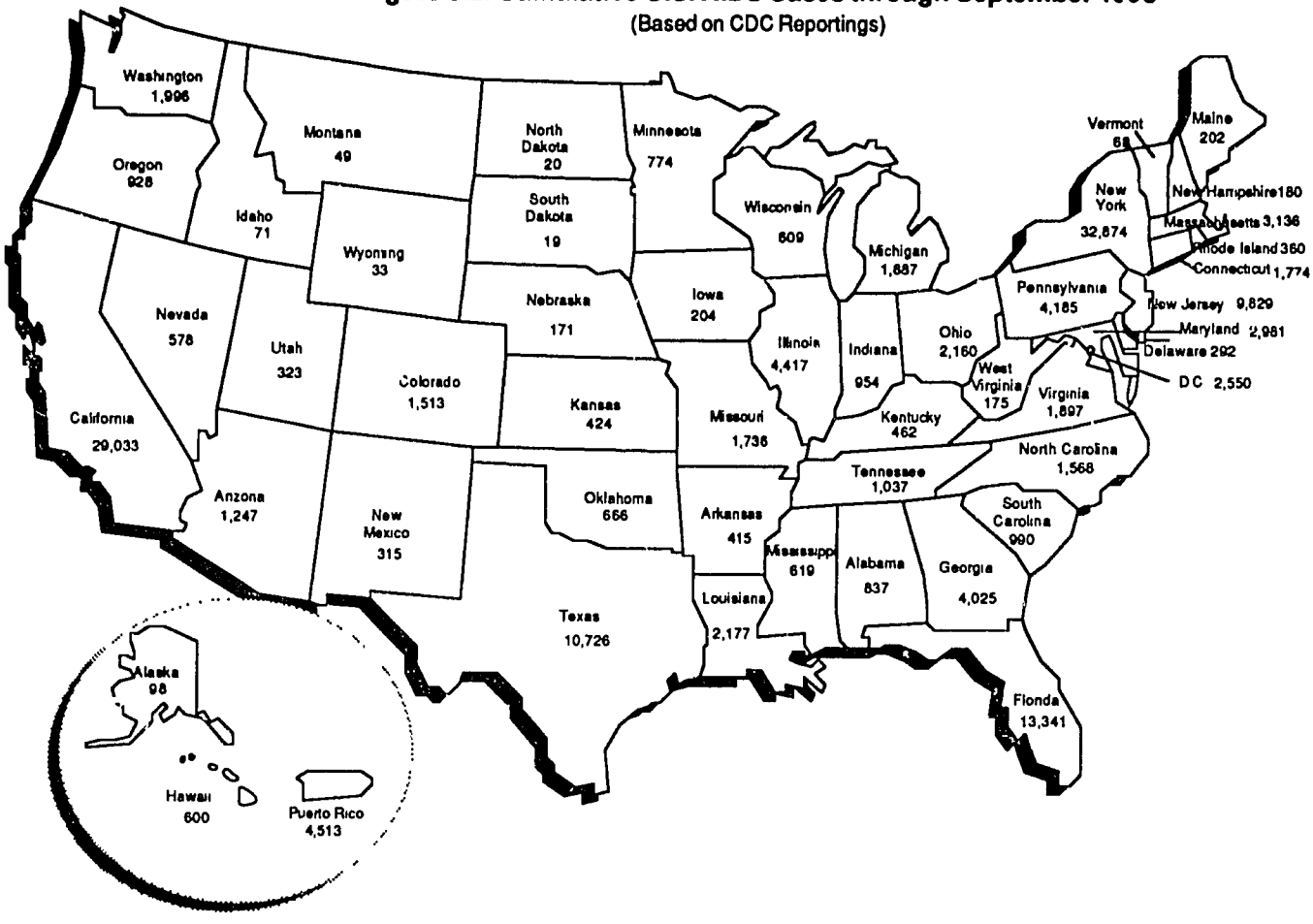
The World Health Organization (WHO) coordinates worldwide AIDS incidence data in an effort to glean information on the global situation and possible changing trends. More than 150 countries are now reporting AIDS cases to WHO, although such reporting is not always regular or current. The number of AIDS cases reported to WHO by continent (as of the end of September 1990) is as follows:

Continent	No. of Cases
Africa	75, 642
Americas	183,266
Asia	790
Europe	39,526
Oceania	2,293
Total	301,517

The United States reports the most number of AIDS cases (152,126), followed by Uganda (15,569), Zaire (11,732), Brazil (11,070), and France (9,718). Thus far, Asia, Oceania, Eastern Europe, the Middle East, and North Africa account for only about 1% of all reported AIDS cases in the world.

Because only diagnosed AIDS cases—and not cases of HIV infection—are reportable, it is difficult to evaluate epidemiological and demographic changes of at-risk and infected populations on a national, regional, or global scale. Although HIV seropositivity is not a reportable condition, most people in the public health field believe that seropositivity—rather than AIDS incidence—should be monitored since AIDS surveillance reflects the epidemiology of HIV infection a few years earlier; it is not an accurate reflection of the present status of the epidemic. Furthermore, fragmented epidemiological studies from discrete populations (whether defined by risk group or by geographic locus) generally cannot be extrapolated to the general population. Nevertheless, such studies provide invaluable information and can be used as a basis for predicting emerging trends and to identify opportunities for intervention.

Figure 3.2. Cumulative U.S. AIDS Cases through September 1990
(Based on CDC Reportings)



AMERICAS

Of 183,266 AIDS cases reported to WHO from the Americas as of September 30, more than 89% (152,126) were from the United States. Countries with the next highest numbers of cases, in order, are Brazil (11,070), Mexico (4,941), Canada (4,402), Haiti (2,456), and Dominican Republic (1,368).

Although all the Americas are categorized as Pattern I countries, several Conference presentations pointed out that a shift appears to be occurring in the predominant routes of HIV transmission in Mexico and some Central and South American countries—particularly, Brazil and Honduras. An increasing number of heterosexual contact cases are being reported in these countries whereas, in the early years of the epidemic, homosexual/bisexual men accounted for the largest proportion of AIDS cases. Analysis of continued surveillance, of AIDS incidence and HIV seroprevalence data, will indicate whether a transition from Pattern I to Pattern II is truly occurring—and perhaps, more importantly, how such a change was able to occur.

United States. Dr. Alan Lifson (San Francisco Department of Public Health) pointed out that in the United States, ethnic and racial minorities continue to be the hardest hit by the AIDS epidemic. Several studies noted increased seroprevalence among blacks and Hispanics in various risk categories compared to that among whites. Based on data from seven states, Marta Gwinn (Centers for Disease Control, Atlanta) reported that the prevalence of HIV anti-

Table 3.2. Cumulative Canadian AIDS Cases through September 1990
(Based on data prepared by Federal Centre for AIDS, Ottawa, Ontario)

Province/Territory	# Cases
British Columbia	809
Alberta	267
Saskatchewan	39
Manitoba	46
Ontario	1,734
Quebec	1,388
New Brunswick	26
Nova Scotia	66
Prince Edward Island	3
Newfoundland	19
North West Territories	3
Yukon	2
Total	4,402

body (an indicator of exposure to HIV) was 5 to15 times higher in black women than in white women. Many Conference speakers also called attention to the disturbingly high seroprevalence rate in the adolescent/young adult population in several states.

Whereas numerous seroprevalence studies show that a large percentage of homosexual/bisexual men are HIV-infected, 1989 statistics reveal that, when compared to the early years of the epidemic, the rate of new AIDS cases reported for this population is much lower (increasing only 8% between 1988 and 1989, in contrast to a 70% rate of increase for the period 1987 to 1988). Dr. Timothy Green and coworkers (Centers for Disease Control, Atlanta) reported a substantial drop, since 1987, in the increase of AIDS cases among white homosexual/bisexual men and among nonwhite and white heterosexual IVDU. The authors offered that a decline in new HIV infections in the early to mid-1980s as well as a lengthening of the incubation period for HIV may account, in part, for this observation; possible reasons for these changes are discussed in the "Modelling" section of this chapter. The Centers for Disease Control (CDC) announced earlier this year that cases attributable to heterosexual contact increased by 27% for 1989 compared with the previous year.

Regionally, an increase in new AIDS cases for 1989 was observed in the South, which accounted for 31% of all new cases (a 22% increase over 1988), compared to 31% for the Northeast, 24% for the West, and 10% for the Midwest. Some of the larger metropolitan areas are reporting a leveling or even decrease in AIDS incidence. Pauline Thomas and colleagues (New York City Department of Health), for example, reported declines in the total number of AIDS cases in New York City for 1989 compared to 1988. However, CDC statistics revealed that smaller urban areas showed increases for the same period: some with populations between 100,000 and 500,000, had new cases increased by 32%; some with populations under 100,000, by 35%.

Canada. Approximately 74.6% of all adult AIDS cases in Canada are attributable to homosexual/bisexual activity, IV drug users account for 0.1% of all cases, while homosexual/bisexual IVDUs account for 3%. The geographic distribution of AIDS cases, by province, shows that Ontario has the highest number of cases (1,734), followed by Quebec (1,388) and British Columbia (809). Using HIV seroprevalence data rather than AIDS incidence may be the better way to monitor the AIDS epidemic; changing trends can be observed much earlier. So argues Dr. Michel Alary and colleague (Hôpital du St. Sacrement, Quebec) who reported new data from 4 anonymous HIV-testing centers in Quebec (3 in Montreal and 1 in Quebec City). A trend in monthly HIV seroprevalence was noted among individuals reporting IV drug use as a

risk factor. Seroprevalence among this group was 19% (17 of 91) for 1989, compared to 4% (2 of 54) for 1988. IVDUs accounted for 9% of all seropositives in this study, compared to 2% observed for the previous year. The authors pointed out that this trend among IVDUs has not yet been detected by AIDS surveillance.

Latin America. As a whole, Latin America has been categorized as Pattern I (with homosexual/bisexual men as the major risk group). However, recent dramatic increases in AIDS cases among women (due to heterosexual transmission) in parts of Latin America have alerted public health officials to further analyze the situation. Dr. Laurent Zessler (Pan American Health Organization, Washington, D.C.) reported that in some Latin American countries, the number of AIDS cases diagnosed among women in 1988 multiplied 6-fold compared to 1986.

Brazil. The AIDS epidemic in Brazil is in a dynamic phase. A glance at the change in AIDS incidence rates (per million population) over time clearly illustrates this point: in 1982 (when the first cases were reported), the rate was 0.05; in 1985, 3.6; in 1987, 15; and in 1989, 26. Dr. Lair Rodrigues (Ministry of Health, Brasilia) reported that the rise in number of AIDS cases could be attributed, in part, to increases within areas previously identified as affected and to a continuing spread of HIV infection from metropolitan centers to surrounding (previously unexposed) communities.

A shift in the distribution of AIDS cases has also been observed: the number of cases is declining in the homosexual/bisexual population while increasing in IVDUs and the heterosexual population (although the increase in IVDUs now appears to be leveling off somewhat). Among women, the number of cases is increasing (primarily attributable to either IV drug use or sex with an IVDU) along with a concomitant rise in perinatal transmission cases. Furthermore, an enormous change in the male-to-female ratio for AIDS cases has been noted from 1984 to 1989—from 120:1 to 9:1.

Mexico. Not unlike some other Latin American countries, Mexico is showing signs of rapid transition from Pattern I to Pattern II. In contrast to the early years of the epidemic when homosexual men accounted for approximately 65% of all AIDS cases (and bisexual men, 24%), only 37% of all AIDS cases in 1989 were among homosexual men (bisexual men showed little change, 20.5%). Dr. Jaime Sepulveda (Ministry of Health, Mexico) provided statistics illustrating the dramatic rise in heterosexual contact cases in Mexico: in 1986, less than 4% of all AIDS cases were attributed to heterosexual transmission, whereas in 1989, slightly more than 20% are in this category. The proportion of transfusion-related cases is quite high in Mexico (about 23% in 1989), which is thought to be related to the commercial sale of blood (now illegal). Interestingly, IVDUs are not a major risk category (in 1989, still less than 2% of all AIDS cases). Bisexual men and contaminated blood are cited as the probable conduits for rapid increase in heterosexual transmission.

Dr. Jose Luis Mora and colleagues (Ministry of Health, Mexico) reported that annual HIV seroprevalence rates (based on 700,000 individuals in 70 cities and in 19 community groups), revealed a recent rapid increase in the spread to women and children. Dr. Carlos Magis and coworkers (Ministry of Health, Mexico) reported that women now represent 12.6% of all AIDS cases; 60% of these are transfusion-associated and 30% attributable to heterosexual transmission.

Honduras. In comparing the epidemiology of Honduras with neighboring Central American countries, Dr. Richard Beach (University of Miami School of Medicine, Florida) and associates, using statistics through September 1989, found that the male-to-female ratio of AIDS cases (1.3:1) indicates that this country exhibits Pattern II—not Pattern I—transmission. (Guatemala had a male-to-female ratio of 12:1 and Costa Rica, 27:1.) Ongoing seroprevalence studies in all 3 countries, however, show that HIV infection is spreading rapidly in the general population, with the large number of bisexual men possibly providing the "bridge" from homosexual to heterosexual transmission.

Table 3.3. Cumulative AIDS Cases for Mexico, Caribbean, and Central and South America through September 1990

(Reported by Dr. Jaime Sepulveda, Directorate of Epidemiology, Ministry of Health, Mexico, and updated with figures provided by Pan American Health Organization)

Country	# Cases	M:F Ratio	Country	# Cases	M:F Ratio
Mexico	4,941	6:1	CENTRAL AMERICA	1,448	
CARIBBEAN	5,896		Belize	11	3:1
Anguilla	4	1:1	Costa Rica	199	11:1
Antigua	3	n/a	El Salvador	192	5:1
Bahamas	507	1:1	Guatemala	92	8:1
Barbados	127	4:1	Honduras	743	2:1
Cayman Islands	5	4:1	Nicaragua	8	6:1
Cuba	63	4:1	Panama	203	15:1
Dominica	121	5:1	SOUTH AMERICA	14,363	
Dominican Republic	1,368	2:1	Argentina	710	19:1
Grenada	17	4:1	Bolivia	11	n/a
Guadeloupe	195	3:1	Brazil	11,070	9:1
Haiti	2,456	2:1	Chile	178	13:1
Jamaica	166	2:1	Columbia	643	13:1
Martinique	135	3:1	Ecuador	81	12:1
Montserrat	1	n/a	French Guiana	191	2:1
Netherlands Antilles	26	6:1	Guyana	108	5:1
St. Kits and Nevis	18	1:1	Paraguay	16	n/a
St. Lucia	16	2:1	Peru	352	9:1
St. Vincent and the Grenadines	22	1:1	Suriname	67	3:1
Trinidad and Tobago	648	3:1	Uruguay	133	10:1
Turks and Caicos Islands	8	2:1	Venezuela	870	19:1

Dr. Roxane Gonzalez and coworkers (Pan American Health Organization, Washington, D.C.) reported that The 1988 incidence of AIDS among women (per million population) in Honduras was 20.76, which is approaching that calculated for the Caribbean (34.33). Furthermore, these authors noted that coastal areas of Honduras (bordering the Caribbean) had the highest case rates.

The Caribbean. Categorized as Pattern II (with heterosexual contact as the predominant transmission mode), Suriname and the Caribbean islands have provided the only regional exception to the Pattern I transmission seen in the Americas.

Trinidad. In 1989, heterosexual contact cases represented 43% of all new AIDS cases in Trinidad, with a male-to-female ratio of 3:1. Dr. Farley Cleghorn (The University of the West Indies/CAREC, Port of Spain) and colleagues reported that 90% of all cases are among Afro-Trinidadians, although they represent less than half (42%) of the island's population. Indo-Trinidadians, the island's other major ethnic group (at 41% of the population), account for only 8.7% of the AIDS cases. The authors suggest that this finding may be related to cultural differences as well as a genetic difference: a protein associated with Kaposi's sarcoma is absent in the Indo-Trinidadian group.

Haiti and Dominican Republic. A study of 397 Haitian and Dominican sugar cane workers, presented by Dr. Mirtha Capellan (University Technologica de Santiago) and associates, showed that HIV seropositivity was significantly higher among Haitians (15%) than Dominicans (4.4%). The male-to-female ratio was 1:1. Samples were also screened for

syphilis, HTLV-1, and malaria, but no evidence for a cofactor role was found. Self-reported risk factors, however, suggested that sexual and ritualistic practices may influence HIV transmission.

Cuba. Although Cuba reported its first AIDS case in 1987 and only 63 total cumulative cases have been reported to WHO, a presentation by Dr. Carlos Moas (Finlay Medical Society, Miami) suggested that the true scope of the AIDS epidemic in Cuba is unknown due to political circumstances. Seroepidemiological data reported by the Cuban government indicate that there are 333 HIV-positive persons in Cuba. The government, nevertheless, has noted dramatic increases in the incidence of gonorrhea and syphilis, as well as HIV-related diseases, such as *Pneumocystis carinii* pneumonia. Annual studies conducted by the Cuban Health Ministry, in which approximately 2.2 million Cubans have been tested, reveal a 0.001% HIV seropositivity in the general population. Dr. Moas stressed that these official numbers are unlikely to be correct.

Refuting the official numbers is a study of 25,000 Cuban emigrants at Opa Loca, Florida, conducted by the Finlay Medical Society in 1980: the overall seropositivity rate (determined from samples collected at this time) was found to be 0.4% to 0.6%. Based on similarities in demographics and hepatitis B seroprevalence rates between the Cuban populous and the Opa Loca Cuban emigrants, Dr. Moas argued that the emigrant group could be considered representative of the Cuban population. Fuelling expectation of higher than reported numbers—both of HIV seropositives and AIDS cases—is the extensive exchange between Africa, where HIV is endemic, and Cuba: more than 400,000 Cubans have been to Africa and returned, and more than 250,000 young Africans have been trained in Cuba.

EUROPE

Of the total number of AIDS cases reported in Europe, more than 90% are reported from Western Europe. Except for the outbreaks reported from the USSR in 1989 and in Romania earlier this year, little has been heard about AIDS in Eastern Europe. The opening of the East to the West, however, has allowed an unprecedented exchange of qualitative information regarding the HIV epidemic in this region.

Eastern Europe. AIDS was first reported in Eastern Europe in 1985; with a relatively small number of cases reported since, these countries are categorized as Pattern III. Surveillance data continue to show the spread of HIV to be markedly slower than that observed in Western Europe. In Czechoslovakia, GDR, Poland, and Hungary, most AIDS cases (69-89%) occur among homosexual/bisexual men, as reported by Professor Sieghart Dittmann (Central Institute of Hygiene, Microbiology, & Epidemiology, Berlin, GDR). In Poland and Yugoslavia, IVDUs represent the major emerging risk group. In Bulgaria, a high percentage of heterosexual contact cases has been reported.

USSR. Since the 1989 report of nosocomial (hospitalization-associated) transmission of HIV to more than 230 children and 12 mothers, much interest has been focused on HIV infection and AIDS in USSR. The infection in the children was traced to the repetitive use of contaminated needles in a hospital in Elista. Of particular interest were reports of HIV transmission from some of these seropositive infants to their mothers during breastfeeding; the details are discussed in "Women" in this chapter.

Dr. Vadim Pokrovsky (Central Institute of Epidemiology, Moscow) reported on HIV seroprevalence among Soviet citizens: 57,397,200 have been tested since 1987; of these, 510 were found to be HIV-positive, with 40.5% attributed to use of contaminated syringes. Heterosexual contact was identified as the major risk factor in 19.35% of cases. HIV-positive persons were further characterized as follows: 402 among the aboriginal population; 11 of 22,083,871 blood donors; and 9 of 10,565,181 pregnant women. Although disputed, the USSR has officially reported only 40 cases of AIDS to WHO as of October 1, 1990.

Figure 3.3. Cumulative AIDS Cases in Europe
(Based on WHO Reportings through September 1990.)

Romania. With the establishment of a national AIDS surveillance system in early 1990, an alarming number of HIV-infected children and children with AIDS was reported. By June 1990, 670 AIDS cases had been reported for Romania, with 92% (617) occurring among children. (Note: by September 1990, the number of AIDS cases had risen to 999.) Pediatric AIDS cases—identified in hospitals and public homes for abandoned children—were almost exclusively attributable to transfusions (41%) or injections (56% had a history of multiple hospitalizations, each presumably with multiple injections); approximately 2% were shown to be perinatal transmission cases. (Only 47% of the mothers of children with AIDS have been tested; tracking mothers of abandoned children is often difficult.) Since proper sterilization techniques were introduced, no new injection-related infections have been reported.

At the Conference, Nicolae Beldescu (Ministry of Health, Romania) reported that because of the policies of the former government, HIV testing was not available in Romania—neither for blood screening nor for diagnosis. Now all children under 3 years of age living in institutions are being tested for HIV. Approximately 12,500 children live in these institutions; 85% of the children had been tested by June 1990 and 10% show unconfirmed HIV seropositivity (repeatedly reactive ELISAs without Western blot results).

Poland. Homosexual/bisexual men still account for the majority of AIDS cases in Poland; however, Dr. Andrzej Stapinski and colleagues (Institute of Venereology of Medical Academy, Warsaw) showed that HIV infection is now spreading rapidly among IVDUs. From January 1986 through December 1989, a total of 5,351 IVDUs were tested for HIV antibodies; 292 (5.5%) proved seropositive. Interestingly, no seropositives had been identified in this population in 1986 or 1987, and only 16 of 1,282 were identified in 1988. In 1989 alone, 276 of 2,471 (11.2%) tested positive. The authors also reported findings from studies conducted from January 1990 through May 1990: 135 new cases of HIV infection were detected among 1,134 IVDUs tested (11.9%).

Western Europe. Dr. Angela Downs (WHO Collaborating Centre on AIDS, Paris) and colleagues reported that although AIDS incidence is still increasing among homosexual/bisexual men, the *rate* of increase among this group continues to show definite signs of slowing in the 12 Western European countries. IV drug users show a much more rapid increase in AIDS incidence (almost equalling that observed for homosexual/bisexual men in 1989), but recent signs indicate that the rate of increase in this group is slowing. Several presenters remarked that the slowing could reflect saturation of the IVDU population. In Italy, for example, high but stable seroprevalence rates (roughly 30–40%) were reported from IVDU cohort studies between 1985 and 1989. And in Spain, although certain regions are known to have large IVDU populations with relatively high seroprevalence rates, decreases have been noted: Fernando Merino (Universidad del Pais, Vasco) and coworkers reported an overall rate of 41.29% (based on 1,504 IVDUs in Basque country during 1984-89); a drop in seroprevalence was observed from 45.41% in 1987 to 26.93% in 1988, which remained relatively stable at 29.8% in 1989. Because IVDUs show a higher rate of increase, the number of new cases among IVDUs will almost certainly exceed that of homosexual/bisexual men in the next few years.

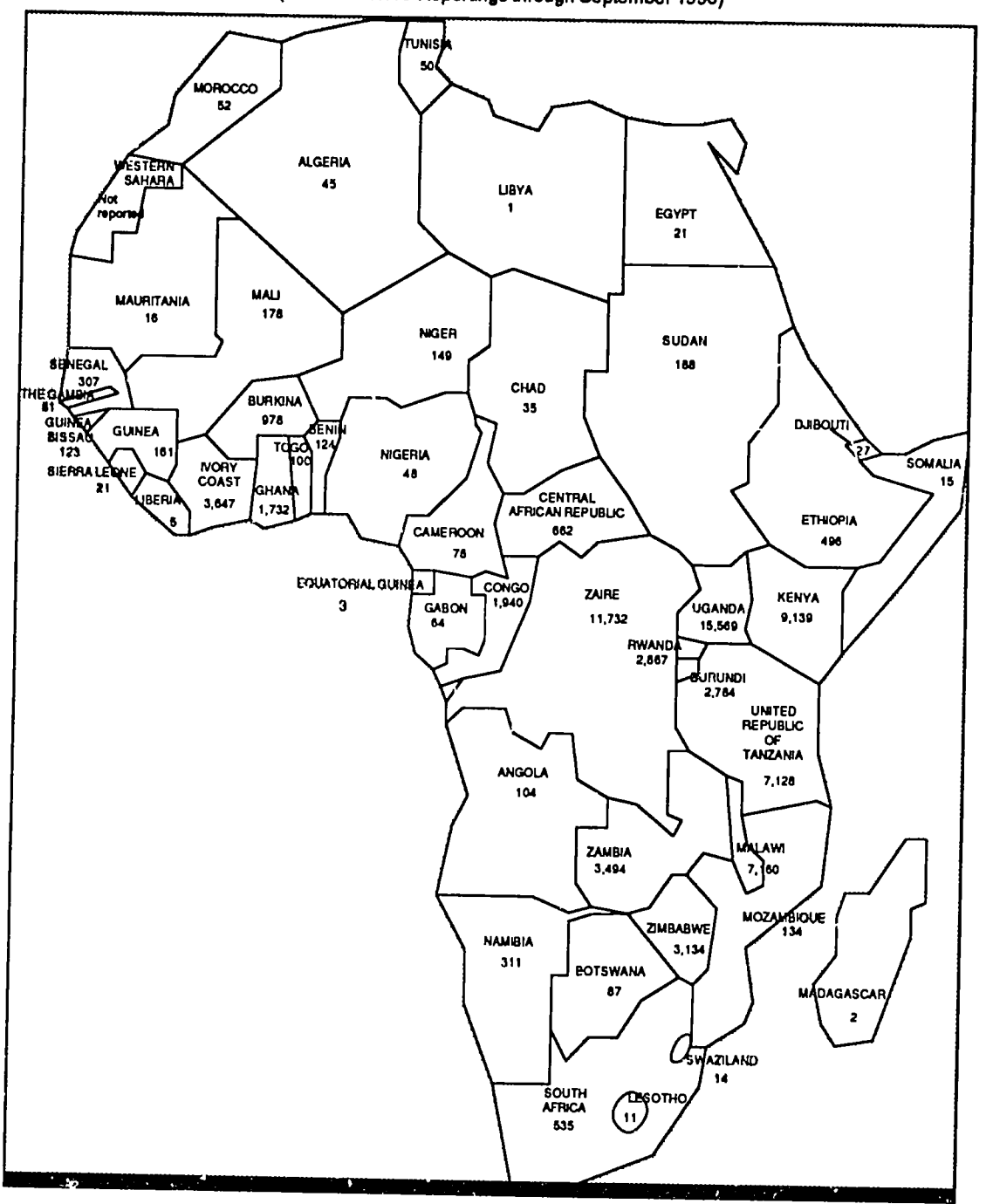
Dr. Downs and colleagues noted that although predictings AIDS incidence based on interpretation of data on heterosexual contact cases is inherently difficult (partially because of the small numbers involved), it is likely that the number of AIDS cases due to heterosexual transmission will continue to rise. Western European countries with the highest numbers of AIDS cases in order, are: France (9,718), Italy (6,701), Spain (6,210), FRG (5,266), and U.K. (3,798).

AFRICA

Very few AIDS cases have been reported from countries in North Africa although ongoing serostudies have been extensive. Epidemiological studies in sub-Saharan Africa continue to reveal widespread HIV infection in sentinel populations, such as hospital patients, STD clinic attendees, and high-risk groups in urban centers—notably prostitutes and clients of prostitutes. Populations not labelled at particularly high risk, however, also show disturbingly high seroprevalence rates. Dr. Marie Laga (Centers for Diseases Control, Atlanta) reported that in many urban areas of Uganda, Malawi, Rwanda, Zambia, and Zimbabwe, seroprevalence among pregnant women exceeds 20%. But the situation may actually be more complicated than this: Dr. Laga contrasted these high seroprevalence rates with those observed in pregnant women in Kinshasa, Zaire, rates that have remained at 5–6% for at least 6 years. From these data alone, it appears that the speed and efficiency of HIV transmission varies tremendously within sub-Saharan Africa

In rural areas, studies suggest that HIV seroprevalence rates are low, but increasing. Heterosexual contact is the predominant mode of transmission in sub-Saharan Africa, and it is estimated that 20% to 30% of all sexually active adults (between 20 and 40 years old) in urban areas are infected. In a July 31, 1990 statement from Dr. Michael Merson (WHO, Geneva), new estimates of HIV seroprevalence for all of sub-Saharan Africa have

Figure 3.4. Cumulative AIDS Cases in Africa
(Based on WHO Reportings through September 1990)



reached 5 million persons (an alarming increase from the 2.5 million estimate determined in 1987). Of 51 countries reporting AIDS cases to WHO, Uganda has the highest number (15,569), followed by Zaire (11,732), Kenya (9,139), and Malawi (7,160).

Although not restricted to this continent, the incidence of HIV-2-associated AIDS has risen considerably in Africa. HIV-2 (a second human immunodeficiency virus) was isolated from female prostitutes in Senegal in 1985, and is now known to be endemic to West Africa. (See "Other Retroviruses," below.) Seroepidemiological studies in West Africa and bordering countries are screening for HIV-1 and HIV-2, as well as HIV-1/HIV-2 dual infection.

AUSTRALIA

The majority of AIDS cases in Australia are localized on the Eastern seaboard. At the Conference, Dr. Brian Mulhall (University of Sydney) cited a figure of 12,000 HIV-infected persons but cautioned that, due to the difficulties inherent in HIV surveillance, this figure is likely to be an underestimate. Dr. John Kaldor (National Centre in HIV Epidemiology & Clinical Research, Sydney) and colleagues reported that the AIDS incidence rate in Australia is 92.8 per million, and that homosexual/bisexual men constitute the largest risk group (93% of all cases). Furthermore, the growth seen among IVDUs in other Pattern I countries has not been observed in Australia. The cumulative number of AIDS cases, at the end of September1990, was 2,040.

ASIA

A total of 790 AIDS cases had been reported to WHO by 25 Asian countries as of October 1, 1990. Because of the relatively recent introduction of HIV (Asia reported its first AIDS cases to WHO in 1985), Asian countries are categorized as pattern III countries. As data from regional seroepidemiological studies are being reported, however, it appears that the demographics of HIV-infected populations from individual Asian countries differ considerably.

In Japan, for example, which has the highest incidence with 290 cases, AIDS surveillance reveals that hemophiliacs constitute the largest risk group (49.1%), followed by homosexual men (29.1%), and heterosexual contacts (13.6%). Dr. Kenji Soda (Yokohama City University School of Medicine) and colleagues reported that seroprevalence data reveals a much different proportionate distribution—of 1,130 testing positive for HIV, 86.6% are hemophiliacs; 6%, homosexual men; and 5.4%, heterosexual.

In Hong Kong and Singapore, HIV infection among local IVDUs appears to be very low, whereas in Thailand IVDUs have the highest seroprevalence rate of any risk category. And although Asia only accounts for 0.2% of the cumulative AIDS cases worldwide, several speakers remarked that Asia is experiencing a rapid growth in HIV infection—it is no longer a problem of foreigners. Looking at currently available data, Dr. Eng-kiong Yeoh (Hong Kong Department of Health) predicted that in the 1990s Asia will likely see an increase in the number of HIV infections due to IV drug use and heterosexual contact. Indeed, WHO announced in late July that the current estimated total number of HIV-infected persons in Asia is approximately 500,000—up from virtually zero just 2 years ago.

Heterosexual contact appears to be the major mode of transmission in India, sex with a prostitute being reported in most cases. The Indian Council of Medical Research reported in July 1990 that 30% of female prostitutes in the city of Bombay were HIV-infected. A disturbing finding reported by Dr. S. Apte and colleagues (Institute of Immunohaematology, Bombay) revealed that the rate of HIV infection among blood donors in the city of Bombay is significantly higher for paid (versus voluntary) donors: 24 per 1,000 compared to 1.4 per 1,000.

HIGH-RISK GROUPS

HOMOSEXUAL/BISEXUAL MEN

The male homosexual/bisexual population, as the longest-affected group, has been the focus of numerous epidemiological studies. Indeed, it was this group in which the clinical symptomology of what is now known as AIDS was first recognized (in 1981). Widespread reduction of high-risk behaviors among this population is believed by many to have contributed to the recent decline in new AIDS cases in many Pattern I countries. Some new studies have raised concern, as they suggest a relapse in high-risk behaviors among some homosexual/bisexual men.

AIDS Incidence. Virtually all Pattern I countries have reported significant drops in the number of new AIDS cases among homosexual/bisexual men. In the United States, this trend has been observed since mid-1987. Whether this is attributable to behavior modification or therapeutic intervention or both, is under study. Several research teams, using statistical analyses, are testing whether such variables as availability and use of therapeutic agents for HIV infection (and HIV-related conditions) could account for the abrupt decline in AIDS cases among homosexual/bisexual men (see "Epidemiological Modelling," below).

In the United States by the end of September 1990, a cumulative total of 89,155 homosexual/bisexual males were reported with AIDS, representing 60% of all U.S. adult AIDS cases. This is in contrast to the early years of the epidemic, when homosexual/bisexual men accounted for close to 80% of all reported adult/adolescent cases (in 1985). Nonetheless, this is still the largest at-risk group to have been diagnosed with AIDS in all Pattern I countries except Spain and Italy.

Seroprevalence. Declines in HIV seroprevalence rates among homosexual/bisexual men during the mid- to late 1980s have been noted in the United States and Western Europe. However, reports and data presented at the Conference show that when these rates are analyzed by age group, increases can be seen among younger homosexual/bisexual men. Timothy Kellogg (San Francisco Department of Public Health) and coworkers reported results from their 1989 serosurvey conducted at STD clinics in San Francisco and surrounding counties. Of 816 homosexual/bisexual men seeking care for a new STD episode, 376 (46.1%) were HIV-positive. A disturbingly high HIV-infection rate of 40.7% was observed among younger men (less than 25 years old). This study also noted that HIV infection rates among black and Latino men were higher than among white men: 53.2% versus 43.9%. And seroprevalence rates were higher in San Francisco (53.7%) than in surrounding counties (30.9%).

Findings from a 5-year study with the multicenter AIDS cohort study (MACS), reported by Dr. Lawrence Kingsley (University of Pittsburgh) and associates, revealed an overall trend of declining seroconversion rates over time in Chicago, Los Angeles, and Baltimore/Washington, but not in Pittsburgh. Among men 29 years of age or younger, significantly higher seroconversion rates were noted throughout the study.

Risk Factors. Deloris Koziol (National Institutes of Health, Bethesda) and colleagues outlined a number of independent risk factors associated with HIV infection from a cohort of 89 homosexual men (not vaccinated for hepatitis B) followed prospectively for 2.5 years:

- duration of homosexual practice
- receptive anal intercourse
- history of gonorrhea
- rectal douching
- sex with person who developed AIDS
- lifetime number of male sex partners
- lifetime number of STDs

According to several Conference reports, receptive anal intercourse, rectal douching, and number of sex partners appear to be the most significant factors influencing HIV transmission among homosexual men. Other risk factors, including history of syphilis (specifically), sex in bath houses, and lack of (or inconsistent) condom use, are being studied.

Although genital ulceration has been demonstrated to facilitate *heterosexual* transmission, studies do not show this to be the case for homosexual transmission. Dr. Ireneus Keet (Municipal Health Services, Amsterdam) and coworkers reported results from a cohort of 1,000 homosexual men evaluated over the period 1985–88, in which 53 men seroconverted. When compared to their seronegative counterpart, the total number of genital ulcerative infections was not significant in HIV-positive homosexual men: 12 of 53 (23%) homosexual men reported at least 1 ulcerative infection compared to 8 of 54 (15%) HIV-negative homosexual men. The authors concluded that genital ulcers contribute little to the already high risk of unprotected anogenital intercourse.

Behavioral Change. Even though the predominant risk factors for this population have not changed qualitatively during the course of the epidemic, they have undergone a dramatic change quantitatively. Once transmission-risk factors were identified and awareness campaigns initiated, drastic declines in the number of partners and the frequency of anal intercourse were reported; increased use of condoms among homosexual/bisexual men was also noted. Increased condom use—within both primary and casual relationships—was reported by numerous conference presenters, including Gail Hughes (San Francisco Department of Health) and coworkers who compared risk-behavior survey results from 1989 (of 401 homosexual/bisexual men) with findings from similar surveys in 1984 and 1987.

Dr. Brian Mulhall (University of Sydney) reported on behavioral changes adopted in a cohort of 96 homosexual and 4 bisexual men living in Melbourne (which has a relatively low AIDS incidence) who were observed and counselled from the time of recruitment in 1983 to 1990. A significant drop in the number of sex partners was consistently reported at each 6-month interval. The sharpest reduction was among the HIV-seropositive group, which also had the highest number of partners at entry. An increase in condom use and decrease in unsafe sex practices, such as anal intercourse, was noted but did not achieve statistical significance. Rather encouraging was the finding that no seroconversions have been detected in this group since 1985.

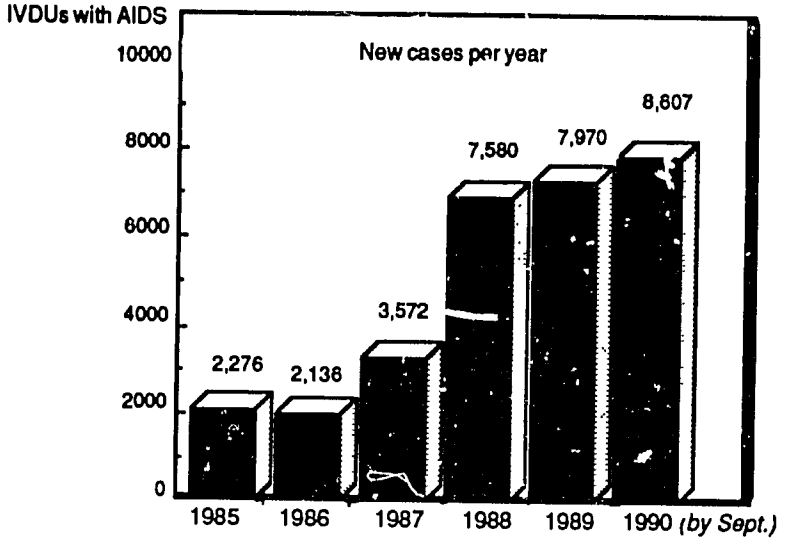
Dr. Richard Wolitski (California State University, Long Beach) and associates showed that sampling different groups of homosexual/bisexual men does not yield uniform behavioral changes. Their sample of 152 gay bar patrons, for example, reported more condom use, a decrease in number of sex partners, and abstinence from high-risk sexual practices than did three other samples of homosexual/bisexual men (new members of a cohort study, participants in a gay-oriented community recreation program, and enrollees in an AIDS prevention/health enhancement program). From their findings, Dr. Wolitski 's team concluded that changes in sexual practices within the gay community may not be as pervasive as once thought.

Although marked behavioral change has been consistently reported from numerous cohort studies of homosexual men in Pattern I countries, relapse to unsafe sex practices has been noted. (Chapter 10: EDUCATION & COUNSELLING discusses this issue in detail.) Several researchers pointed out that while seroconversion rates have fallen among homosexual/bisexual men, a percentage continue to become infected; in some instances, despite participation in cohort studies (which implies exposure to behavior modifying influences). Data presented by Dr. Brian Willoughby (University of British Columbia and St. Paul's Hospital, Vancouver) demonstrate that recent seroconverters could be characterized as younger, less educated, of lower socioeconomic status, and more likely to use illicit drugs. Marie-ange Schiltz and colleague (CNRS-GSPM, Paris) compared annual surveys (from 1985 to 1989) conducted among 1,200 French homosexual men. Their findings reveal that after rapid reduction of high-risk sexual behavior, since 1988 there has been a slowing of additional change. In fact, although a steady increase in regular condom use was noted, so were slight increases in the number of partners and frequency of anal intercourse.

INTRAVENOUS DRUG USERS

Sharing or reusing discarded intravenous needles presents a significant risk for acquiring HIV infection through direct blood-to-blood transmission. When IV drug users (IVDUs) share needles, residual blood usually remains in the needle as it is passed from one user to another. If the needle has been used by an HIV-infected person, the residual blood can harbor the virus and serve as a vector for the transmission of the virus to the next needle user, even hours later as shown by previous studies.

Figure 3.5. AIDS Cases among IV Drug Users in the United States



Figures provided by the Centers for Disease Control (Atlanta) demonstrate the rapid growth of newly diagnosed AIDS cases among the IV drug using population. By the end of September 1990, new AIDS cases among IVDUs had already surpassed the total for the year 1989.

Collecting and analyzing epidemiological data on IVDUs is fraught with difficulties: People who inject drugs are part of an extremely heterogeneous population. As was evidenced by the many studies presented at this year's Conference, varying patterns of drug-injecting behavior can be distinguished when considering such variables as geographic locus, gender, and, in the United States, ethnicity. For example, female IVDUs in the United Kingdom show higher levels of needle sharing than their male counterparts. One San Francisco cohort study of seropositive black IVDUs revealed that women tended to inject more frequently than men. And according to Ellie Schoenbaum and colleagues (Montefiore Medical Center, New York), whites are more likely to share with close friends and relatives, whereas blacks and Hispanics are more likely to share with acquaintances or strangers.

AIDS Incidence and HIV Seroprevalence. Several speakers at the Conference emphasized the growing incidence of AIDS and HIV infection among IVDUs in Pattern I countries. Already the second-largest population with AIDS in the United States and most Western European countries, drug injectors also show the most rapid increases in new cases of AIDS and HIV infections in parts of the United States, Western Europe, and several South American countries—notably Brazil and Argentina. In Italy and Spain, AIDS incidence among IVDUs has surpassed that among homosexual/bisexual men. HIV seroprevalence studies from several Pattern III countries, including Thailand and Poland, suggest that IVDUs are emerging as their major risk category.

While many regions report rapid increases among IVDUs, some cities exposed to HIV early in the epidemic show a leveling of infection rates in this group. Saturation of the population as a possible contributing factor to this observed leveling has been theorized by some; others, such as Dr. Gerald Stimson (Charing Cross and Westminster Hospital, London) point out that, not surprisingly, changes in risk behaviors coincide with a flattening of the seroprevalence curve (see "Behavioral Change," below).

Many factors influence HIV seroprevalence rates for a given study population. Local drug-injecting practices and the levels of high-risk behavior have been shown to correlate with

seroprevalence. Movement patterns of drug-injecting individuals also appear to be important: as they move into, out of, and within geographic areas, they engage in high-risk behavior with populations of various seroprevalence rates.

United States. Dr. David Allen (Centers for Disease Control, Atlanta) reported results from surveys of more than 15,000 IVDUs entering drug treatment clinics in 27 metropolitan areas. For the year 1988-89, HIV seroprevalence rates ranged from 0% to 47.7%, with most rates hovering around 5.0%. (Rates were highest in the Northeast at 18.2%.) Racial/ethnic differences could be distinguished, but these varied from city to city. Analyses of changes in rates over time suggest that in some metropolitan areas, rates are stabilizing. In a series of surveys conducted from 1987 to mid-1989, Dr. Robert Battjes (National Institute on Drug Abuse, Rockville, Maryland) and colleagues found no change in seropositivity rates in 6 cities. Dr. John Watters (University of California, San Francisco) reported similar findings on examination of seroprevalence rates and risk factors from 2,114 IVDUs from the second half of 1986 to 1989. From a 6.1% in 1986, the seroprevalence rate peaked at 12.8% in the second quarter of 1987 and has remained steady at about 12% since. Another San Francisco study revealed declining seroconversion rates in the period 1985 to early 1990: rates dropped form 4.5% to 0.6% for this cohort. A decline in seroconversion rates was also noted among IVDUs in Baltimore followed from 1985 through the end of 1989.

Italy. While AIDS incidence data for 1989 shows that IVDUs now account for the majority of AIDS cases in Italy, seroprevalence studies reveal that rates have plateaued at about 30-40%. Mauro Zaccarelli (AIDS Unit USL, Rome) and colleagues reported on their cohort of 1,000 IVDUs followed from 1985–89: for 1985, the annual seroprevalence rate for new entrants into a methadone program was 33.9% and in 1989, 32.1%. Rates determined for IVDUs already in the program were slightly higher: 40% in 1986 and 45% in 1989.

Spain. Conference reports showed rather varying seroprevalence rates for Spanish IVDUs, ranging from 27% to 69.1%. Roberto Muga (Hospital "Germans Trias i Pujol," Barcelona) and coworkers found a seroprevalence rate of 69.1% for IVDUs who came into a hospital for either detoxification or for non-drug related illnesses during the period 1984–88. Of 382 IVDUs, 246 were HIV-positive; heroin was reported as the main drug used in 95% of the cohort. From a 1989 study of 1,233 IVDUs in drug treatment centers throughout Spain, Miguel Delgado-Rodriquez (State Office for National Plan on Drugs, Madrid) and associates reported a seroprevalence rate of 41%. Several other groups reported similar rates—in the range of 30% to 45%.

FRG. A seroprevalence study of 784 heterosexual IVDUs in West Berlin conducted from 1984–1989 and reported by Klaus Stark (Landesinstitut fur tropenmedizin, Berlin) and colleagues, revealed an increasing trend in HIV seroprevalence rates. The overall (5-year) rate was found to be 23.2%.

Brazil. The estimated seroprevalence has been cited at about 15%, but already 13.8% of cumulative AIDS cases in this country are attributable to IV drug use. Annual AIDS incidence data shows that AIDS in IVDUs has risen from 4% in 1986 to 19% in 1989. A cohort study of 541 IVDUs in Sao Paulo found seroprevalence rates to range from 78% (for heterosexual IVDUs) to 58% (for homosexual IVDUs).

United Kingdom. An estimated 52.6% of all HIV-infected individuals in Scotland are heterosexual IVDUs. Dr. David Goldberg (Ruchill Hospital, Glasgow) and colleagues, however, presented widely discrepant HIV seroprevalence rates among IVDUs in 2 cities in Scotland just 44 miles apart: a 6% seroprevalence rate was obtained for IVDUs in Glasgow, compared to rates of up to 50% in Edinburgh. (By contrast, in England, Wales, and Northern Ireland, the large majority of seropositives are homosexual/bisexual men and only 8.2% of all seropositives are IVDUs.)

Risk Factors. Several risk factors for acquiring HIV are peculiar to the IVDU population, all of which are directly or indirectly related to needle sharing:

- duration of abusing injectable drugs
- frequency of injection
- frequency of injecting with shared needles
- needle sharing with strangers
- injecting in "shooting galleries" (where needles are commonly shared or rented)

The number of sexual partners who inject drugs and history of imprisonment have also been shown to present some risk for HIV transmission for IVDUs. Some studies demonstrated that the infection rates vary with the drug injected (heroin, amphetamine, or cocaine, for example). Martin Iguchi (University of Medicine & Dentistry of New Jersey) and colleagues reported that in their study of 1,397 IVDUs in Newark and Jersey City (of whom 55.8% and 46.9% were HIV-infected, respectively), three risk factors were statistically significant with respect to HIV seropositivity: years of IV drug use, frequency of injecting heroin and cocaine together (speedball) in the past 6 months, and frequency of injecting cocaine in the past 6 months. Christina Hartgers (Municipal Health Service, Amsterdam) and coworkers found "mainly heroin and cocaine" use as a new independent risk factor in their study of 386 IVDUs followed from 1985 to 1989.

In the United States in particular, higher rates of infection have been seen in cocaine injectors. Data from 1987 and 1988 showed IV use of cocaine and speedball to be the strongest predictors of HIV seropositivity among IVDUs in New York City. Dr. Wayne Wiebel and colleagues (University of Illinois at Chicago) offered that because it is a group activity for most IVDUs (in which sharing of injection equipment is the rule), cocaine injection in and of itself is an important independent risk factor for acquiring HIV. Smokable cocaine, or crack, also appears to be a risk factor. (Note, however, that perhaps more than half of IVDUs who report crack use previously routinely injected cocaine.) The true influence of crack may be in the spread of HIV to non-IVDU heterosexuals.

IVDUs and the Heterosexual Spread of HIV. Sexually active heterosexual, as well as homosexual/bisexual, IVDUs are seen as the major conduit for the flow of HIV into the general population in Pattern I countries—primarily through known high risk sexual behaviors including a large number of sex partners and infrequent condom use. With increasing, or stably high, seroprevalence rates among IVDUs in many parts of the world, the potential for spread into the wider (non-IVDU) population has become a concern.

HIV transmission rates from IVDUs to their sexual (non-IVDU) partners appear to be very high. (Because tracing sexual partners of IVDUs is an arduous task, only a few studies actually presented figures on transmission rates.) A Centers for Disease Control (CDC) study showed a significant increase in the number of AIDS cases among sex partners of IVDUs in six Southeastern states (where AIDS incidence has increased in recent years) for 1987–89 compared to 1984–86. Dr. Ruth Berkelman and associates presented data showing that the increase in new AIDS cases among sex partners (15.5% to 27.9%) was greater than that observed among IVDUs (9.2% to 13.8%). Several disturbing Conference reports gave evidence of persistent high-risk sexual behavior despite knowledge of HIV seropositivity. One study reported by Luis de la Fuente (State Office for the National Plan on Drugs, Madrid) found that of 789 IVDUs who knew they were HIV-positive, only 324 (41%) adopted risk-reducing sexual behaviors.

Several reports focused on the drugs-for-sex barter system, and especially the "crack-for-sex" trade. Such activity is likely to enhance the heterosexual transmission of HIV, presumably by increasing the frequency of engaging in high-risk sexual behaviors. One study, conducted by Dr. Hannah Wolfe and colleagues (University of California, San Francisco, and San Francisco General Hospital), found crack use associated with high-risk sexual

behavior, but not with HIV seropositivity. However, researchers are concerned that HIV-infection rates among crack users and their partners may eventually parallel the increased use of crack and the growth of the crack trade in general.

Behavioral Change. A practical understanding of the forces driving IVDUs had to be incorporated into efforts promoting the adoption of risk-reducing behaviors by this group, namely:

- needle-exchange programs (which seek to eliminate needle sharing)
- instruction in needle cleaning techniques (bleaching)
- drug abuse treatment centers (methadone maintenance programs)
- instruction on safe sex practices

A large number of surveys have shown, however, that the IVDU population as a whole is slow in making the necessary behavioral changes. And, overall, changes in sexual behavior lag behind changes in drug use. Researchers appear not to be in agreement regarding the effect that the availability of free sterile needles, bleach, or condoms has on limiting the spread of HIV. (Chapter 10: EDUCATION & COUNSELLING discusses educational interventions in detail.)

In the United States, where intervention programs are not as prevalent as in Western Europe, several reports suggest relatively little progress in risk reduction by IVDUs. (U.S. policy-makers have generally been concerned that government-funded programs for IVDUs would be equated with governmental sanctioning of drug use.) From their study of seven U.S. cities, Dr. Robert Battjes and coworkers (National Institute on Drug Abuse, Rockville, Maryland) reported that although seropositivity rates are not increasing, increases in risk behaviors persist.

A particularly strong argument for providing IVDUs with clean needles came from a Johns Hopkins University (Baltimore) study on diabetic IVDUs. Dr. Kenrad Nelson presented his team's data demonstrating that among 702 HIV-infected IVDUs (out of a cohort study of 2,921 IVDUs), only 4 (0.57%) had diabetes. Dr. Nelson pointed out that diabetics have unrestricted access to sterile needles (and are not arrested for carrying injection equipment), which is likely to account for the significantly lower rate of HIV infection in this subpopulation of IVDUs.

Needle/Syringe Exchange Programs. In a number of European and Australian cities, needle/syringe exchange programs have been in place for several years. Such programs usually establish a central facility at which used needles and syringes can be exchanged for free sterile needles and syringes. Katie Dolan (Charing Cross and Westminster Hospital, London) presented her group's findings on such a program in England. Of 86 "attendees" at exchange sites recruited between April and September 1989, 62% reported that they were receiving treatment for their drug abuse (compared to 40% of non-attendees). Furthermore, almost half of the attendees had been referred to drug treatment centers through the exchange program, demonstrating that exchange programs are an avenue for entering treatment. Attendees were more likely to have been tested for HIV than non-attendees; 60% said they reduced or stopped needle sharing after being tested compared to 33% of non-attendees. The seroprevalence rate for attendees was 1.2% (1 of 82), compared to 3.6% (4 of 110) for non-attendees.

In Canada, needle-exchange programs in Montreal, Toronto, and Vancouver were established in 1989. The first U.S. needle/syringe-exchange program was implemented in Tacoma, Washington. Now Portland, Oregon; Boulder, Colorado; and Honolulu also have programs in place; New York City's program has been shut down by the mayor, David Dinkins. And community-based exchange programs exist in San Francisco.

An interesting observation was noted by Atnaf Keffelew and colleagues (University of California, San Francisco) in their survey of local methadone treatment programs, which

also offered anonymous screening: in this group with a persistently high rate of needle use, 28% had used a needle-exchange program, and those who knew they were HIV-positive had a higher overall rate of exchange (42%) than did their HIV-negative counterparts (26%). This finding is encouraging in that it may mean that anonymous screening plus needle-exchange programs may reduce HIV transmission by IVDUs.

Bleach Distribution and Education Programs. One method for disinfecting used needles is to clean them with bleach, which inactivates HIV. Many behavioral change programs for IVDUs emphasize bleach use as a means of reducing HIV transmission. Dr. Graham Hart (University College & Middlesex School of Medicine, London) and associates suggested that the provision of bleach at needle/syringe exchange centers may enhance risk reduction. In Bangkok, Dr. Pralom Sakuntanaga (Bangkok Metropolitan Administration) reported that 6 months following the implementation AIDS education, the number of IVDUs who reported needle sharing dropped from 66.5% to 52.2% and the number who reported bleach use rose from 8.4% to 15.3% in the same period.

SEXUALLY ACTIVE HETEROSEXUALS

The World Health Organization estimates that about 60% of all HIV infections worldwide have resulted from heterosexual intercourse, and that the rate is slowly increasing on all continents. By the year 2000, WHO projects that 75-80% of all HIV infections will result from heterosexual relations. While heterosexual transmission is the predominant mode in Pattern II countries (sub-Saharan Africa and The Caribbean), Pattern I countries are seeing increasing numbers of AIDS cases and HIV infections among the so-called heterosexual contact risk category—a trend that was first detected in 1986. This category applies to persons born in Pattern II countries or reporting heterosexual contact with an HIV-infected person as their only high-risk behavior. (Heterosexual contact cases *exclude* persons who report same-sex sexual contact, IV drug use, or history of blood transfusions.)

In Pattern II countries, HIV is believed to be widespread in the general population, as demonstrated by approximately equal infection rates among males and females. In urban centers of sub-Saharan Africa, it is estimated that 20–30% of all sexually active adults (aged 20–40) are infected. Pattern I countries, where homosexual/bisexual men and IVDUs have accounted for the vast majority of AIDS cases since the beginning of the epidemic, are now reporting a steady climb in the numbers of heterosexual contact cases. In the United States and Western Europe, many believe that the spread of HIV outside the original high-risk categories has been largely mediated by IVDUs. (A noteworthy exception is Pattern I countries that have large emigrant populations of persons from Pattern II countries; Canada may be such an exception.)

At this year's Conference, researchers from Central and South America pointed to bisexuality as an important route of transmission to the general heterosexual population. (A few anecdotal reports from the United States and the United Kingdom suggest that a not-insignificant proportion of homosexually active men engage in heterosexual sex; furthermore, it was noted that these men tend not to practice safe sex with women.)

AIDS Incidence. AIDS cases attributable to heterosexual contact in Pattern I countries still represent only a small percentage of the total: approximately 5% of the cumulative number of adult cases; specifically, 4.4% for Western Europe, 5% for the United States, and 6.6% for Canada. The number of cases in Australia is significantly less (2.7%). Among Pattern I countries, the highest incidences are reported by Mexico (14%) and Brazil (10%).

Annual U.S. statistics reveal that AIDS cases attributable to heterosexual contact rose from 5% in 1988–89 to 6% for 1989–90. Dr. Nancy Padian (University of California, Berkeley) told Conference participants that, among IVDUs and persons with low socioeconomic status, the heterosexual AIDS epidemic already exists.

Figures from Miami reveal incidence rates substantially higher than the national rates. Data collected by Janet Masiero and colleagues at the University of Miami AIDS Clinical Research Unit and reported to the Public Health Department (Miami) for the period 1987–89 showed annual incidence rates of 20% (1987), 24% (1988), and 55% (1989). The authors also gave details of these heterosexual-transmission cases: Of the 70 heterosexual contact cases for 1987, for example, 53 were "first generation" transmission cases—that is, HIV was acquired through sex with a person belonging to a high-risk category. Second generation heterosexual transmission refers to cases in which the virus was acquired through sexual contact with a person who did not report any high-risk behaviors and who presumably acquired the virus through sex with a member of a high-risk group.

Risk Factors. Numerous research teams are actively looking into risk factors that may facilitate the (hetero)sexual transmission of HIV. Although each may carry a different degree of risk, the following have been found to be associated with heterosexual transmission:

- origin in Pattern II country
- sexual contact with a high-risk individual (including persons from Pattern II countries)
- trauma and/or exposure to blood during sex
- genital ulceration (typical in syphilis, chancroid, and herpes)
- lack of condom use
- lack of circumcision (in African males)

Other factors, which are still controversial, include disease status of the HIV-infected partner, engaging in receptive anal intercourse, the number of sex partners (lifetime), number of penile-vaginal contacts with an infected person, and sex with a prostitute. Interestingly, data from the Italian Partners' Study (which followed 368 women who were steady partners of HIV-infected men) showed that the use of an intrauterine device actually increased the risk of heterosexual transmission.

Numerous studies examining risk factors associated with HIV transmission in heterosexual adults in Central Africa found additional independent risk factors to be statistically significant, some of which appear peculiar to Africa. These include cervical ectopy in women; history of STD (both ulcerative and nonulcerative); gonococcal infections (independent of other STDs); 5 or more lifetime sex partners (for women only); having sex during menses (for men only); genital bleeding due to sex; genital bruising due to sex (for men only). Gina Dallabetta (Johns Hopkins University, Baltimore) and colleagues reported a novel association between HIV seropositivity in Malawi women and the use of stones (silica gel, potassium permanganate, and pumice-like stone) as a vaginal tightening agent. The researchers offered that the stones have an irritating and erosive effect on the vaginal mucosa and may thereby facilitate viral entry.

Transmission Dynamics. Studies on heterosexual HIV transmission rely on the use of monogamous couples in which one of the partners is seropositive and one is seronegative, so-called discordant couples. Such studies allow for careful analysis of individual risk factors, such as the non-use of contraceptive barriers. Heterosexual transmission rates using discordant couples have been reported to range from 8% to as high as 44%. The relative efficiency of male-to-female versus female-to-male transmission can be studied by looking at couples in which the female partner is infected and the male partner uninfected, and vice versa. Ongoing studies are investigating the seroconversion rates of both types of discordant couples.

Dr. Federica Tacconi and colleagues (University of Pavia, Italy) presented data on 142 discordant couples followed from 1986-1989. At the beginning of the study, there were 36 couples with an HIV-positive female IVDU and non-IVDU seronegative male partner and 106 couples with an HIV-positive male IVDU and non-IVDU seronegative female partner.

HIV testing was carried out at 6-month intervals. By the end of the study in 1989, 6 seroconversions were noted—all among female partners of seropositive men. All the seroconverters reported that they never used contraceptive barriers. All Conference presentations examining this issue reported similar findings—that is, female partners of HIV-infected men showed higher seroconversions than did male partners of HIV-infected women.

Dr. Nancy Padian (University of California, Berkeley) reported that, based on her team's study of 58 male sex partners of HIV-infected women and 269 female partners of HIV-infected men, female-to-male transmission is less efficient than male-to-female transmission. (In this study less than 2% of the male partners of infected women seroconverted, compared to 20% of the female partners of infected men.) Dr. Padian said that, although difficult to recruit, sample sizes with larger pools of infected women are needed to study the question of why HIV transmission appears to be more heavily weighted in one direction. Alfredo Nicolosi (National Council of Research, Milan), reporting for the Italian Partners' Study, showed that the difference in susceptibility in his study group—8.9% seroconversion for partners of infected females versus 27% for partners of infected males—could neither be explained by differences in sexual behavior nor by different characteristics of the infected partner (such as disease stage).

From a biological perspective, it is curious that such a difference exists. In attempting to address the issue of transmission direction, Dr. Yvette Henin (Institut Pasteur, Paris) and collaborators examined vaginal secretions from seropositive women for presence of HIV. One could argue that female-to-male transmission is relatively inefficient because HIV is rarely present in vaginal secretions of infected women. In their small study, Dr. Henin's team did not find this to be the case. Of 46 study subjects, HIV was isolated from secretions of 14 women (5 were asymptomatic or had lymphadenopathy; 9 had AIDS). Again, larger studies are needed to obtain statistically significant data; the possible association between presence of HIV in secretions and disease status may prove to be particularly important. Also, further studies will need to correlate the presence of HIV in secretions of women whose sex partners remain seronegative. It would seem likely that for women with no detectable HIV in vaginal secretions, their sex partners would remain negative.

HEMOPHILIACS AND OTHER TRANSFUSION RECIPIENTS

Prior to implementation of blood-screening tests for HIV, recipients of blood and blood products were at high risk for acquiring HIV infection. Current statistics on the number of transfusion-associated AIDS cases give some idea of the level of that risk. In industrialized countries, approximately 3–6% of the total number of AIDS cases are transfusion-related. France reports the highest number of transfusion-associated AIDS cases in Europe. Dr. Dominique Costagliola (INSERM, Paris) reported that the cumulative number of transfusion-associated AIDS cases in France will eventually reach between 2,300 and 4,500. (Through the end of 1989, France had reported 576 cases, approximately 6% of all AIDS cases.)

Although the percentage of AIDS cases attributable to transfusion(s) continues to fall in most industrialized nations, developing countries still see high AIDS incidence rates among members of this risk category. Roughly 10% of all AIDS cases in Africa are related to blood transfusion. However, as Dr. Peter Lamptey (Family Health International, Durham, North Carolina) pointed out, the incidence of transfusion-associated AIDS in children is even higher—in Kinshasa, Zaire, for example, 25% of all pediatric AIDS cases. In countries where paid blood donors are (or were) used—like India and Mexico—transfusion-associated cases are, perhaps not surprisingly, extraordinarily high: 16% for Mexico, for example. Interestingly, 18 Caribbean countries show only 1.1% of all AIDS cases from this region to be transfusion-related.

Hemophiliacs. The discovery of AIDS in hemophiliacs was one of the first clues leading to the identification of HIV as a blood-borne virus; members of this well-defined group require multiple blood transfusions or receive blood products isolated from large pools of donated blood to cope with frequent blood loss.

In the United States, by the end of September 1990, a total of 1,323 adults/adolescents and 132 children with hemophilia had been diagnosed with AIDS. Linda Augustyniak (National Hemophilia Foundation, New York) and colleagues reported that of 7,214 patients with hemophilia tested between 1985 and 1989 in the United States, 3,644 (50%) were found to be HIV-infected.

Data on the clinical course of HIV-related disease among hemophiliac patients (known to have received contaminated blood products) appear to yield conflicting conclusions. British and American studies have found that progression to AIDS is correlated with age—that is, older patients with hemophilia (over age 25) undergo a more rapid disease course. Dr. Nicola Schinaia, speaking for the Italian Cooperative Hemophilia-HIV Study Group, reported that, based on 103 Italian hemophiliacs, a significantly faster rate of progression was observable among patients over age 35. However a study of 161 French hemophiliacs, reported by Dr. Yves Laurian (International Hemophilia Training Center, Kremlin-Bicetre, France) and associates, found that progression to AIDS occurs at a faster rate in children than adults.

Other Transfusion Recipients. In reporting AIDS statistics, some countries make a distinction between hemophiliacs (a group known to require multiple or regular transfusions due to a blood clotting deficiency) and persons who have received blood transfusions or blood components. Through the end of September 1990, the CDC had reported that in the United States a total of 3,512 adult/adolescent AIDS cases and 243 pediatric AIDS cases were attributable to transfusion of infected blood or blood products. Based on these cumulative figures, transfusion-associated AIDS cases represent approximately 2% of all U.S. adult/adolescent AIDS cases and about 9% of all pediatric cases.

OTHER GROUPS UNDER STUDY

Several groups within the general population, such as STD clinic patients, prostitutes, and prisoners, were identified in the early years of the AIDS epidemic to have higher than "normal" seroprevalence rates—that is, rates above those observed for the rest of the general population. Epidemiological studies on these groups indicated that a significant percentage engaged in HIV-risk behaviors (high risk sexual and/or drug-injecting behaviors) at least some of the time. Ongoing demographic analyses show steadily increasing numbers of new HIV infections among women, adolescents and children, and, in the United States, racial/ethnic minorities and the homeless.

Based on the potential for HIV transmission, other "sentinel" populations are being followed—primary care hospital patients, emergency-room patients, dialysis patients, and health care personnel, for example. (Chapter 9: WORKPLACE ISSUES discusses the epidemiology of healthcare workers.)

STD CLINIC ATTENDEES

Sexually transmitted disease (STD) clinics receive a relatively large number of patients who are HIV-positive. This finding is not particularly surprising since certain STDs—notably ulcerative STDs—are thought to facilitate HIV transmission and since STD clinic patients generally engage in high-risk sexual behaviors.

General seroprevalence figures for STD clinic attendees in the United States were presented by Dr. Eugene McCray (CDC, Atlanta). The overall seroprevalence, based on 115,989 STD clinic attendees from 31 metropolitan areas, was 2.5% (the range was 0–39%). The rate for

homosexual/bisexual men among this group was 38% and for IVDUs, 7%. The highest rates for men were in the 35–39 age bracket and for women, the 30–34 age bracket. Similar seroprevalence rates were reported from several other studies, including studies from California; however, two notable exceptions were from Dade County, Florida (which includes the city of Miami), and New York City.

In the Dade County study, Dr. Mac Otten (CDC) and associates reported a seroprevalence rate of 11.3% (among 2,402 STD clinic attendees who agreed to be tested). Perhaps the most poignant finding was that approximately half of the clinic patients who did not accept testing could be identified as having high-risk behaviors: 59% of these patients were positive for syphilis and 56%, for gonorrhea. (Since December 1987, HIV testing has been offered to every patient; a total of 47,453 patients had attended the clinic by the end of 1989.) Mary Ann Chaisson (New York City Department of Health) and coworkers found an overall seroprevalence rate of 14% (249 of 1,834) among STD clinic patients in New York City. When analyzed by risk behavior, the following seroprevalences were calculated:

Risk Category	Seroprevalence	
	%	HIV+ / # tested
IVDUs	49.0	(88/180)
Homosexual men	39.7	(56/141)
Sex partner of bisexual men or IVDUs	14.0	(54/394)
"Other risk"	0.10	(7/73)
No identified risk	0.04	(44/1,046)

They also found crack-related sexual behaviors to be strongly associated with HIV infection in men (3.7%) and women (5.6%) for whom no identifiable risk behavior could be found. The authors offered that in regions of the city where crack use and HIV infection are common, a drug-for-sex barter system (as well as a money-for-sex trade) may enhance the spread of HIV into the heterosexual population.

In Europe, the highest rates for STD clinic populations came from Italy: Dr. Roberto Zerboni and colleagues (University of Milan) found an overall seroprevalence rate of 18.4%; 7,271 patients (2,859 homosexual men; 1,001 IVDUs, 3,411 heterosexuals) were evaluated during the period 1985–89. When analyzed by risk behavior, the rates were 22.3% among homosexual men, 58.6% in IVDUs, and 3.5% among heterosexuals. Dr. Zerboni's team was also able to examine these rates over time: seropositivity increased among homosexual men from 20.3% in 1985 to 33.4% in 1989; for heterosexuals, from 3.2% in 1985 to 5% in 1989. No changes (from about 60%) were observed for the IVDUs.

Seroprevalence rates from STD clinics in the Caribbean and Africa (Pattern II countries, where heterosexual contact is the primary mode of transmission) were not very different from those observed in the United States: 2.5–2.7%. Nigel O'Farrell (City Health Department, Durban, South Africa) and coworkers reported that, among a Zulu population visiting an STD clinic, higher seroprevalence rates were observed in women (3.2%, or 30 of 937) than in men (1.9%, or 33 of 1,745). Of the HIV-positive women, 50% were in the 15–19 age group. Among the seropositive men, HIV infection was associated with ulcerative STDs, but not with gonorrhea or genital warts.

PROSTITUTES

Although female "sex workers" have been studied more thoroughly as an HIV risk group, male prostitutes are also being studied by epidemiologists. Because of their high-risk sexual behaviors, the numbers of people they interact with, and, in many cases, IV drug abuse, both male and female prostitutes are not only highly vulnerable to HIV infection but are recognized as key disseminators of HIV to the general population.

Seroprevalence rates among prostitutes appear to vary widely. In areas where prostitution is legal, registered prostitutes tend to have much lower rates than street prostitutes. Brothel prostitutes tend to be regular users of condoms. In some cohorts, cultural differences may account for relatively lower rates, particularly where IV drug use is not a significant factor, as in Mexico.

Female Prostitutes. Cohort studies of female prostitutes in Pattern I countries—where IVDUs are a primary risk group—show that seroprevalence rates differ considerably when IVDU prostitutes are excluded. (Note, however, that identification of IVDUs among this population depends almost exclusively on self-reporting, which may not always be reliable.) In a 3-year study (1985 to 1988) of 3,137 registered female prostitutes working in the Frankfurt area, a yearly seroprevalence rate of 2.2% was observed. Dr. Hans Doerr (University of Frankfurt) and coworkers pointed out that German studies of prostitutes with drug-related behaviors report rates that are 20 times higher: 20–27%. Similar findings were reported by Dr. Celso Granato (Institute Adolfo Lutz-Sao Paulo) and associates in their 1989 study of prostitutes in Santos; a seroprevalence rate of 18.8% was noted for IVDU prostitutes, compared to 3% for their non-IVDU counterparts.

Reports from India (a Pattern III country with 48 cumulative AIDS cases) indicate that appreciable increases of HIV infection among females from the red light districts of Bombay have been seen in the last 2 years. Dr. Geeta Bhave (Seth G.S. Medical College & KEM Hospital, Bombay) and colleagues reported that the number of newly identified seropositives rose from 0 (for the period September 1986-March 1987) to 133 (out of 730 screened for the period April-May 1990). Between September 1986 and May 1990 in Bombay, 290 seropositive results were identified out of 2,740 female prostitutes tested. In Bombay, only recipients of blood and blood products show a higher seroprevalence rate:16.2% for blood/blood product recipients versus 10.6% among prostitutes. The authors proposed a model for the transmission dynamics between Indian prostitutes and other identifiable segments of the general population; see Figure 3.6.

Prostitutes in sub-Saharan Africa, where heterosexual contact has consistently been the primary transmission mode, are considered a significant reservoir for HIV. In fact, in studies of seropositive heterosexual adults, sex with a prostitute is frequently their only reported HIV-risk factor. One study of prostitutes in Kinshasa, Zaire, revealed an HIV seroprevalence rate of 35%. Drug-injecting behaviors are not seen in African prostitutes; their primary risk factor appears to be incidence of sexually transmitted diseases. Dr. Marie Laga (CDC/Institute of Tropical Medicine, Antwerp) and colleagues found that non-ulcerative STDs—but not ulcerative STDs—are significantly associated with HIV seropositivity in prostitutes from Kinshasa. A comparison study of HIV-positive and HIV-negative prostitutes revealed that the incidence of genital ulcers was similar for both groups (5% and 4%). However, seropositive prostitutes had a higher incidence of:

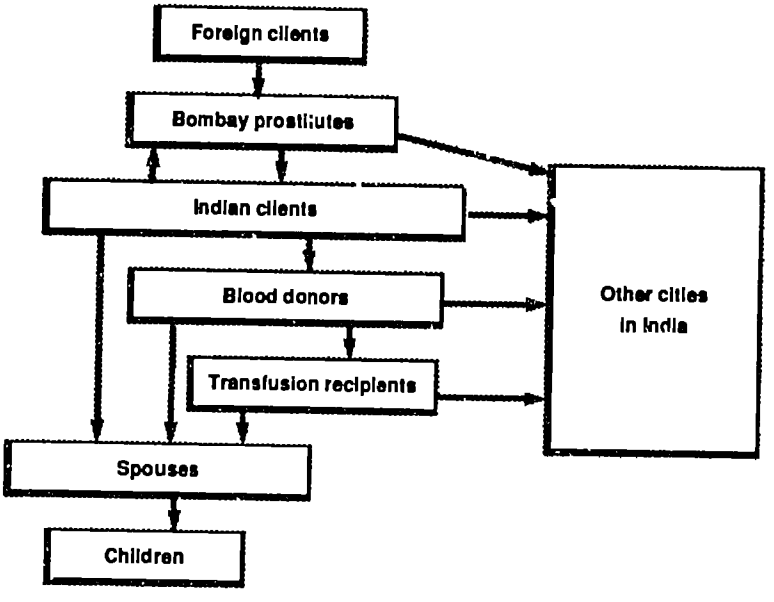
- purulent cervicitis (24% versus 10%, for seronegative prostitutes)
- leukocytes on vaginal smear (31% versus 9%)
- gonorrhea (52% versus 19%)
- chlamydia (29% versus 6%)
- trichomoniasis (43% versus 18%)

These findings suggest that transmission of HIV is also facilitated by STDs that are not ulcerative. (Data from numerous studies of risk factors for HIV infection in the general population in Africa have shown that ulcerative STDs—due to actual skin breaks in the genital region—are associated with HIV seropositivity.)

Male Prostitutes. Several Conference reports on male prostitutes indicated that, like female prostitutes, this population has significantly higher HIV-seroprevalence rates than the general populous. Furthermore, a Brazilian study found that seroprevalence

Figure 3.6 Model of Chain of HIV Transmission in India

(Adapted from a Conference report by Dr. Geeta Bhave and Usha Wagle at Seth G.S. Medical College & KEM Hospital, Bombay, in collaboration with Srikant Tripathy of Indian Council of Medical Research, New Delhi.)



rates are not dropping: a rate of 68.6% was observed for 1989, compared to 43% for 1988. Dr. Kirk Elifson (Georgia State University, Atlanta), in collaboration with the CDC, reported that in Atlanta, seroprevalence rates were found to be much higher among male transvestite prostitutes (68.9%) than among non-transvestite (male) prostitutes (25.3%). Only 7% of the seropositive transvestite prostitutes reported IV drug use, suggesting that high-risk sexual behaviors are largely responsible for HIV transmission. A high incidence of sexually transmitted diseases among seropositive Italian and South American male transvestite prostitutes was reported by Dr. Spinello Antinori (University of Milan) and coworkers: 27 of 48 (56.3%) subjects were HIV-positive, and 83.3% of these were also positive for syphilis.

PRISONERS

For several reasons, prison inmates are included in sentinel population studies of HIV infection. A significant percentage of prisoners are current or past IVDUs and many self-report multiple risk behaviors, including same-sex contact, receptive anal intercourse, history of STDs, and high numbers of sexual partners. Luisa Martin (Ministerio Justicia, Madrid) and colleagues reported a March 1989 cross-sectional study of approximately 79% of the total prison population of Spain. In identifying HIV-risk factors, they found that tattooing and acupuncture held significant relative risk value—exceeded only by syringe use—as did receiving a blood transfusion before 1987 and having more than two sex partners per year.

In a 3-year study of 1,484 IVDU arrestees in Stockholm, Kerstin Kall and coworkers (Karolinska Institute, Sundbyberg) found an HIV seroprevalence rate of 11.1%. When IVDU prisoners were studied according to the main type of drug used, heroin users were found to have a higher seroprevalence rate (36.8%) than those who reported using mainly amphetamines (5.3%). The majority—82%—of this cohort population reported using mainly amphetamines whereas 18% reported using mainly heroin.

WOMEN

Virtually all Pattern I countries show that the proportion of female AIDS cases is growing as the epidemic continues to expand. The World Health Organization estimates that one in 700 women are infected in North America, one in 500 in South America, and one in 1,400 in Western Europe. Women who are IV drug users as well as women who are heterosexual contacts of HIV-infected persons account for the great majority of the female AIDS cases.

AIDS Incidence and Seroprevalence. In the United States, surveillance data reveal that 9.5% of all adult and adolescent AIDS cases are female. Projections estimate that this figure will rise to 11% by the end of 1990. The CDC reported that the proportion of heterosexual contact cases among females rose slightly from 1988–89 (32%) to 1989–90 (34%) for the same 12-month period; IVDU cases dropped slightly for the same period (from 52% to 48%).

Issues have been raised surrounding the collection of seroprevalence data among women in general. Most epidemiological studies are based on risk factors rather than gender; thus much of the data on women is subsumed under risk categories such as IVDUs and STD clinic attendees. Recently epidemiologists have turned to blind screening of newborn blood samples to estimate the seroprevalence rate among women. A major drawback to relying on such data is the finding (by researchers at Walter Reed Army Institute of Research, Washington, D.C. and others) that HIV-positive women are more likely to abort spontaneously or have obstetrical complications, resulting in fewer live births than their uninfected peers. Such seroprevalence studies then may yield lower estimates of HIV infection in women since not all pregnancies among HIV-infected women come to term or result in live births.

Several seroprevalence studies on women in the United States found rates ranging from 0.03% to 3.7%. (Not included in that range was a study of 61 women at Harlem Hospital Center, New York, for which a seroprevalence rate of 18% was found; the overall seroprevalence among women delivering at this hospital was 3.5–5%.) Patricia Sweeney (CDC) and colleagues reported data gathered from more than 100,000 women from 130 family-planning, prenatal, and abortion clinics in 32 metropolitan areas since 1988: a seroprevalence rate of 0-2.6% was found. (Data was expressed as units per 1,000, so that this rate was equivalent to 0-26 HIV-infected women out of a total of 1,000 women.) Ms. Sweeney said that, not surprisingly, areas with the greatest number of AIDS cases—such as the Northeast—also had the highest seroprevalence rates. However, it was surprising that in some Midwestern cities, rates as high as 0.2% (2 per 1,000) were found. This study noted that women 20–29 years of age had the highest rates, and the median seroprevalence rate among black women was higher (0.5%; range 0–3.5%) than white (0%; range 0–4.3%) or Hispanic women (0%; range 0–2.5%).

Other U.S. studies reporting seroprevalence rates for women also noted racial/ethnic differences when data was available: In a state-by-state nationwide survey, analysis of the findings from 6 states providing data on race showed that the rate for black women was 5 to 15 times that for white women. (In this study the overall seroprevalence rate, determined by anonymous testing of infants delivered during the period 1988–89, was 0.15%, or 1.5 per 1,000 childbearing women.)

In a study of 135,762 childbearing California women conducted in late 1988, Hispanic women were found to have rates twice that of white women and black women had rates more than 12 times that of white women. The authors offered that the high rate in black women might reflect the extent of infection among black IVDUs in California. AIDS cases in the state are increasing more rapidly among women than among men and, unlike other areas of the country, HIV acquisition among women in California was equally attributable to IV drug use and heterosexual contact.

In sub-Saharan Africa, where the primary mode of transmission in the general population is heterosexual and the male-to-female ratio is roughly 1:1 in all countries, seroprevalence rates typically range from 20% to 40%. Dr. Christopher Ndugwa (Makerere University, Kampala, Uganda) reported his team's cohort study of pregnant women who came to the prenatal clinic at Mulago Hospital in Kampala between December 1988 and May 1990. Of 3,612 women screened for HIV, 1,032 (28.6%) were seropositive. Analysis of demographic information for seropositives and seronegatives revealed essentially no differences. (In this population an association between HIV infection and a history of herpes zoster and syphilis was found.)

Risk Factors. Cohort studies of pregnant women or women of childbearing age reveal that risk factors are generally the same as those identified for sexually active heterosexuals—multiple sex partners or history of STDs, for example. Several studies identified independent risk factors as statistically significant among the women studied:

- cocaine/crack use with no history of IV drug use (Harlem Hospital study, New York City)
- history of hepatitis B—antibody reactivity to hepatitis B core antigen (Ohio Department of Health study)
- history of genital warts—but not genital ulcers (in a Malawi study by Johns Hopkins University)
- smoking (in studies conducted in Haiti and the Dominican Republic; see below)
- breastfeeding and cracked nipples (in the USSR, see below)

A novel type of HIV transmission from infected children to their breastfeeding mothers has been demonstrated in the Soviet Union. Dr. Irina Kuznetsova (Central Research Institute of Epidemiology, Moscow) reported that 230 children (who had been infected through reuse of syringes while hospitalized) and 12 mothers were found to be HIV-infected in 1988. Of these 12 mothers, 7 reported no known HIV-risk factors, but acknowledged that they breastfed their infants until age 2. Among 94 women who didn't breastfeed, none were HIV-positive. Blood-to-blood contact between the infected infants and their mothers was identified as the transmission route: the suckling infants had severe stomatitis (inflamed and bleeding mouths) and the mothers had cuts and cracks on their nipples.

A follow-up study on the possible association between smoking and HIV infection in women in Haiti was reported by Jacqueline Coberly (Johns Hopkins University, Baltimore, Maryland) and colleagues. Analysis of risk behaviors for Haitian women who smoked, compared to former smokers and non-smokers showed that smokers were found to exhibit high-risk sex behaviors: They had their first intercourse earlier, had more lifetime sex partners, and were less likely to be married. In an independent study presented by Ernesto Guerraro (Centro de Orientacion e Investigacion Integral, Santo Domingo), smoking in female sex workers in the Dominican Republic was correlated with higher rates of STDs and high-risk sex practices, including anal sex (8.6%) and sex during menses (30%).

ADOLESCENTS/YOUNG ADULTS

Several Conference reports this year emphasized the need to look at AIDS incidence data and seroprevalence rates in Pattern I countries with respect to age. New AIDS diagnoses among young adults in 1989 or 1990 suggests that HIV acquisition probably occurred during their adolescent years. Studies of adolescents and young adults reveal a high percentage engage in risk behaviors associated with HIV transmission. IV drug use, high-risk sexual behavior (notably, multiple partners and lack of condom use), and a history of STDs are major identifiable risk factors. Non-IV drug use was also found to be associated with high-risk sexual behaviors by some investigators.

In a study of seropositive adolescents and young adults in England, Wales, and Northern Ireland, Sian Clarke (Communicable Disease Surveillance Centre, London) and associates reported the following data, accumulated through the end of 1989:

Age Group	No. Infected	Major Exposure Category
10–14	86	93% hemophiliac
15–19	269	44%, hemophiliac; 35%, homosexual; 9% heterosexual
20–24	1,318	57%, homosexual; 19%, IVDU; 10% heterosexual

Of those infected through heterosexual contact, 82% in the 15–19 group were female; as were 66% in the 20–24 age group.

In Spain, where IVDUs accounted for more AIDS cases in 1989 than homosexual/bisexual men, IV drug use appears to be the major risk factor in adolescents and young adults (15-20 years of age). In a Madrid study, Perez Alvarez and colleagues (Health Department, Madrid Council) identified 725 out-patient clinic attendees with HIV-risk behaviors; 64% were IVDUs and 81% of these reported sharing syringes. Of the 725 at-risk individuals, 273 (38%) were found to be HIV-positive, of whom 57.4% were IVDUs; 6.5%, homosexual; and 5.9%, partners of HIV-infected persons.

In the United States, seroprevalence studies of adolescents and young adults (15–24 years of age) conducted during 1988–90 in STD clinics, drug treatment centers, and women's health centers in 44 metropolitan areas (more than 1,000,000 samples were tested), show that the average rate of infection is about 0.5%. In STD clinics, for which the most data has been collected, seroprevalence rates range from 0% to 30.4%, with rates hovering around 1.3% for males and 0.6% for females. Rates were found to vary markedly by sex, age group, geographic region, and clinic type—for example, rates in STD clinics and women's health centers were highest in the Northeast and South.

Very different results for a supposedly low-risk population were reported by Dr. Steven Keller and colleagues (University of Medicine & Dentistry, New Jersey). Among 407 healthy, heterosexual, non-IVDU, inner-city adolescents who received routine medical examinations (generally, for school or work), 1.24% were found to be seropositive. The researchers pointed out that these adolescents are from an AIDS epicenter (Newark); analysis of risk behaviors actually revealed substantial levels of high-risk sexual behaviors with the strongest predictor of high-risk sexual behavior being moderate use of alcohol or marijuana.

U.S. studies of adolescent homeless populations show much higher rates. A blind study of runaway and homeless youths in four states from October 1987 to September 1989 revealed a disturbing seroprevalence rate of 4.13% among 4,383 in the study population, as reported by Rachel Stricof (New York State Department of Health, Albany). When this rate was analyzed by state and by racial/ethnic background, significant differences could be distinguished:

State	Seroprevalence Rate
Florida	2.33%
Louisiana	2.19%
New York	5.69%
Texas	2.10%

Racial/Ethnic Background	Seroprevalence Rate
Hispanics	5.95%
Blacks	4.70%
Whites	2.63%

The CDC reported that as of September 1990 in the United States, 585 AIDS cases had been diagnosed among adolescents (13–19 years of age), and approximately 6,371 cases had been diagnosed in the 20–24 age group.

CHILDREN

Pediatric HIV infection is a growing problem in virtually every part of the world. HIV-infected children (13 years of age or younger) have acquired the virus, for the most part, either by perinatal transmission (from their mother) or by transfusion of contaminated blood or blood products. A few anecdotal case studies of HIV infection by sexual abuse have also been reported. The majority of pediatric cases—in all countries—are attributable to perinatal transmission (see "Perinatal Transmission," below). And most industrialized countries are now seeing significant declines in the percentage of cases due to transfusion with concomitant increases in perinatal AIDS.

In the United States, from 1988 to 1989, a 40% decrease in transfusion-related cases among children less than age 13 was noted and a 17% increase in perinatal cases. Based on data through December 1989, Dr. Blake Caldwell and coworkers (CDC) reported the following distribution of pediatric AIDS cases by mode of transmission: 81%, perinatal; 5%, hemophilia; 11%, transfusion-associated; and 3%, unknown. (Pediatric AIDS cases represent approximately 2% of total U.S. AIDS cases.)

The incubation time for pediatric HIV infection is generally shorter than that for adults; furthermore, children who have acquired HIV perinatally appear to have the shortest incubation period. Some of these children develop AIDS within 18 months after birth (about 11% of all perinatal cases); other perinatally acquired cases follow a much slower course (similar to that seen in adults), with an average incubation time of 6.4 years. Children with hemophilia or transfusion-related infection also tend to develop AIDS on a time course similar to that seen in infected adults. In Africa, HIV-associated mortality in infants is much higher than in industrialized countries—and death occurs much earlier. (See "AIDS-Associated Mortality," below.)

THE HOMELESS

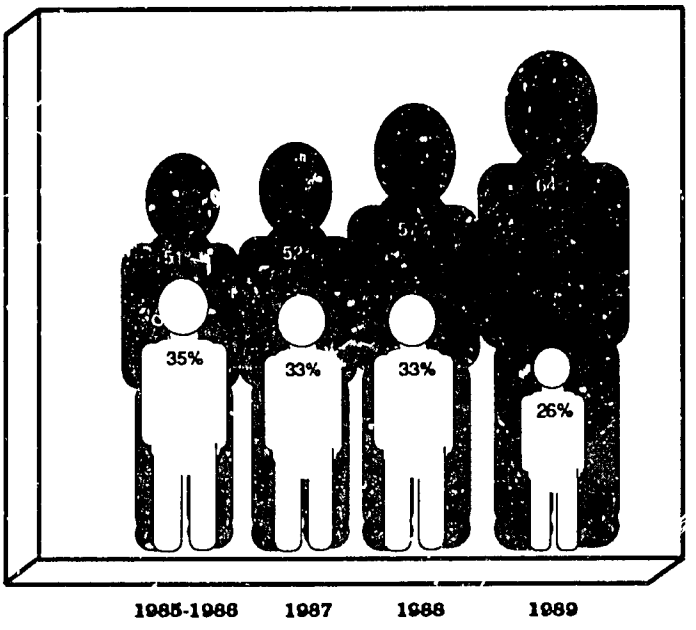
Although only a small number of epidemiological studies on homeless individuals have been conducted, it is suspected that HIV seropositivity among this group is very high. (See also "Adolescents," above.) Substance abuse and the very circumstances of homelessness (malnutrition, exposure to the elements, exposure to many infectious agents in "shelters," and lack of medical care), each of which contributes to an immunosuppressed state—plus being subjected to frequent acts of violence, including rape—are believed to place homeless persons at risk for HIV infection. In New York City alone, it is estimated that of the 70,000-90,000 homeless men, women, and children, 20% to 30% are HIV-positive and 10,000 are believed to have HIV-related illnesses.

U.S. MILITARY PERSONNEL

All applicants to the U.S. military are screened for HIV and all branches routinely retest personnel. Data from these tests has provided much information on seroprevalence rates, risk factors, and demographics. However only cautious extrapolations to the general population can be made since the military is not representative of the general populous (for example, individuals entering or serving in the military are less likely to be IV drug users).

Based on the number of seroconversions among active duty soldiers during the period 1985–89, a consistent and significant downward trend in new HIV infections was noted. When HIV infection trends are studied by race and gender, however, black men show

Figure 3.7 HIV-Infection Trends among Men in the U.S. Military by Race
(Based on data reported by Dr. Patrick Kelley of Walter Reed Army Institute of Research, Washington, D.C., and colleagues.)



increasing HIV seroprevalence over time (see Figure 3.7). A seroprevalence study of more than 2 million civilian applicants for military service showed overall declining rates for the years 1985–89. An increase in seroprevalence among black females, however, was noted in 2 of 3 years. Rates were consistently lower over time in metropolitan areas with high AIDS caseloads, but not in locations outside these "AIDS epicenters."

Dr. Thomas Peterman and coworkers (CDC), in collaboration with Walter Reed Army Institute of Research (Washington, D.C.), reported on risk factors identified in 21 of 43 recent male seroconverters. Risk behaviors included sex with men, sex with IVDU women, and sex with women from Haiti or Africa. Of 18 men who reported same-sex contact, 13 also had sex with women. Of the recent seroconverters for whom *no* risk was identified, 7 (32%) reported that their partner bled during sex, 4 (18%) reported having had an STD since their last negative HIV test, 3 (14%) reported having sex with a prostitute, and 2 (9%) reported having anal sex with a female partner.

PERINATAL TRANSMISSION

Perinatal or vertical transmission refers to the transmission of HIV from mother to child: HIV infection can be acquired while *in utero*, at the time of labor and delivery, or during the postpartum period. (Although all children of seropositive mothers are considered to be at high risk for acquiring HIV, not all become infected. Roughly one-third of all children of seropositive mothers become infected; see "Transmission Rates," below.) Apparently the at-risk fetus can become infected at any time during the pregnancy; HIV has been detected in the fetus, placenta, and amniotic fluid. Transmission can also occur during labor and delivery. Dr. Patrick Donegan (Boston City Hospital) and coworkers identified HIV-infected cells in the cervix, but not vaginal tissue, of HIV-positive women—a finding that may explain why delivery by cesarean section does not work as a preventive strategy for neonates of seropositive mothers. The authors suggested that the cervix is a likely reservoir of HIV, and that transmission could be taking place at this site at any time during the pregnancy, including labor and delivery.

Table 3.4. Factors Associated with Perinatal Transmission Rates
(Based on a presentation by Dr. Francois Boue, Hôpital Antoine Beclare, Clamart, France.)

Study	Maternal status (during pregnancy)	HIV+ mothers (Total = 88)	Perinatally infected children (Total = 29 or 33%)
CD4 cell count	$>150/mm^3$	73	19 (26%)
	$<150/mm^3$	15	10 (66%)
p24 antigen	negative	64	17 (26%)
	positive	24	12 (50%)
Viremia	low replication	38	6 (16%)
	high replication	14	11 (78%)

(Note: Only children found to be HIV-positive after 15 months of age were considered to be HIV-infected in this study.)

Although thought to be relatively rare, breastfeeding can also account for some cases of perinatal transmission. HIV infection by breast milk has been documented in cases where children, born to HIV-negative women who seroconverted sometime after childbirth, themselves seroconverted at 20–22 months with breastfeeding as the only risk factor.

As pointed out by Dr. Janet Mitchell (Harlem Hospital Center, New York), the efficiency of HIV transmission from mother to child may be affected by the mother's stage of disease at the time of pregnancy and, perhaps independently, by the mother's immunological status. One exemplary study, presented by Dr. Francois Boue (Hôpital Antoine Beclare, Clamart, France), showed that the incidence of perinatal transmission is significantly higher when the infected mother has a low CD4 cell count (less than $150/mm^3$), has p24 antigen, or has positive HIV culture with a high rate of replication (see Table 3.4).

Similar findings were reported by others, including Dr. Ann-Britt Bohlin (Danderyds Hospital, Stockholm) and colleagues who noted that none of 10 children born within the first 2 years of maternal HIV infection were infected, compared to 5 of 11 children who were infected and were born to mothers who had been infected for longer than 2 years at time of birth. In a study of 46 HIV-infected pregnant women, however, Dr. G. V. Zuccotti and coworkers (University of Milar, San Paolo Hospital, Italy), could not find a correlation between maternal immunological status and development of HIV infection in their children.

Transmission Rates. Although HIV-transmission rates from infected mothers to their children are generally reported to be about 30-33%, some conference reports based on specific populations found rates as low as 19% and as high as 42%. In Africa, where HIV seroprevalence in the general population is very high, the number of HIV-infected women of childbearing age is probably higher than anywhere else in the world. This fact translates into correspondingly high numbers of HIV-infected newborns. Not surprisingly, studies based on specific African populations—in Rwanda, Burkina Faso, Kenya, Zambia, Zaire, and Congo—reported the highest rates (42% in Congo). In one representative presentation Dr. Clement Mwale (University Teaching Hospital, Lusaka, Zambia) and coworkers, followed 109 seropositive mothers and their children for 3 years and found 42 children to be infected—a transmission rate of 38.5%.

Most presentations from Europe, the United States, and many industrialized countries quoted an overall (approximate) perinatal transmission rate of 30%; the majority of perinatal-transmission cases are from HIV-infected mothers who are or were IVDUs. (See also Chapter 4: DIAGNOSTIC TESTS FOR HIV INFECTION.)

BLOOD SUPPLY SAFETY

Since the identification of HIV as the etiologic agent of AIDS, major steps have been taken to ensure that worldwide blood supplies remain free from contamination with the virus. Mandatory screening of all donated blood for HIV antibodies (one established indicator of HIV infection) has been particularly effective in ensuring blood supply safety. In the United States, Canada, and most Western European countries, routine screening was instituted in 1985.

Although results obtained from screening tests for the detection of HIV antibodies are generally 98-100% accurate, there have been reports of contaminated blood "missed" by such tests—the so-called "false negatives." Based on their experience with a high-risk population, one research team concluded that special circumstances may dictate supplementing antibody tests with other HIV-detection methods. Dr. Thomas Quinn (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) and colleagues were able to identify HIV infection in 6 IVDU patients attending an inner city emergency room (who were seronegative by antibody testing); in these 6 patients, HIV status was determined by either virus culture or a test that detected HIV DNA (polymerase chain reaction, or PCR). Chapter 4: DIAGNOSTIC TESTS FOR HIV discusses the issue of false negatives in full detail, along with discussions on sensitivity of the various types of screening tests.

In addition to screening tests, the cooperation of the blood-donating public is essential to ensuring the safety of the blood supply. On careful interviewing of donors for high-risk behaviors, those identified as having risk factors are generally dissuaded from making an initial donation; such individuals are referred to HIV-testing sites. Carol White and colleagues (CDC), in cooperation with 20 U.S. blood centers, interviewed 457 HIV-positive heterosexual donors identified in the period May 1988-September 1989. Seventy-nine (61%) had no risk factor other than heterosexual contact with a person at risk for AIDS. The authors found that despite knowledge of the partner's risk, many individuals who had heterosexual contact did not perceive themselves to be at risk. In an independent study, Debra Kessler and coworkers (The Greater New York Blood Program) identified 15 HIV-positive blood donors who donated again within 2-48 months following notification of their seropositivity; 5 of the 15 reported that they donated again because they wanted to be retested.

In northern Brazil, a study of three types of blood centers (government, university, and private) revealed overall high seroprevalence rates for donors, but particularly disturbing was the highest rate (2.2%), which was observed for a private transfusion center. Dr. Carlos Alves (Federal University of Bahia, Salvador) and colleagues reported that The State Blood Center, with a seroprevalence rate of 0.29%, excluded 40% of potential donors. Risk factors identified from all three center types included homosexual contact (1.4% of male donors at the private center), contact with a prostitute, and IV drug use.

Dr. Peter Lamptey (Family Health International, Durham, North Carolina) related the particularly poor state of affairs with respect to the blood supply in developing countries, and specifically in Africa. While public health officials have acknowledged that the blood supply is best protected by testing all blood donors, this is not an easy task in African countries. Dr. Lamptey pointed out that blood banks do not exist in most African countries and that transfusions occur only in life-threatening situations, in which family or friends (and even paid donors) donate blood for an urgent transfusion, often without screening or cross-matching. Furthermore, because HIV in Africa is primarily spread by heterosexual contact, the seroprevalence rate in the general population is relatively high. Finding safe blood donors has become increasingly difficult. The issue of economics is another major part of the problem in developing countries: HIV testing adds appreciably to the cost of a transfusion and most hospitals cannot afford to implement routine testing. Transfusion-related transmission in Africa accounts for approximately 10% of all AIDS cases.

INCUBATION TIME

The length of time between initial infection with HIV and the time of diagnosis with AIDS—referred to as incubation time—is difficult to trace in most populations because the time and/or source of infection is unknown. In transfusion recipients, however, the exposure to HIV is usually traceable to the date of a single transfusion with contaminated blood, enabling epidemiologists to study the length of the incubation period for this population. Compared to infection by other means (by sexual contact, for example), HIV infection acquired through transfused blood or blood products generally correlates with a shorter incubation period: 80 months compared to 96-108 months. Patricia Volkow (Hospital PEMEX, Mexico City) and coworkers reported a maximum incubation time of 46 months for a small population of adult transfusion cases; 36 cases were included in the study and all were recipients of only one transfusion: 75% were diagnosed with AIDS within 36 months; 50% after 27 months; and 20%, within 12 months. Based on data from 42 cases of transfusion-associated AIDS patients who have died, Katherine Swanton and colleagues (Transfusion Related AIDS Support Unit, Sydney, Australia) reported that the average time from infection to death (resulting from AIDS-related causes) was 35 months. Last year, at the Montreal Conference, a mathematical model of the incubation time, based on European transfusion-associated HIV infections presented by WHO, found the average time to be 6.4 years. (See also "Epidemiological Modelling," below.)

HIV-ASSOCIATED MORTALITY

AIDS is still recognized as a uniformly fatal disease, but investigators have documented several changes in mortality trends relating to HIV disease. In industrialized countries, people with AIDS are surviving longer after their diagnosis, at least in part because of the availability of effective drug therapy. However, the increase in survival time is not enjoyed by all subgroups of patients. And even though some people with AIDS are living longer, AIDS is accounting for an increasing proportion of deaths worldwide.

In the United States, national surveillance data, reported by Bradley Hersh and coworkers (CDC), on more than 37,000 homosexual/bisexual men with AIDS show a striking increase in survival time between 1984 and 1987. Of homosexual/bisexual men diagnosed in 1984, 40% survived at least 12 months; of those diagnosed in 1987, the proportion increased to 56.6%. The average survival time after diagnosis increased from 10 to 16 months. Average survival for men diagnosed in 1987 was greater in whites (17 months) than in blacks or Hispanics (12 months), and greater in men living in New York City, San Francisco, or Los Angeles (17 months) than in men living elsewhere (12 months). Studies from San Francisco, Massachusetts, and London also showed increased overall survival in homosexual/bisexual men and in all adult patients with AIDS. In several of these studies, the prolonged survival occurred mainly in patients who were initially diagnosed with *Pneumocystis carinii* pneumonia (PCP); it was less striking in patients presenting with other opportunistic infections. In patients whose initial AIDS diagnosis was Kaposi's sarcoma, mortality trends were either inconsistent or unfavorable—that is, average survival time has decreased.

Dr. George Lemp (San Francisco Department of Public Health) reported that antiviral and other drug therapy accounted for much of the increase in survival times. Almost 1,300 homosexual men who received drug therapy at some point in their disease course, even if treatment was discontinued, were compared with 582 patients untreated at the time of their AIDS diagnosis. Those who began treatment with zidovudine (the antiviral AZT) or aerosolized pentamidine (as prophylaxis or treatment for PCP) had a median survival time of 23 and 24.8 months, respectively, and the effect of combined or sequential therapy with the two agents was even greater. In contrast, the median survival time for the untreated-

patient group was 13.7 months. A proportional hazards analysis showed that an initial diagnosis of PCP, as opposed to any other AIDS-defining condition, had the strongest influence on survival, followed by treatment with AZT. A smaller study from England of 63 patients diagnosed in 1987 also showed that AZT contributed to prolonged survival: for those taking AZT, median survival times exceeded 24 months, versus 7 months for those not using the drug. However, prophylaxis against PCP was also offered more aggressively in the AZT-treated group, reported Barry Stephen Peters (St. Mary's Hospital, London) and associates.

AIDS continues to increase in standing as a cause of death in some demographic groups worldwide. In the developed world, AIDS is affecting the mortality of groups in which it previously had little impact. U.S. statistics show that AIDS accounted for 2% of *all* deaths and was the fourth leading cause of death in black men and women aged 20–24. Dr. Barbara Kilbourne (CDC) and coworkers reported that HIV-related disease ranked fifth among causes of death in white men in their early 20s, and it is becoming an important cause of death among women of reproductive age, especially those between ages 25 and 34. Susan Chu (CDC) and colleagues presented data showing that AIDS accounted for 11% of all deaths in American black women and 3% of deaths in white women in this age group. HIV-related disease now accounts for almost 7% of deaths in children under age 15 in California, and in Canada AIDS has become the leading non-violent cause of death in men aged 30–39.

In some developing countries, particularly in Africa, AIDS is taking an even greater toll. In Central Africa, AIDS was the leading cause of death in middle-aged factory workers in Kinshasa, Zaire, accounting for about 50% of all deaths in male workers and 33% of deaths in their spouses. A study conducted in a Kinshasa general hospital, reported by Dr. Bila Kapita (Mama Yemo Hospital) and colleagues, showed that AIDS was the leading cause of hospitalization (27% of hospitalized patients had AIDS) and of in-hospital deaths (about 66% were attributed to AIDS). In Abidjan, Ivory Coast, AIDS was also the leading cause of death in adults. Kevin DeCock (Projet RETRO-CI, Abidjan) and colleagues reported results that are particularly disturbing because HIV was introduced relatively recently in Abidjan in comparison to many other African cities: An autopsy study showed that 41% of male and 31% of female adults were seropositive for HIV. AIDS accounted for 15% of deaths in men and 13% of those in women.

In general, compared to adults, children with AIDS have shorter survival times. Although this period seems to vary according to the first clinical manifestation, reported survival times ranged from 7 months to 6 years. Perhaps not surprisingly, HIV-infected children in the developing world do not survive as long as their counterparts in industrialized countries. In Brazzaville, Congo, according to a study conducted by Marc Lallemant (ORSTOM S/URB, Paris/Brazzaville) and colleagues, about half of a group of children born to infected mothers (27 of 64) were infected. One-third of these infants did not survive 1 year, and almost half died before reaching age 2. In a study in Rwanda, Philippe Lepage (Centre Hospitalier de Kigalie, Rawanda) and coworkers reported that about 33% of infants of seropositive mothers were infected, and 14% of children of infected mothers died in their first year. High death rates were also reported in infants and young children born to HIV-infected mothers in urban Zaire, in rural Uganda, and in an urban slum in Haiti. In the latter study, Reginald Boulos (Centers for Development and Health, Port-au Prince) and colleagues showed that a significant decline in total infant mortality was reversed by HIV-related disease: Various interventions had resulted in decreasing infant mortality—from 23.5% in 1976 to 8.5% in 1982. However, in the Haitian neighborhood studied (an urban slum), where almost 10% of pregnant women are seropositive for HIV, infant mortality had increased to 11% in 1989.

OTHER RETROVIRUSES

HIV-2

Large-scale screening for infection with HIV-2 (a second human retrovirus associated with AIDS-like symptoms) has been limited for the most part to West Africa. At the Conference, Senegal, Ivory Coast, Burkina Faso, and Guinea Bissau reported ongoing surveillance data, confirming the endemicity of this virus in West Africa. (Regional differences in HIV-2 seroprevalence among West African countries and even within a given country were not uncommon.) HIV-2 infection is primarily found in those who

- are of West African descent
- have lived in or traveled to West Africa
- have had sexual contact with a West African
- have received blood transfusions in West Africa

However, there have been a small but increasing number of reports of HIV-2 infection (and HIV-1/HIV-2 double infection) in persons with no seeming West African connection. HIV-2 infection is believed to have been introduced to Europe through countries with historical relations with West Africa—notably, Portugal and southern France. In a 1987–89 study in which 9,305 sera were evaluated, Dr. Ana Benito-Garcia and colleagues (Instituto Nacional de Saude, Lisbon) reported finding an HIV-2 seroprevalence of 1.1% among Portuguese with no known contacts with Africa. Interestingly, this seroprevalence rate approximated that seen in Portuguese *with* African contacts (0.8%). In this same study, 32% of the 348 AIDS cases attributable to HIV-2 were among Portuguese with no known African contacts. Continuing studies will examine how extensive HIV-2 infection is among the non-African Portuguese population and the rate of spread into this population.

Data presented by Dr. Catherine Tamalet (Hôpital de la Timone, Marseilles) and coworkers revealed a seroprevalence rate of 0.32% for HIV-2 infection in Marseilles: 18 of 88,482 sera collected between 1987 and 1989 were found to be HIV-2 positive, with 12 of the infected individuals from Africa, 4 from Marseille, and 2 of unknown origin.

The United States, Canada, and Brazil have reported a small number of cases of HIV-2 infection. Dr. J. Peralta (Fundacao Oswaldo Cruz, Rio de Janeiro) and colleagues, in collaboration with the U.S. Centers for Disease Control, have also documented HIV-1/HIV-2 double infection in Brazil. HIV-2 infection appears to be rare among U.S. residents: 18 cases of HIV-2 infection have been detected. Dr. Thomas O'Brien (CDC) reported that 13 of these are individuals originally from West Africa, 1 is an American who has traveled to West Africa, 1 is from Europe, and 3 are of unknown origin. Ida Onorato (CDC) and colleagues presented data showing no evidence of HIV-2 infection from their sentinel surveillance studies established in 1988 in 5 metropolitan areas. More than 10,614 serum samples from attendees of STD clinics or drug treatment centers were tested.

The transmission routes and transmissibility (relative ease of transmission) of HIV-2 are apparently not identical to those of HIV-1. High-risk behaviors for HIV-1 infection (namely, intravenous drug use and homosexuality), for example, do not appear to factor into the transmission of HIV-2. Several African studies examined risk factors for HIV-2: Dr. Lee Harrison (CDC) and coworkers found that among 23 HIV-2 infected hospitalized patients in Guinea Bissau, sex with a prostitute was the principal risk factor for men and, for women, a history of blood transfusions.

An interesting finding on the lack of mother-to-child transmission for HIV-2 was reported by Dr. Jean-Elie Malkin (G.E.E.P., Villeneuve-Saint-Georges, Burkina Faso) and coworkers: A screening program of 1,400 pregnant women at Banfora Hospital (Burkina Faso) was initiated in which the rate of infection among this group, as well as the rate of perinatal transmis-

sion, was determined. Among 41 seropositive women, 51% were HIV-1 positive, 19% were HIV-2 positive, and 24% had HIV-1/HIV-2 dual infection. Of the 21 children born to seropositive mothers who were followed in this study, 4 have been confirmed positive—HIV-1 positive. No case of HIV-2 or HIV-1/HIV-2 transmission was observed. Similar results were reported by Georgette Adjoriolo (Projet RETRO-CI, Abidjan, Ivory Coast) and colleagues who also found that the mean age of HIV-2 seropositive individuals was higher than that of HIV-1 seropositives. The authors also noted that evaluation of 1,003 hospitalized and well children revealed HIV-2 and HIV-1/HIV-2 infection to be rare in children, with the seroprevalence rate for both types of infection being 0.5%, compared to 9% for HIV-1 infection.

HTLV-I AND HTLV-II

Two other human retroviruses, the human T-cell leukemia viruses, HTLV-I and HTLV-II, are also being studied by epidemiologists in an effort to determine transmission patterns, geographical areas of endemicity, and possible correlations with HIV transmission. Some research teams have speculated that infection with either HTLV-I or HTLV-II may influence transmission of HIV (and vice versa). Indeed, it is known that in the laboratory, in environmentally controlled cell culture experiments, the presence of HTLV-I can speed up the rate of HIV replication.

HTLV-I. Infection with HTLV-I occurs via sexual contact, blood contamination, or vertical (congenital or perinatal) transmission. An interesting epidemiological phenomenon is the finding that HTLV-I infection seems to be correlated with age—in Japan, the United States, and Caribbean. No explanations or hypotheses for this correlation have been offered.

Known geographical pockets of HTLV-I endemicity include southern Japan, the Caribbean, the southeastern United States, southern Italy, and parts of Africa and South America. So far, countries bordering HTLV-I endemic regions show very low seroprevalence rates. Because HTLV-I has also been detected in healthy individuals, blood-supply screening measures have been implemented in various parts of the world. Persons emigrating from endemic areas may be introducing HTLV-1 into the blood supply in nonendemic areas. For example, Dr. Joliette Coste (Centre Regional de Transfusion Sanguine, Montpellier, France) and coworkers presented a study of 45,033 blood donors from 12 areas in France; the data revealed a significantly higher HTLV-I seroprevalence rate among donors who emigrated from endemic regions: 61% (2 of 325) among blacks from endemic regions compared to 0% (0 of 93) for blacks/Asiatics from nonendemic regions and .007% (3 of 44,615) for whites native to France.

From worldwide surveillance data, HTLV-I is known to be associated with an adult form of leukemia (adult T-cell leukemia, or ATL) and also with a neurological disorder inducing varying degrees of motor and/or sensory loss called HTLV-I associated myelopathy (HAM) or tropical spastic paraparesis (TSP). Dr. Michel Dumas (Institut de Neurologie Tropicale, CHU Limoges, France) reported findings on a study of neurological disorders in West Africa—Ivory Coast, Senegal, Togo, and Burkina Faso. Although seroprevalence of HTLV-I in this part of Africa is relatively high (about 2%), infection with this virus does not appear to be tightly associated with paraparesis, in stark contrast to associations reported from other countries. Of 82 cases of spastic paraplegia, only 13 (15.85%) were HTLV-I infected. In the United States, Dr. Michel Canavaggio, from Abbott Laboratories in Chicago, reported that 39% of a patient population with TSP were infected with HTLV-I.

HTLV-I/HIV Dual Infection. Reports on findings of HTLV-I/HIV dual infection provided no clear pattern. In the Dominican Republic, Dr. Evelyn Rodriquez (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) and colleagues found HTLV-I seroprevalence among STD clinic attendees to be 4.1% (24 of 583), with HTLV-I/HIV dual infection to be 0.5% (3 of 583). Dr. Mirtha Capellan (University Technologica de Santiago) and colleagues

found an HTLV-1 seroprevalence rate of 5.8% among 397 Haitian and Dominican Republican sugar cane workers; only 1 case of HTLV-I/HIV dual infection was identified. In contrast, Dr. Jean-Pierre Allain (Abbott Laboratories, Chicago) and colleagues found a significant association between HTLV-I and HIV in an outpatient population of rural Haiti. Dual infection was observed in 18.3% of patients manifesting symptoms of immune deficiency (57 of 643); infection with HTLV-I alone, in 4.5% (15 patients); and infection with HIV alone, in 49% (312 patients).

HTLV-II. The retrovirus HTLV-II was first identified in a leukemia patient in the United States in 1982, but since then scant data has been available on its association with disease. Transmission routes, presumed to be sexual contact and blood contamination, are still under investigation as is whether this virus is associated with any clinical manifestation. HTLV-II has rarely been reported outside the United States and is rarely found in non-IVDU populations. However, Dr. Jonathan Kaplan (CDC, Atlanta) and colleagues reported that HTLV surveillance studies in Panama revealed an unusually high incidence of HTLV-II infection among Guaymi Indians in Panama. Seropositivity was not associated with disease but was associated with age (particularly in subjects age 15 and older).

HTLV-I/II seroprevalence. In a broad-based study on HTLV seroprevalence rates among U.S. blood donors, Helen Lee (Abbott Laboratories, Chicago) and coworkers reported an HTLV-I seroprevalence rate of 0.023% (28 out of 484,000). The overall (HTLV-I/II) seroprevalence rate determined for this sample was 0.043%—3 times higher than the overall seroprevalence rate for HIV in the general U.S. population. Demographic data revealed HTLV-II infection to be highly correlated with IV drug use or sex with an IVDU, whereas HTLV-I was linked to endemic areas; numerous investigators reiterated these findings.

Several reports from major metropolitan areas in the United States cited disturbingly high HTLV-I/II seroprevalence rates among IV drug using populations—and, to a lesser degree, in persons having sexual contact with IVDU. Differential rates for HTLV-I and -II were not always reported in these studies, partially because of their scope and the need for highly specific tests to distinguish between the two. A study of drug treatment centers and STD clinics in six major cities, conducted by the CDC and state/local health departments, found the HTLV-I/II seroprevalence rate for IVDU populations to range from 0.6–9.2% (1,593 total samples). Seroprevalence rates were found to be significantly higher in nonwhites (8.6% versus 2.9%) and highest when heroin was the primary drug injected (7.5% versus 2.7%). Among the STD population (3,487 total samples), the seroprevalence range was 0–2.1%, with seropositivity being associated with a history of IV drug use (6.5% versus 0.9%).

Blood Screening. In the United States, all blood and plasma collected for transfusion and blood product manufacture must be screened for both HTLV-I and HTLV-II. Antibody tests for the detection of HTLV-I and -II are currently in place; but, as was evident from the numerous presentations at the Conference, these tests require further technical fine-tuning to achieve more accurate results. Whether screening tests can accurately and consistently discriminate between the two HTLVs seems to be a major concern. (Much discussion also focused on the sensitivity of screening tests—that is the ability to detect low levels of virus or antibodies to viral proteins.)

The use of DNA technology (specifically, polymerase chain reaction, or PCR), for differential detection will likely be adopted for special situations in the near future. PCR-based tests are being evaluated for their ability to confirm infection in samples that yield indeterminate results with antibody tests. Dr. Mathew Longiaru and colleagues (Roche Diagnostic Systems, Inc., Nutley, New Jersey) reported on the development of a rapid (4-hour) detection format that uses PCR but does not use radioactively-labeled DNA probes and can process a relatively large number of samples. Results indicated that the HTLV-I–specific capture probe employed detects only HTLV-I DNA, not HTLV-II DNA.

EPIDEMIOLOGICAL MODELLING

Available data on AIDS incidence can be mathematically processed in various ways to estimate such unknown quantities as the actual number of people infected with HIV and the future size of the AIDS epidemic. At past International AIDS Conferences, epidemiological modellers presented major new methods with which to make these predictions and to model the dynamics of the epidemic. The simplest approach to predicting epidemic growth—extrapolation from past growth curves (in this case, annual incidence rates)—was augmented by techniques such as back-calculation, multistage models, and rather complicated simulation models of transmission dynamics that incorporate parameters such as sexual mixing and infectivity.

At this year's Conference, several investigators described comparison studies showing that the various modelling approaches tend to validate each other and produce estimates that agree with current seroprevalence data. Others applied these methods to populations not previously studied in this way. A few described innovations, including an eight-stage HIV infection model, a method for quick estimation of HIV seroprevalence in a population, and the use of an earlier disease stage, rather than the onset of AIDS itself, as a basis from which to make projections.

In a poster discussion session on modelling, discussants touched on the controversy surrounding the treatment effect—the effect of AZT, aerosolized pentamidine, and other treatments that appear to postpone the onset of AIDS. The widespread use of these treatments in people who are HIV-infected but have not yet progressed to AIDS almost certainly contributed to the unexpected decline in the growth rate of the AIDS epidemic that occurred in mid-1987. Also contributing to the slowed growth were sexual behavior changes that occurred earlier in the decade, resulting in a decline in the HIV-transmission rate, and a shift from hospital to outpatient care for patients in early disease stages, possibly resulting in a time lag in case reporting. Whatever the reasons, the slowing of growth has made accurate prediction of future AIDS incidence much more difficult. The existence and size of each of these effects, unknown or unproven at present, will continue to have a major impact on epidemiological models of the AIDS epidemic.

INCUBATION TIME

In the back-calculation method, the number of HIV-infected individuals in the general population is calculated from the number of reported AIDS cases, using known distributions of incubation times—that is, the time interval from initial detection of HIV infection to the onset of clinical AIDS (which may vary from population to population). The incubation distributions are based on lengthy observation of people infected with HIV, but because of the treatment effect, the estimations based on these distributions are probably no longer accurate. Dr. Mitchell Gail (National Cancer Institute, Bethesda, Maryland), who first proposed this "treatment hypothesis," noted that if back-calculation fails to take the treatment effect into account, the result may be a significant underestimation of the number of people currently infected. Since the treatment effect results only in a postponement of the development of AIDS among already infected people, the ultimate result would be a higher-than-expected incidence of AIDS, until such time as a new incubation period can be derived that takes the treatment effect (current and improving) into account.

Dr. Dominique Costagliola (INSERM, Paris) and coworkers presented their model of the incubation time for two groups: In homosexual men, the incubation time was calculated to be 9.9 years. In children who acquired HIV perinatally, two patterns could be distinguished: they either develop AIDS within 18 months after birth (about 11% of all perinatal cases) or they follow a slower course, more akin to that seen in adults, with an average incubation time of 6.4 years.

NEW APPROACHES TO MODELLING

Most AIDS projections are based on the number of AIDS cases reported in a population; however, this practice is likely to result in inaccurate projections in populations where the number of AIDS cases is low—less available data results in less-precise estimates. Using an earlier, more inclusive epidemiologic definition of HIV-related disease can increase the accuracy of these estimates by increasing the number of "cases" on which estimates are based, indicated Dr. Graham Bird (University of Edinburgh).

Based on seroepidemiological studies, the two major cities in Scotland (Glasgow and Edinburgh) experienced an epidemic of IVDU-associated HIV transmission in the early to mid-1980s. However, the incidence of AIDS in Scotland lags behind HIV transmission: only 114 AIDS cases were reported by late 1989, and the majority were homosexual men. Since Scotland has centralized laboratory monitoring of HIV-infected persons, investigators had access to data that allowed them to use an earlier marker of HIV-associated immunologic impairment: the number of CD4 cells, which declines as disease progresses. By late 1989, they had identified nearly 500 "CD500" patients—those with CD4 cell counts below $500/mm^3$—including about 150 "CD200" patients. Patients enter the CD200 stage about 2 years before they develop AIDS, on average. By applying back-calculation to these figures, the investigators estimated with some confidence that about 3,000 people in Scotland were infected with HIV in late 1989. They were also able to demonstrate that the epidemic among IVDUs in the two Scottish cities are out of phase; Edinburgh is about 1 year ahead of Glasgow. The data also suggest that this risk group has an unusually long AIDS incubation period—12 years from infection to CD200 and another 2 years to AIDS—and that prophylactic treatment is beginning to delay the onset of AIDS even longer.

Simple back-calculation typically uses a model based on two stages of AIDS: pre-AIDS HIV infection and clinical AIDS. Dr. Ira Longhini (Emory University, Atlanta) described an eight-stage model of HIV infection that was applied to data from a cohort of San Francisco homosexual men. The eight stages of the model are :

STAGE	DESCRIPTION
1	One post-infection, pre-antibody stage
2–3	Two asymptomatic antibody-positive stages
4–6	Three symptomatic pre-AIDS stages
7	AIDS
8	Death

On the basis of past experience, the average AIDS incubation time was expected to be 9.8 years. However, beginning in mid-1986 the data showed a substantial treatment effect in patients in stages 4–6 (the conditions under which prophylactic treatment is usually begun), and the average incubation time was increased to 12.7 years.

AIDS PROJECTIONS IN THE UNITED STATES

Dr. John Karon (Centers for Disease Control, Atlanta) described the U.S. Public Health Service's efforts to evaluate the accuracy of past AIDS predictions in the United States and to revise these predictions on the basis of current knowledge. The decline in the overall rate of increase of AIDS cases first observed in mid-1987 was mainly due to a decline among white, urban homosexual men—a group in which the treatment effect, behavioral changes, and treatment-related changes in AIDS case reporting are all expected to have their greatest impact. Projections based on a variety of methods all concur that the incidence of AIDS in the United States will continue to increase through the year 1993, although the rate of increase will not be as steep as that predicted before the treatment effect was recognized.

Back-calculation methods that incorporate a treatment effect result in a higher eventual case total because they assume that development of AIDS in infected people is merely being postponed. The Public Health Service now predicts a cumulative total of 390,000 to 480,000 AIDS cases through 1993, with 285,000 to 340,000 deaths from AIDS.

Dr. Philip Rosenberg (National Cancer Institute, Bethesda) described a study that compared seroprevalence data to the results of back-calculation in various U.S. population groups in 1985 and 1987. The results of both methods were fairly consistent with each other and produced a "pretty robust" estimate of 544,000 Americans infected in 1985. The most likely estimate for mid-1987 was slightly over 1 million infected. After that year, the impact of prophylactic treatment makes such comparisons vastly more difficult.

EPIDEMIOLOGIC MODELLING IN EUROPE

Between 30,000 and 40,000 cases of AIDS will be diagnosed in 1990 and 1991 in the 12 member countries of the European Community, according to Dr. Angela Downs (World Health Organization Collaborating Centre on AIDS, Paris). These predictions were based mainly on extrapolation, although they agree with some projections made by back-calculation. The increase in AIDS incidence has slowed to a linear or near-linear rate in homosexual men and is approaching a linear rate in IV drug users. In contrast, the rate of increase in heterosexually transmitted AIDS may be exponential (of a sharper or steeper increase) a full 9% of European AIDS cases are now attributed to heterosexual transmission, versus the 6% of 2 years ago.

A study in homosexual men in Amsterdam by Dr. Jan Hendriks (Amsterdam Municipal Health Service) showed that at a certain stage of the AIDS epidemic, the ratio of HIV-positive individuals to AIDS cases stabilizes for at least a few years, resulting in a simple and quick method to estimate the number of persons infected in a given population if the number of AIDS cases is known. In this particular group, the ratio of infected men to AIDS cases was 12:1, comparable to ratios seen in cohorts of gay men in San Francisco and in England and Wales. Presumably each risk group has its own ratio that is characteristic of the epidemic's behavior in that group, said Dr. Hendriks in a poster session.

MODELS IN PATTERN II COUNTRIES

Dr. Massimo Arco (II Universita, Rome) described a model showing that in Pattern I countries (using Italy as an example), an epidemic of heterosexually transmitted AIDS cannot be self-sustaining, but requires continued exposure to IVDUs. The situation is quite different in Pattern II countries, where approximately 95% of AIDS cases are heterosexually transmitted. Of course, the treatment effect is also irrelevant in the developing countries, since prophylactic drugs are virtually unavailable.

Small differences in crucial variables of HIV transmission could explain the large differences in AIDS incidence among various Pattern II countries—as well as the low rate of heterosexual transmission in developed countries—according to a presentation by Ann Stanley (Los Alamos National Laboratory, New Mexico) and associates. (Indeed, other epidemiologists have commented on the extreme heterogeneous nature of the HIV epidemic in Pattern II countries and even in different regions within a Pattern II country.) Dr. Stanley's team prepared a model that tested the sensitivity of AIDS epidemic growth to various factors, including demographic variables, viral infectivity, and sexual practices. Sensitivity analyses are carried out by simulating the behavior of the epidemic with a set of hypothetical baseline variables, and then varying each of the factors within a realistic range and observing the effect on the simulated epidemic.

The outcome of the model was highly sensitive to small differences in viral infectivity, which could be affected by variables that the authors regarded as cofactors—such as other sexually transmitted diseases, local viral strains, genetic susceptibility, circumcision, or basic health conditions. In addition to these infectivity cofactors, the size of the simulated epidemic was strongly influenced by differences in rates of acquisition of new sexual partners and in the pool size of promiscuous female population. For example, a smaller high-risk female population with higher contact rates was found to have a slower epidemic spread. Observations of large variations proceeding from small differences in multiple factors suggest that predicting the course of the AIDS epidemic in Pattern II countries will be quite difficult. But more importantly, the sensitivity of the epidemic as demonstrated by this model offers hope that control measures, even if only partially effective, can have a great impact on the course of the epidemic.

CHAPTER 4

Page 91 **BACKGROUND**
92 Direct and Indirect Testing Methods
92 Specificity & Sensitivity
96 False Positives and False Negatives
97 Algorithms
98 **ANTIBODY TESTS**
99 Enzyme-Linked immunosorbent Assay (ELISA)
100 Western Blot Test
102 Immunofluorescence Assay
102 Urine Tests
102 Saliva Tests
102 **ANTIGEN TESTS**
103 Antigen EIA
104 Flow Cytometric Assay
104 **DNA-BASED TESTS**
106 **RAPID TESTS**
106 Agglutination Tests
106 Enzyme Immunoassays
106 **SPECIAL TESTING SITUATIONS**
107 Developing Countries
109 Children Born to Seropositive Mothers
112 **QUALITY CONTROL**
112 Problems & Caveats
113 Evaluation Programs

DIAGNOSTIC TESTS FOR HIV

BACKGROUND

Diagnostic testing for any infectious agent is an important aspect of disease control, not only for the individual but for entire populations. Indeed, the safety of the blood supply depends on the capabilities of diagnostic tests that can signal myriad infectious agents, including HIV. During the past decade, advances in molecular biology, immunology, and biotechnology have driven the relatively rapid development of HIV diagnostic tests.

In the United States, Canada, Australia, and Western Europe, routine testing of all donated blood for the presence of HIV was initiated in 1985. Today, scientists are developing tests that can signal a second AIDS virus (HIV-2), which was first identified in 1985 and has since been found to be endemic in West Africa. At the Conference this year, numerous presentations highlighted the spread of HIV-2 throughout Europe, which re-emphasized the need for the differential detection of HIV-1 and HIV-2. (See Chapter 3: EPIDEMIOLOGY for a discussion of HIV-2 seroprevalence and, in this chapter, Table 4.2 for an outline of HIV-2 tests currently in development.) Screening for the other human retroviruses, HTLV-I and HTLV-II, are discussed in the context of their epidemiology in Chapter 3: EPIDEMIOLOGY.

This chapter describes current HIV diagnostic tests, those in development, caveats for their use, and their applicability in special situations. Much research has focused on adaptations for special testing situations, such as those in developing countries—where, because of economic limitations, large-scale HIV screening is not routine—and for use in pediatric patients—since HIV infection cannot be reliably assessed until age 15 months due to the presence of maternal antibodies. In the context of HIV-associated illness, the issue of identifying seropositive individuals extends far beyond technology; practical concerns, such as counselling, must be integrated with notification of test results. These issues are discussed in Chapter 10: EDUCATION & COUNSELLING.

DIRECT AND INDIRECT TESTING METHODS

The presence of HIV infection can be determined through two basic approaches:

- direct detection of the virus itself (or its components) or
- indirect signaling of its presence, as indicated by detection of an immune response to HIV, notably through the identification of HIV-specific antibodies

Direct diagnostic tests identify the virus itself via virus culture, viral antigen assays, viral enzyme assays, or viral RNA/DNA tests.

Direct Methods. Researchers are intent on perfecting or developing new diagnostic tests that detect HIV directly. Currently available direct tests do not lend themselves to large-scale screening because of their highly technical (labor- and time-intensive) protocols. One exception is an HIV antigen test—an enzyme immunoassay (EIA) that detects HIV's major capsid, or core, protein (p24)—but its results are not considered reliable. Although p24 antigen can be detected in infected persons before seroconversion, this appears to be a transient phenomenon; generally, p24 can only be detected again in late-stage disease, and only in some patients. A negative antigen test result is therefore not informative: it does not necessarily reflect an uninfected sample.

Virus isolation by culture techniques (using blood lymphocytes or mononuclear cells) may be showing some gains: recent reports show this technique to be at least 90% successful, independent of the subject's clinical status. However, this method requires a "cultivation" period and is therefore not suitable for large-scale screening. Isolation methods employ biochemical (reverse transcriptase activity) assays or antigen assays to detect virus, or electron microscopy to directly view viral particles released from or harbored within infected cells; (Electron microscopy is used for research purposes only and is not adaptable to broad-based studies.)

Direct detection of HIV by a relatively new gene amplification technique—polymerase chain reaction (PCR)—is receiving an enormous amount of study and research dollars. Many scientists view PCR as having the potential for being the definitive HIV test because of its extreme (theoretical) sensitivity. Although technical fine-tuning is required, it is hoped that PCR will be in place as a confirmatory test in the next few years. (See "DNA-Based Tests," below.)

Indirect Methods. Outside the research environs, testing for HIV generally refers to testing for antibodies to HIV. Because they detect a product of an immune reaction rather than the causative agent itself, the results of an antibody test reveal whether an individual's immune system has been challenged by HIV. Although in widespread use, and generally the basis for a diagnosis of HIV infection worldwide, a significant problem is associated with HIV antibody tests: the so-called seroconversion window. Seroconversion is the point at which the body begins producing and secreting antibodies in response to an infection. There is, however, a time lag between exposure to the infectious agent and inception of antibody production. Estimates on the length of time between exposure and antibody production (the seroconversion window) generally range from 2 to 14 months, with an average of 6 to 12 weeks. Individual differences in immune responsiveness may account for the lengthier time periods; seroconversion has been reported to be delayed by as long as 28 months. (Note that once antibody production begins, detection can still be difficult because the antibodies are not in high enough concentration in the circulation.)

SPECIFICITY & SENSITIVITY

As our understanding of the biology of HIV (and the body's immune response to the virus) grows, the strengths and shortcomings of current diagnostic tests become more apparent. Two parameters by which the usefulness of a test is rated are specificity (how well it can identify a given substance, distinguishing it from similar or interfering substances) and

Table 4.1. Diagnostic Tests for HIV

	Test Type	Test Parameters
INITIAL SCREENING TESTS		
Antibody enzyme-linked immunosorbent assay (ELISA), or Enzyme immunoassay (EIA)	Indirect	Detects antibodies to HIV
Antigen EIA	Direct	Detects HIV antigens
Flow cytometry	Direct/Indirect	Detects HIV-infected cells; detects antibodies to HIV
Passive agglutination	Indirect	Detects HIV antigen-antibody complexes
CONFIRMATORY TESTS		
Immunofluorescence assay (IFA)	Indirect	Detects antibodies to HIV
Radioimmunoprecipitation (RIA)	Direct/Indirect	Detects HIV antigens; detects antibodies to HIV
Western blot	Indirect	Detects individual antibodies to HIV
FOR RESEARCH USE ONLY *		
DNA-based tests	Direct	Detects HIV DNA (proviral) sequences
Virus isolation	Direct	Enables use of antigen and enzyme assays and viewing of viral particles by electron microscopy

* These tests are not used for blood testing per se, but are employed in basic research as definitive evidence of viral presence.

sensitivity (how well it detects the substance at low concentrations; the smallest quantity perceived). In HIV testing, the substance to be detected may be the virus itself, components such as proteins or RNA/DNA, or substances indicating an immune response to the agent.

Increasing the sensitivity of current HIV diagnostic tests should mean that earlier detection will be possible, based on the presumption that viral load (the amount of virus) is low early in infection because the virus has not had a chance to replicate in large numbers. The specificity of diagnostic tests for HIV infection has steadily improved since the first tests were licensed (1985). In many instances, the first generation tests resulted in a falsely "positive" reading (see below) because the sample being tested contained cellular proteins closely resembling those of the virus. In Africa and tropical countries, for example, multiple infection with various endemic microorganisms is relatively common, presenting a highly complex pattern that makes accurate diagnosis difficult in the absence of highly specific tests. Furthermore, pregnant women possess antibodies that cross-react with HIV antigens, making interpretation of their antibody test results difficult (see "Western Blot Tests," below).

Epidemiological and molecular studies have also shown that viral isolates can vary antigenically by geographic region. While increasing specificity is a desired property of any diagnostic test, HIV tests must also be able to recognize all variants of the virus. Thus it is necessary to use HIV-infected sera from different geographic regions when evaluating HIV assays.

Table 4.2. Antibody Tests that Detect HIV-2 (and/or HIV-1) Infection (In Development)

Note: Some assays signal HIV-2 or HIV-1 infection or HIV-1/HIV-2 dual infection; only a subset distinguish the three types of infection.

Test Name (Manufacturer)	Description	Ingredients	Conference Report(s)
Anti-HIV-1/ HIV-2 EIA (Hoffmann-La Roche, Switzerland)	• Combination assay that signals HIV-2 and/or HIV-1 infection • For use as initial screen • Confirmatory testing (e.g., Western blot) needed to distinguish HIV-1 from HIV-2	Uses a recombinant fusion protein consisting of immunoreactive regions of HIV-1 *env* and *gag* antigens and HIV-2 *env* antigen	Simultaneous detection of antibodies to HIV-1 and HIV-2 was found to be highly efficient using this recombinant fusion-protein–based assay, reported Michael Deckmann (Hoffmann-La Roche, Basel) and coworkers. Testing of 634 HIV-1 positive sera, 77 HIV-2 positive sera, and HIV-1 early seroconversion samples yielded a sensitivity of 100% and specificity of 99.6–99.9%.
Anti-HIV-1/ HIV-2 ELISA (Abbott Laboratories, North Chicago)	• Combination assay that signals HIV-2 and/or HIV-1 infection • For use as initial screening test • Confirmatory testing (e.g. Western blot) needed to distinguish HIV-1 from HIV-2	Uses three recombinant antigens: HIV-1 p24, HIV-1 gp41 and HIV-2 p41	Capable of detecting IgM as well as IgG antibodies, this recombinant ELISA was reported by Ping Wu (Abbott Laboratories, North Chicago) and coworkers to be more sensitive than Abbott's second generation HIV-1/2 EIA. The new assay—when compared with the first generation viral-lysate–based ELISA and second generation recombinant assay by testing a series of early seroconversion samples—detected HIV antibodies in samples that were antibody-negative by other ELISAs but positive on p24 antigen assay. The authors suggest that the enhanced sensitivity may be directly related to IgM detectability.
Anti-HIV-1/ HIV-2 assay (Ortho Diagnostics Systems, Raritan, New Jersey)	• ELISA format that signals HIV-2 and/or HIV-1 infection • For use as initial screen • Confirmatory testing (e.g., Western blot) needed to distinguish HIV-1 from HIV-2	Uses three recombinant antigens (HIV-1 *env*, *gag*, and *pol*) and one synthetic peptide (representing HIV-2 *env*)	Based on results obtained from testing 300 HIV-1-positive and 55 HIV-2-positive samples (Western blot confirmed), Todd Hill and coworkers reported this assay's sensitivity as 100%. A specificity of 99.84% was calculated using 1,220 samples from blood donors; 2 of the samples were reactive but indeterminate by Western blot. Performance evaluations included comparison with commercial viral-lysate–based tests on a panel of early seroconversion samples: the combination assay was found to be more sensitive.
HIV-2 ELISA (Behringwerke AG, Marburg, FRG)	• Detects antibodies to HIV-2 only; for research purposes, can identify HIV-1 cross-reactive antibodies • For use as discriminatory screening test or confirmatory test for HIV-2 infection	Uses a synthetic peptide representing HIV-2 gp36 (*env* protein)	This HIV-2 specific ELISA was found to correctly identify 107 confirmed HIV-2-positive sera from Ivory Coast and Europe, Stefan Brust and colleagues (Behringwerke AG, Marburg, FRG) reported. Specificity was demonstrated by testing of known HIV-1-positive sera: only 1 sample gave borderline reactivity.
HIV-1/HIV-2 EIA (IAF BioChem International, Montreal)	• Combination EIA that signals HIV-2 and/or HIV-1 infection • For use as initial screening test • Confirmatory test (e.g. Western blot) need to distinguish HIV-1 from HIV-2	Uses three cyclic synthetic peptides corresponding to HIV-1 gp120, HIV-1 gp41, and HIV-2 gp36	Martial Lacroix (IAF BioChem International, Montreal) and colleagues reported that this assay showed greater specificity than viral-lysate–based EIAs: of 344 outpatient clinic patients, 19 false positives obtained with viral-lysate–based tests were negative with IAF BioChem assay. Using 258 Western blot–indeterminate samples, 128 were identified as true positives; 127, true negatives. Sensitivity, evaluated using a seroconversion panel, was very high—infection was detected even in samples only weakly reactive for gp160 on Western blot.

Table 4.2. Antibody Tests that Detect HIV-2 (and HIV-1) Infection (In Development) *Continued*

Test Name (Manufacturer)	Description	Ingredients	Conference Report(s)
HIV-1/HIV-2 GENIE (Genetic Systems, Seattle)	• "Spot blot" EIA that signals HIV-2 and/or HIV-1 • Distinguishes HIV-1 infection from HIV-2 infection from HIV-1/HIV-2 dual infection • For use as an initial screen or confirmatory test	Uses synthetic peptides representing conserved (shared) regions of HIV-1 gp41 and HIV-2 gp36	Researchers at Genetic Systems, Inc. (Seattle) have developed a rapid (10-minute) assay capable of detecting dual HIV-1/HIV-2 infection as well as discriminating between HIV-1 and HIV-2 infection. Patrick Coleman reported data demonstrating the test's sensitivity and specificity as 100% and 100%. Testing with a seroconversion panel revealed GENIE to be more sensitive than 7 commercial (FDA-licensed) ELISAs and equal to that of Western blot.
Inno-LIA HIV-1/HIV-2 (Innogenetics NV, Antwerp, Belgium)	• Combination test that differentially detects antibodies to HIV-1 and HIV-2 • "Line" ELISA enabling identification of individual antibodies; in contrast to Western blot, no electrophoresis required • For use as confirmatory assay primarily; can be used as initial screen also	Recombinant HIV-1 antigens (p31, p24, p17) and synthetic peptides (corresponding to HIV-1 gp41 and HIV-2 gp36)	Performance characteristics of this single-strip line immunoassay were presented by Anne Van den Abeele and associates (Innogenetics, Antwerp). Using sera from healthy blood donors, sera yielding indeterminate Western blot results, and early seroconversion panels, results obtained by Inno-LIA were compared with Western blot results: None of the 599 samples deemed negative or indeterminate were reactive with Inno-LIA. All confirmed HIV-2-positive samples were detected by Inno-LIA: 7% reacted with gp36 only, 93% reacted with gp36 in combination with some other antigens. Based on early seroconversion samples, Inno-LIA proved more sensitive than Western blot.
OPUS HIV 1 + 2 (PB Diagnostic Systems, Westwood, Massachusetts)	• Combination EIA that signals HIV-2 and/or HIV-1 infection • For use as as initial screen • Confirmatory testing (e.g., Western blot) necessary to distinguish HIV-1 from HIV-2	Uses synthetic peptides that correspond to conserved (shared) regions of transmembrane envelope glycoproteins of HIV-1 and HIV-2	Philipe Wong and colleagues (PB Diagnostics Systems, Westwood, Massachusetts) presented test results obtained with this combination assay: Of 211 HIV-1-positive and 40 HIV-2-positive samples, OPUS HIV 1+2 identified all 251 as positive; furthermore, the test correctly identified 1,053 samples as negative. In seroconversion panel testing, OPUS HIV 1+2 detected antibodies as early as 7 commercial ELISA kits.
Peptest 1 + 2 (Manufacturer not specified, USSR)	• ELISA format that signals HIV-2 and/or HIV-1 infection • For use as an initial screen • Confirmatory testing needed to distinguish HIV-1 from HIV-2	Uses a mixture of synthetic peptides: two corresponding to HIV-1 gp41 and one to HIV-2 gp36	Alexander Kozhich (USSR Academy of Sciences, Moscow) and colleagues reported that this peptide-based assay correctly identified 223 HIV-1-positive sera and 27 HIV-2-positive sera and 1,026 negative sera from healthy blood donors.
Peptilav 1-2 (Diagnostics Pasteur, Paris)	• ELISA format that signals and distinguishes HIV-1, HIV-2, and dual infection • For use as an initial screen • Confirmatory testing recommended	Uses synthetic peptides corresponding to transmembrane antigens: HIV-1 gp41 and HIV-2 gp36	Anne Porter (Projet RETRO-CI, Abidjan, Ivory Coast) and associates reported that this synthetic peptide-based assay performed remarkably well on sera from Ivory Coast, showing extensive HIV-1/HIV-2 cross-reactivity. Of 1,289 sera collected from tuberculosis treatment centers, 589 were correctly found to be HIV-1-positive, HIV-2 positive, or HIV-1/HIV-2 dually infected. Compared to a viral-lysate–based ELISA, this assay had a higher specificity, identifying 69 samples as false positives. This assay identified 69 sera as HIV-2 positive, of which 67 (97%) were confirmed by HIV-2 Western blot analysis.

(Continues on next page)

Table 4.2. Antibody Tests that Detect HIV-2 (and HIV-1) Infection (In Development) *Continued*

Test Name (Manufacturer)	Description	Ingredients	Conference Report(s)
Peptoscreen-2 (Institute of Immunology, Moscow)	• ELISA format that signals presence of HIV-2 and/or HIV-1 • Suggested for use by authors as both a screening and confirmatory test	Uses four synthetic peptides: two from a region of HIV-1 gp41, one from HIV-1 gp120, and one from HIV-2 gp32	Dr. Rakhim Khaitov and colleagues (Ministry of Public Health, Moscow) reported that the Peptoscreen-2 ELISA performed better than recombinant protein- and viral-lysate–based ELISAs when used on known HIV-positive sera, on sera from healthy blood donors, and on "potentially" false positive samples (which included sera from pregnant women, persons with autoimmune disorders, and patients with other viral infection). Based on a total of 6,507 sera samples tested, sensitivity was found to be 99.7% and specificity, 99.8%.
UBI HIV-1, 2 Combo PHA (UBI/Olympus Corporation, Lake Success, New York)	• Combination hemagglutination assay that signals HIV-2 and/or HIV-1 infection • For use as initial screen • Confirmatory testing (e.g., Western blot) necessary to distinguish HIV-1 and HIV-2	Uses synthetic peptides corresponding to conserved (shared) regions of HIV-1 and HIV-2 *env* proteins	Edward Kang and coworkers (United Biomedical, Inc., Lake Success, New York) reported a specificity of 99.14% for this new combination agglutination assay, based on testing of random blood donors (13 of 1,505 reactive samples were not confirmed as positive by commercial EIAs or Western blot). A sensitivity of 100% was obtained using sera from blood donors, confirmed HIV-2-infected individuals, and patients with adult T-cell leukemia or autoimmune disease.

Furthermore, the emergence of HIV-2 has complicated matters: diagnostic test manufacturers are seeking to pinpoint reliable differences between HIV-1 and HIV-2 in their effort to design type-specific tests. Significant advances have been made in this area and many presenters reported different strategies for dealing with this problem (see Table 4.2).

New technologies have meant significant improvements in both specificity and sensitivity for diagnostic tests for HIV infection. Nevertheless, basic science research is intent on eliminating (or at least reducing to statistically insignificant numbers) the idiosyncrasies of the currently used tests, which can yield indeterminate or ambiguous results. As information regarding HIV infection and the body's immune reaction to it accumulates, substances with the potential for interfering with conventional assays will be recognized. For as yet undetermined reasons, inconclusive results tend to be an especially common finding in large low-risk populations, such as blood donor populations in industrialized countries.

False Positives and False Negatives

Inherent to all diagnostic tests is the possibility of obtaining incorrect results—so-called false positives and false negatives. In the HIV setting, where infection invariably results in disease, just emphasis is placed on minimizing such errors. A false positive refers to a result that indicates exposure to HIV although subsequent (confirmatory) testing does not support such a finding. Conversely, a false negative refers to a result that indicates the absence of infection even though viral indicators are in fact present, as determined by subsequent, confirmatory, testing.

The problem of false negatives constitutes a significant threat wherein blood that is wrongly labeled "uncontaminated" may enter the blood supply. False negative results are frequently obtained with antibody tests when exposure to HIV has been recent and the

body has not yet produced detectable levels of antibodies; see "Indirect Tests," above. While the consequences of a false positive result are much narrower in scope, personal rather than global, they are nonetheless considerable given the nature HIV-associated disease and the ethical and medical responses ensuing from a positive test result.

ALGORITHMS

In most industrialized countries, HIV testing algorithms include retesting of initially reactive specimens followed by confirmatory testing. Typically, screening assays are antibody enzyme immunoassays (ELISAs), and confirmatory tests are Western blot. (Immunofluorescence assays and radioimmunoprecipitation assays are also used as confirmatory tests, but to a much lesser degree.) As the sensitivity and specificity of diagnostic tests continue to improve, and as detection of HIV-2 has become increasingly necessary (with attendant costs skyrocketing), variations on the original algorithms are being considered.

Dr. Gaby Vercautern (Institute of Tropical Medicine, Antwerp) and colleagues reported on a strategy in which samples are screened with two commercial tests from different manufacturers; confirmatory testing is performed only when contradictory results are obtained. Samples that are reactive on one test are then retested using a second manufacturer's test. Only samples reactive on both screenings are considered positive. Sera reactive on the first screen and nonreactive on the second are subjected to confirmatory testing. In this study using 353 fresh serum samples, evaluation was made of six pairings from among three manufacturer's assays: Abbott's recombinant HIV-1/HIV-2; DuPont's HIVCHEK; and PBS-Orgenics' Immunocomb. Results showed that four of six test combinations were able to detect all true positives and true negative samples (sensitivity = 100%; specificity = 100%). The four most successful combinations were as follows:

Initial Screen	Second Screen
Abbott's recombinant HIV-1/HIV-2	DuPont's HIV Chek
Abbott's recombinant HIV-1/HIV-2	PBS-Orgenics' Immunocomb
PBS-Orgenics' Immunocomb	DuPont's HIV Chek
PBS-Orgenics' Immunocomb	Abbott's recombinant HIV-1/HIV-2

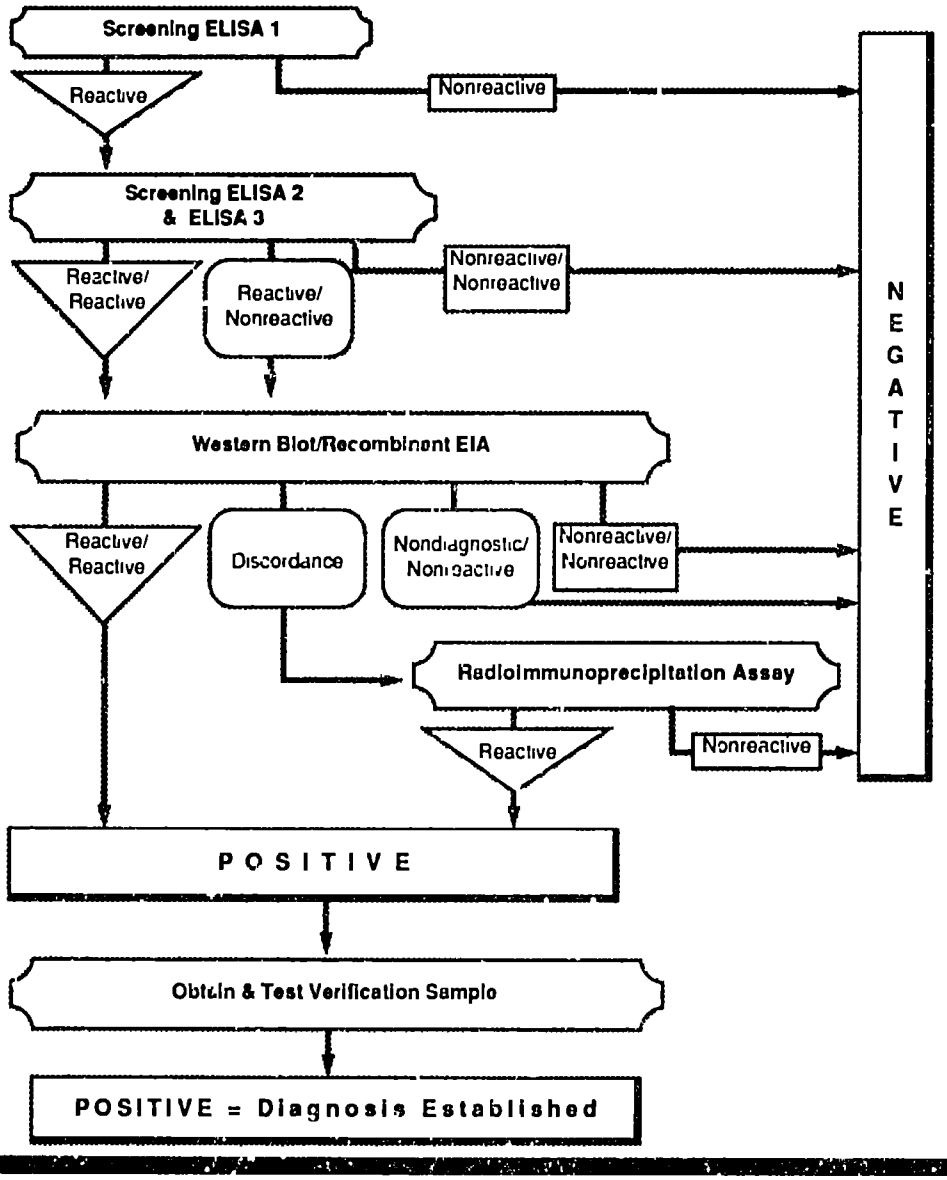
The pairings revealed that the best results were obtained when the most sensitive assays were used as primary screen. The authors concluded that this new algorithm, which obviates the need for expensive Western blot confirmatory testing of each initially reactive sample, is an attractive, cost-saving alternative to classical algorithms.

P. B. McGreevy and coworkers (Brazilian Army Institute for Biology, Rio de Janeiro) reported similar findings based on testing 10,027 samples from Brazilian soldiers: When two different screening tests are used for primary and secondary HIV screening—prior to confirmatory testing—costs are reduced, without loss in sensitivity. Samples were tested with DuPont's (whole virus) ELISA as a primary screen, then were retested either with DuPont's ELISA or with Cambridge BioSciences' (env) ELISA as a secondary screen. All ELISA-reactive sera were then subjected to confirmation by Western blot, and for comparison, with immunofluorescence assay (IFA). As a screening strategy, the DuPont/Cambridge BioScience combination proved to yield fewer false positives. Furthermore, confirmatory testing results using IFA were comparable to those of Western blot.

Barbara O'Bryen and colleagues (Walter Reed Retroviral Research Group, Washington, D.C.) reported that, based on results obtained from screening 4.66 million sera from military recruits over a 5-year period, the Department of the Army's HIV-1 testing algorithm represented an accurate and cost-effective large-scale screening program (see Figure 4.1). Average direct and indirect costs per specimen tested were calculated to be $4.55 (U.S.).

Figure 4.1. Department of the Army HIV-1 Screening Algorithm

Using this large-scale screening program, Barbara O'Bryen and colleagues (Walter Reed Retroviral Research Group, Washington, D.C.) reported a false-positive rate of 1 in 1 million and an average cost of $4.55 per specimen tested.



ANTIBODY TESTS

Antibody tests (specifically, ELISA and Western blot) were the first diagnostic tests licensed for use in screening the blood supply. Today for large-scale screening programs, these tests remain the most reliable, time efficient, and easiest to perform, technically. Antibody tests identify HIV-infected specimen samples by signaling the presence of antibodies that react with HIV antigens. Several types of antibody tests have been developed, including:

- enzyme-linked immunosorbent assay (ELISA or EIA)—used as a screening assay
- Western blot—used as confirmatory test
- immunofluorescence assay (IFA)—used as confirmatory test
- radioimmunoprecipitation assay (RIPA or RIA)—not used as frequently, primarily because it requires radioactive material and a high level of technical expertise

(Most antibody tests are based on detection of antibodies of the immunoglobulin class IgG, the most abundant antibodies found in the serum. Although IgM and IgA serum antibodies appear sooner than IgG after infection, they are in much lower concentrations in the blood. Special testing situations may prove IgM and IgA antibody screening to be diagnostically warranted.)

Advances in biotechnology and the HIV knowledge base spur corresponding modifications to the prototype antibody assays. Tests using synthetic peptides (strings of amino acids) that contain amino acid sequences identical to those of discrete HIV antigens and recombinant (genetically engineered) HIV proteins are gradually replacing those using purified viral antigens (antigens from disrupted whole virus). Use of synthetic peptides or recombinant proteins minimizes contact with potentially infectious material in the laboratory.

A novel fusion protein for use in antibody tests was described by Dr. Yihshing Shih and coworkers (Cetus Corporation, Emeryville, California). They fused a common bacterial enzyme (alkaline phosphatase) that naturally effects a color-change reaction to a recombinant HIV envelope antigen (gp41). This "chimeric" (hybrid) protein was recognized by antibodies from HIV-infected blood and by HIV-specific monoclonal antibodies. Two other chimeric proteins, alkaline phosphatase-gp120 and a second alkaline phosphatase-gp41 (with different amino acid sequence), were analyzed for their immunoreactivity and found to be less reactive. Nevertheless, these fusion proteins represent a safe method for the manufacture of HIV antigens as well as providing (through the color-change effect) a built-in detection method.

Antibody tests are also being evaluated for their ability to signal the presence of HIV antibodies in body fluids other than blood—notably, saliva and urine. Such noninvasive antibody tests, requiring neither needles nor lancets, would be ideal for certain testing circumstances (particularly those in which venipuncture is not feasible). As Dr. Jean-Pierre Allain (Abbott Laboratories, Chicago) pointed out, a positive result based on saliva or urine is highly indicative of true HIV infection, while a negative result remains questionable. Although the technology is evolving at a rapid pace, at this time, confirmation by serum assays is still recommended.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA)

The most widely used HIV screening test is the enzyme-linked immunosorbent assay (ELISA, or EIA), an antibody test that is easy to perform technically and can handle a large volume of specimen samples simultaneously. Adopted for routine large-scale use in 1985, this assay was designed to be over-sensitive, to catch even suspicious samples. ELISAs use HIV antigens—which can be synthetic peptides, recombinant antigens, or antigens derived from purified disrupted whole virus—to capture any HIV-specific antibodies in a specimen samples. Following an incubation period, these samples (usually serum) are then mixed with an enzyme-labeled probe (usually antibodies to human IgG or to HIV); a color reaction (produced by the enzyme on binding between the probe and antigen) is measured with an instrument called a spectrophotometer. (The newly developed "rapid" ELISAs are self-contained and do not require a spectrophotometer read-out; the color reaction is visible to the naked eye. See "Rapid Tests," below.)

Although the ELISA is an indirect test, signaling infection through detection of antibodies to HIV, it offers a relatively high level of specificity and sensitivity for large-scale purposes. Improvements in both the specificity and sensitivity of ELISAs (compared to the first generation ELISAs in 1985) have been noted in numerous studies; however confirmation of ELISA-positive samples (by Western blot, immunofluorescence assay, or radioimmunoprecipitation assay) is still necessary. With respect to sensitivity, "early seroconversion" studies have documented significant increases in detection capabilities of ELISAs. (Early seroconversion studies use a panel of blood samples that consists of an HIV-positive sample plus earlier

(stored) negative samples from the same individual; it is suspected that the sample(s) immediately preceding the one in which antibodies are first detected contain very low levels of antibodies. Such panels are from patients who are repeatedly tested because of suspected exposure—recipients of blood transfusions, for example. Once antibodies are detected, the stored (previously negative) samples are used to evaluate new assays for their sensitivity.)

In a comparison study of synthetic peptide-based (IAF Biochem, International, Inc., Montreal) and recombinant-based ELISA (Cambridge BioScience, Inc., Massachusetts), Dr. Richard Galli and coworkers (Ontario Ministry of Health, Toronto) looked at a panel of 163 early seroconversion serum samples from 68 cases. When compared against Western blot results, the synthetic peptide-based assay was found to be more sensitive in detecting HIV antibodies in these early seroconversion samples: Although both the synthetic peptide and recombinant-based ELISAs were more sensitive than Western blot, the synthetic peptide ELISA could identify as positive 3 Western blot-negative samples that in subsequent specimen samples were shown to be positive; the recombinant antigen-based ELISA identified 1 such sample.

Several ELISAs have been developed to detect HIV-2 antibodies specifically; these and HIV-1/HIV-2 combination assays are being extensively evaluated in Western Europe and Africa, where the number of cases of HIV-2 infection are significant. Table 4.2 summarizes the ELISAs currently in development, as reported at the Conference.

WESTERN BLOT TEST

The Western blot test is a common laboratory technique used to identify individual proteins. As an HIV test, Western blot is used to identify the presence of antibodies to individual HIV proteins, such as gp120, gp41, and p24 (see Figure 4.2). Thus, in contrast to the "all-or-none" positive/negative reactivity of ELISA, Western blot is a qualitative test, revealing the antibody profile of a specimen. It is for this reason that Western blot is used as a confirmatory test.

Although only in development, several diagnostic test manufacturers are using highly purified recombinant HIV proteins instead of antigens from disrupted whole virus; such "immunoblot" and "dot blot" assays have received high marks for their specificity in preliminary evaluation studies.

Perhaps the greatest drawback with Western blot analysis as a HIV confirmatory testing strategy is the lack of standardization; the banding pattern for any given sample is broadly categorized as positive, negative, or indeterminate.

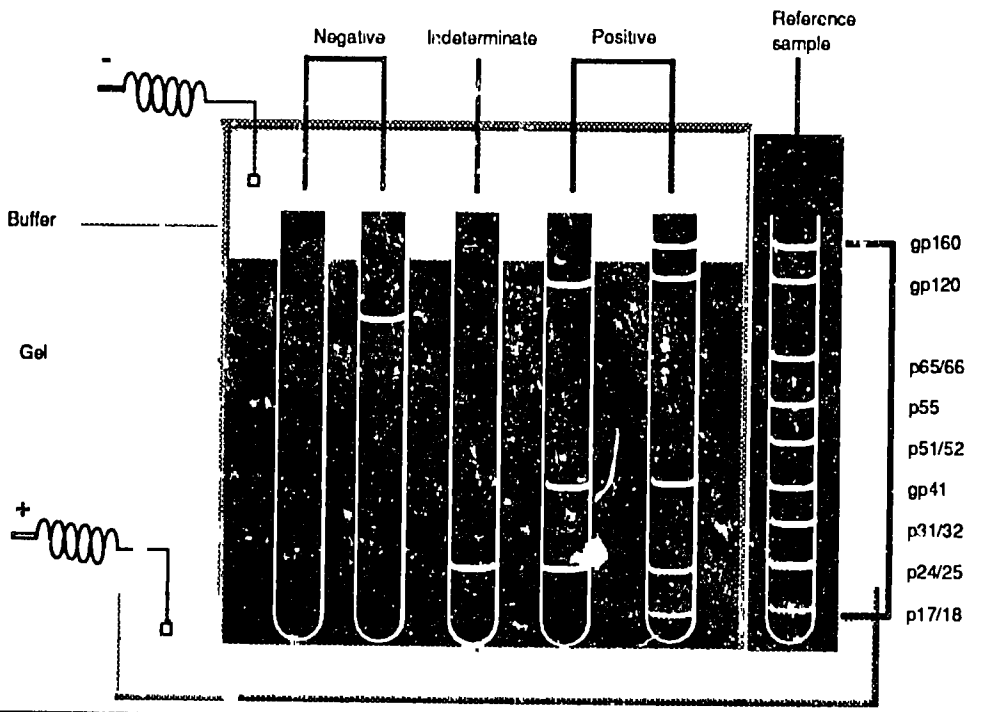
Currently several sets of criteria for interpreting Western blot banding patterns are being used, including those from the manufacturer (FDA/DuPont), Centers for Disease Control, American Red Cross, Walter Reed Medical Center, Association of State and Territorial Public Health Laboratory Directors, as well as those developed in-house by numerous individual blood centers. Generally, Western-blot positive samples are those with bands corresponding to two of the 3 major bands considered diagnostically significant (gp160 or gp120, gp41, and p24).

Several investigators are studying weakly reactive and indeterminate sera samples for possible clues as to what these results signify. In some cases, indeterminate Western blot results are obtained when samples are from HIV-infected individuals who are in the early stages of mounting an antibody response to HIV, as evidenced by an HIV-positive result on testing of later samples (2-6 months later).

Based on her team's findings, Dr. Connie Celum (University of Washington, Seattle) reported that only a small percentage (less than 5%) of indeterminate Western blot tests prove to be true positives, as determined by testing of later samples from the same indi-

Figure 4.2. Western Blot Test

The presence of HIV antibodies can be demonstrated by visualizing their reactivity with purified HIV proteins (antigens) that have been separated by molecular weight during electrophoresis. The antigens are immobilized by "blotting" them onto absorbent, nontransferable paper. Incubating the serum sample with the immobilized antigens allows antigen-antibody binding, if any antibodies are present. Antibodies will be read as discrete bands, corresponding to HIV antigens. Samples are designated positive, negative, or indeterminate according to the banding pattern. (Note that criteria for a positive Western blot result may differ from laboratory to laboratory.) A positive control (reference) sample is shown on the right.



viduals. The great majority do not reflect true infection and are generally the result of immunological cross-reactivities: that is, other antibodies present in the sera reacting with HIV antigens; for example, autoantibodies (in persons with autoimmune disorders) or HLA-DR histocompatibility antibodies (in currently or previously pregnant women). From a group of 152 individuals with indeterminate Western blot results (and repeatedly reactive ELISAs), Dr. Celum's team followed 107 cases for 6 months; 87 HIV-negative-ELISA controls were included in the study. Only 4 (3.7%) indeterminate Western blot cases seroconverted (all 4 had p24 bands on initial Western blot). According to this study, indeterminate Western blot cases were more likely to have had a positive tuberculin skin test in the past, a recent tetanus booster, or a history of autoimmune disease. Women with indeterminate Western blot results were also more likely to have been pregnant when tested or have had a prior pregnancy. Cross-reactivity to other retroviruses could not account for indeterminate results, in most cases. Citing her study's findings, and echoing discussion points from several Conference participants, Dr. Celum emphasized the need for a more reliable confirmatory test.

Other researchers pointed to the test manufacturer's criteria as the problem, adding that less stringent criteria gave better interpretation. When using criteria from the New York Blood Center, for example, Dr. Karen Siller (Cornell University Medical College, New York) and colleagues found that 22 (71%) of 31blinded serum samples categorized as "probably positive"were from AIDS patients. The banding pattern for most was p24/gp41/gp120-160; and p31 was noted to be the last band to appear. If the manufacturer's criteria had been used instead, these 22 would have been indeterminate.

IMMUNOFLUORESCENCE ASSAY

As a confirmatory antibody test, the immunofluorescence assay (IFA) is increasing in use. HIV antibody detection by IFA is achieved by using antibodies to human IgG conjugated to a fluorescent molecule; any antibodies to HIV in the sample will bind to the fluorescent-labeled anti-human IgG antibodies. Unbound fluorescent-labeled antibodies are removed in a wash step; then positive sera samples are identified by their fluorescent signal, which is visualized with fluorescence microscopy.

A comparison study between IFA and Western blot presented at the Conference by Thanne Rose and coworkers (Los Angeles County Department of Health Services) revealed a higher level of sensitivity than Western blot. Of 26,570 specimens (from STD clinics), 923 repeatedly reactive ELISA samples were tested by IFA and Western blot: 2 (0.2%) were "non-specific" by IFA, whereas 75 (8.1%) were Western blot indeterminate (by manufacturer's criteria). A significant discrepancy was noted in the number of IFA-positives (64, or 6.9%) which were Western blot indeterminate. Two repeatedly reactive ELISA samples were negative by both tests. Although a high degree of agreement was observed between IFA and Western blot overall, IFA yielded more positives and fewer non-specific results, suggestive of a higher level of sensitivity.

URINE TESTS

Although considerably diminished in concentration compared to serum or plasma levels, antibodies to HIV can be detected in urine. Two reports at the Conference demonstrated improved sensitivity in detection of antibodies in urine with ELISA assays. Based on matched serum/urine samples, Susan Stramer and associates (Abbott Laboratories, Chicago) noted a sensitivity level of 92% with a purified viral antigen-based ELISA using undiluted (1:1) urine: 304 of 330 urine samples were correctly identified as positive. A recombinant-based ELISA, which was evaluated using undiluted urine, had a sensitivity rating of 94%. Using class-specific (IgG) antibody tests, John Parry (PHLS Virus Reference Laboratory, London) and colleagues found that sensitivity could be maximized, even in diluted (1:5) urine samples. Adaptation of an IgG antibody capture assay and an IgG antibody capture ELISA gave accuracy rates of 99.7% and 99.9%, respectively, for the two tests. Of 711 specimens tests, all 158 true positives were detected by the IgG capture assay and 157 were picked up by the IgG capture ELISA. However, when four commercial serum ELISAs and Western blots (unadapted) were used, markedly lower accuracy rates were obtained (range, 42.9-88.7%). Although the two class-specific antibody tests had high marks, the authors recommended repeatedly positive urine samples be confirmed using serum samples.

SALIVA TESTS

The concentration of antibodies is significantly lower in saliva than in serum. Independent studies conducted by Federal Centre for AIDS (Ottawa) and Chulalongkorn University Hospital (Bangkok) presented evidence that modified ELISAs show excellent specificity (100%) for detection of HIV antibodies in saliva—but lower sensitivity than that achievable with sera. Virtually all HIV (gp160 or gp120/160) reactive salivary antibodies were those of the IgG class. A presentation by Andrew Goldstein (Epitope, Inc., Beaverton, Oregon) and coworkers demonstrated that the observed lower sensitivity correlates with the finding that antibody levels in saliva are, on average, 1% of serum levels.

ANTIGEN TESTS

At the present time diagnostic tests that detect specific HIV antigens, such as the core antigen (p24), include the antigen enzyme immunoassay (EIA) and flow cytometry (a quantitative test that measures cell-associated HIV antigens).

ANTIGEN EIA

Enzyme immunoassays that can detect HIV antigens use purified human anti-HIV antibodies to capture circulating HIV antigens in blood samples. (Laboratory "grown" monoclonal antibodies directed against discrete HIV antigens can also capture HIV antigens, and are used in some commercial assays.) Although originally anticipated to be capable of signaling HIV infection earlier than conventional antibody tests, such has not been the case. Theoretically, antigen tests should be able to side-step the problem of the seroconversion "window"—the length of time elapsed before antibody levels become detectable, about 6–12 weeks after initial infection. However, currently available antigen tests have been found to be no more sensitive than antibody tests.

Dr. John Ward (Centers for Disease Control, Atlanta) presented his team's data obtained from 515,494 serum samples (collected in 1989 from 13 blood centers) that were tested by one of two antigen tests (from Abbott Laboratories or Coulter Immunology, Hialeah, Florida). Of the total, 225 samples were identified as antigen-positive and confirmed by Western blot, giving a seroprevalence rate of .004%—a rate very similar to that obtained by antibody screening. Dr. Ward and co-authors pointed out that antigen testing did not identify any samples that were antibody-negative.

Sera Analyses. Previous studies have shown that HIV's core antigen (p24) appears and disappears in HIV-infected persons throughout disease course. Although a pattern has emerged, it is not invariable. Researchers at Abbott Laboratories (Chicago) report that p24 can appear as early as 2 weeks after infection, but disappears 6 to 12 weeks later, and reappears (in some patients) in late-stage disease. Efforts to correlate p24 levels to clinical status have been largely disappointing (see Chapter 7: CLINICAL MANAGEMENT). One reason for the transient appearance of p24 antigen may be related to its interaction with p24-specific antibodies in the body: the level of circulating p24 could be extremely low (below the limits of detection) because most antigen is bound up in antigen-antibody immune complexes, which precludes detection of these antigens. An interesting study presented by Dr. Luc Kestens (Institute of Tropical Medicine, Antwerp) and colleagues demonstrated that when HIV antigen is dissociated from immune complexes, detectable HIV antigen levels are observed to increase significantly twice that seen in sera from asymptomatic individuals).

HIV antigens other than p24 are being evaluated for their detectability. Dr. Clive Loveday and coworkers (University College and Middlesex School of Medicine, London) reported on the development of an antigen EIA for measuring HIV-1 reverse transcriptase in sera and virus culture supernatants (fluids). Reverse transcriptase–specific antibodies (purified from HIV-positive human sera) were used to capture reverse transcriptase antigens in HIV antibody-positive serum samples. Samples from 57 asymptomatic donors had low levels of circulating reverse transcriptase antigens; samples from patients with symptoms did not show significantly higher levels. Control sera (from 772 HIV antibody-negative blood donors) were found to be unreactive with this assay. Examination of serial samples of 24 seroconverters revealed 12 who had reverse transcriptase antigens; in 11 of these, reverse transcriptase was the first serological marker. In contrast 9 patients had p24 antigenemia, p24 being the first marker in 3 cases. These preliminary results suggest that this assay is valuable as a highly specific and sensitive test for early seroconverters. Further studies will seek to evaluate the assay's specificity and sensitivity as a screening test for different populations.

Virus Culture. HIV antigen can be detected in virus-cell cultures, as well as in sera. Although usually a time-intensive laboratory procedure due to the culturing of samples individually, Arnold Fowler (SRA Technologies, Inc., Rockville, Maryland) and colleagues presented data on a micro-coculture system for whole blood specimen samples. Whole blood added to 96-count microtiter plates containing cultures of normal stimulated (mononuclear) blood cells can be assayed for p24 antigen. In most cases, less than 1 micro-

liter of blood was required to obtain a positive culture by a p24 antigen capture EIA. Although culturing time is unchanged (approximately 7 days before antigen is detectable), this demonstration gave evidence for a semi-automated culturing system that would allow for the processing of many samples simultaneously.

Two independent research teams reported on virus-blood cell cultures that yielded enhanced p24 antigen levels on stimulation. In one study, presented by Patricia Moran and associates (Oncogen Corporation, Seattle), HIV production—as measured by p24 detection in blood lymphocyte cultures—was noted to be increased following stimulation with monoclonal antibodies to the cell surface proteins CD3, CD2, or CD8. Cultures to which soluble anti-CD3 was added were found to yield the highest levels of p24, detectable by day 7. And Dr. Carlos Izaguirre (Federal Centre for AIDS, Ottawa) and coworkers found that monocytes (from asymptomatic HIV-infected individuals), cultured in the presence of recombinantly derived mononuclear colony stimulating factor (M-CSF), granulocyte-mononuclear colony stimulating factor (GM-CSF), and interleukin-3 (IL-3) had increased p24 levels—compared with cultures lacking these growth factors. These and other ongoing studies seek to optimize HIV antigen detectability in virus cultures.

FLOW CYTOMETRIC ASSAY

HIV antigens that are cell-associated (as opposed to circulating) can be detected—and quantified—by flow cytometry, a technique that analyzes and enumerates large numbers of cells in suspension as they traverse a focused laser beam in single file. Lymphocytes from blood samples are tested for the presence of intracellular p24 through the use of a fluorescent-labeled monoclonal antibody to p24. The fluorescence signal is picked up (as a "per cell" unit) by fluorescence detectors. In a study of serial blood samples from 10 late-stage HIV disease patients, Dr. Betsy Ohlsson-Wilhelm (Pennsylvania State University College of Medicine, Hershey) and colleagues used the p24 flow cytometric assay to examine the relationship between p24 levels before and during AZT (zidovudine) treatment. The authors observed episodic detection of p24-positive lymphocytes in all patients, with numbers of p24-positive lymphocytes declining in some individuals after initiation of therapy but persisting in others.

DNA-BASED TESTS

Following the 1985 discovery of a molecular biological technique enabling specific DNA sequences to be amplified (thereby allowing sufficiently large quantities to be collected), numerous research teams set to the task of applying gene amplification to the detection of HIV DNA. (Although RNA encodes HIV's genetic information, once inside a host cell the RNA is copied—through reverse transcription—into DNA. This viral DNA is then immediately integrated into the host cell's DNA; it is these "proviral" DNA sequences that are targeted for detection.) This technique, called polymerase chain reaction or PCR, turns out to be a very powerful diagnostic tool: it allows for the detection of a *single* HIV DNA sequence buried in several million cells. Under highly controlled laboratory conditions, a heat-stable bacterial enzyme, polymerase (specifically, *Taq* polymerase, from the bacterium *Thermus aquaticus*), amplifies targeted DNA sequences. Radioactive or enzyme-labeled DNA probes—that is, complimentary DNA sequences—then signal the presence of specific DNA sequences. Although certain technical aspects are still being worked out, it is thought that PCR will eventually serve as the industry's gold standard.

Standard detection of PCR-amplified HIV DNA sequences involves the use of radiolabeled probes, which are visualized as blots or bands by Southern blot analysis: DNA sequences are separated according to molecular weight through an electric field (electrophoresis) and immobilized on absorbent, nontransferable paper; radiolabeled probes are then added to the

immobilized DNA in a hybridization step—the probes will hybridize, or bind, with any complimentary sequences present. A technique called autoradiography captures any radioactive emissions from hybridized probes onto photographic emulsion paper for visual interpretation.

Several nonradioactive (and, therefore, presumably safer) detection assays are currently being explored. Researchers at Gen-Probe, Inc. (San Diego) presented data on a new DNA probe system that uses acridinium ester-labeled probes that give off a luminescent flash on hybridizing with target DNA sequences. Dale Dondero (California Department of Health Services, Berkeley) and colleagues also pointed out that this assay can be performed in 30 minutes, compared with the 24-hour period necessary for the Southern blot/autoradiography procedures. Dr. Brian Conway (Massachusetts General Hospital, Boston) and coworkers found alkaline phosphatase-linked probes, which allow for colorimetric analysis, achieve results similar to those obtained by the more labor-intensive Southern blot method.

While PCR is, in principle, a simple *in vitro* gene amplification technique, in practice it is a highly sophisticated technological procedure susceptible to significant contamination—and therefore, error. Many investigators acknowledge that further fine-tuning of PCR is needed before PCR results are considered reliable. Evaluations of the practical sensitivity and specificity of PCR are being conducted in numerous studies using samples from different (diagnostically problematic) populations, including pediatric cases and occupationally exposed health care workers. Although a few presenters at the Conference alluded to the use of PCR for large-scale screening, most work focused on PCR for confirmation of indeterminate HIV test results or for suspicious HIV-negative samples (primarily those from high-risk seronegative individuals).

Dr. Hassan Khayam-Bashi (University of California, San Francisco) reported that, using HIV *gag* sequences, his team found 19 Western blot indeterminate samples to be negative by PCR, which was verified by virus culture and HLA coamplification. In addition, of 21 seronegative high-risk individuals, 1 was found PCR-positive although virus could not be cultured from this individual. (A control panel of 17 known positive samples were identified as such by PCR.)

Differential detection of HIV DNA sequences was noted by several independent teams—the implications of which are not clear. Dr. Maurice Moncany (Institut Pasteur, Paris), for example, reported that his team was able to detect HIV regulatory genes *nef*, *vpr*, and *vpu* more frequently than the structural genes, *gag*, *pol*, and *env*. In their study of multitransfused seronegative hemophiliacs and sexual partners of seropositive individuals, Dr. Moncany's team obtained positive PCR results with *nef*, *vpr*, and *vpu*, but not with the complete set of HIV genes (genes of the entire genome of HIV-1 and HIV-2 were used.) Also interesting was that in four of these individuals, PCR yielded positive results 1 to 3 years before seroconversion. Control experiments with HIV-positive lymphocyte cultures demonstrated that all HIV genes were detectable by PCR.

Similar findings were reported for a cohort of 38 seronegative multitransfused beta-thalessemia patients: Panayotis Markoulatos (Institut Pasteur Hellinesque, Athens) and coworkers found only 6 patients to be entirely negative by PCR using genes from the entire HIV genome. The authors pointed out that if only structural genes (*gag*, *pol*, and/or *env*) were used, 14 patients would have been classified as PCR-negative.

The potential for wide-scale use of PCR in the direct detection of HIV remains debatable. Many enthusiasts of PCR technology point to its extreme (theoretical) sensitivity; continued development of PCR, it is believed, will lead to realization of its theoretical limits. Variations on conventional PCR schemes are already emerging and include the testing of urine pellets: Dr. Li Jian Jun and coworkers (New York University Medical Center) compared the results of using PCR in serum and in urine pellets to detect HIV proviral DNA (and to a lesser

extent, viral RNA by reverse transcription/PCR). Although their preliminary results demonstrated significant lack of agreement between urine and serum samples (69% accuracy versus 94%), a noninvasive urine DNA test may eventually prove achievable.

RAPID TESTS

Diagnostic tests capable of signaling HIV infection within minutes (and without instruments) are being extensively evaluated. Such "rapid" tests have application in industrialized countries—in hospital emergency rooms, drug treatment centers, STD clinics, and physicians' offices. And in developing countries, where blood banks may not exist and blood transfusions are frequently performed in life-threatening situations (with little or no time for screening of blood units), these tests are desperately needed. (See also "Developing Countries," below.) Table 4.3 catalogues the rapid tests currently being evaluated for licensing.

AGGLUTINATION TESTS

An agglutination test is a standard clinical microbiology test in which antigen-antibody reactions are observable as a clumping phenomenon. This method is routinely used for blood typing, and is the diagnostic test of choice for such diseases as cholera and typhus. Researchers at Cambridge BioScience Corporation (Worcester, Massachusetts) have developed an HIV agglutination test, called Recombigen, that employs latex beads coated with a recombinant (genetically engineered) HIV envelope protein, gp160. The latex beads serve as a vehicle for antigen presentation; furthermore, because they are inert, they do not interfere with antigen-antibody reactions. Although others have also developed HIV agglutination tests—SERODIA HIV (Fujirebio, Inc., of Japan) and PHA-CHEK (United Biomedical, Inc., of Lake Success, New York)—their assay times total 1–2 hours, so they are not classified as rapid tests.

For HIV testing, a sample of whole blood is mixed with the HIV-coated latex beads; if the sample contains antibodies to HIV, these will bind to the coated beads (indicating a positive result) within 5 minutes. A positive finding signals the need for confirmatory testing (generally with Western blot or IFA).

ENZYME IMMUNOASSAYS

Modifications to the standard HIV enzyme immunoassays (ELISAs) by several test manufacturers have allowed for the development of rapid, self-contained HIV antibody test kits. Visual interpretation of colored immunoreactive bands—as in the Testpak assay (Abbott Laboratories, Chicago)—or spots—as in the GENIE assay (Genetic Systems, Seattle)—is possible within 10 minutes. As with standard ELISAs, this generation of rapid ELISAs uses purified or recombinant HIV antigens (or synthetic peptides corresponding to HIV antigens) to signal any anti-HIV antibodies in a sample. Performance evaluations of these rapid tests have yielded sensitivity and specificity ratings in the range of 94.3–100%.

SPECIAL TESTING SITUATIONS

Adaptations of conventional HIV screening tests and strategies have become necessary in certain circumstances and for certain populations. In developing nations, logistical and economic problems—such as timely transportation of blood samples to reference laboratories—continue to hinder efforts aimed at ensuring blood supply safety. In neonates, diagnosis of HIV infection also requires special considerations: conventional HIV antibody tests are currently unable to differentiate between maternally derived antibodies and those produced by the newborn; therefore, true HIV infection cannot be reliably established—in the absence of symptoms—until 15 months of age.

Table 4.3. Rapid Tests

Several enzyme immunoassays and agglutination tests are now capable of signaling HIV infection within minutes. The tests included here were found to be of comparable specificity and sensitivity in presentations at the VI International Conference.

Test Name	Manufacturer	Test Detects	Time in Minutes
GENIE	Genetic Systems (Seattle)	Antibodies to HIV-1 and HIV-2	10
HIVChek HIV-1	DuPont (Wilmington, Delaware)	Antibodies to HIV-1	10
HIVChek 1 + 2	DuPont (Wilmington, Delaware)	Antibodies to HIV-1 and HIV-2	10
Immunofiltration ELISA	Clonatec (Paris)	Antibodies to HIV-1 and HIV-2	3
OPUS HIV 1+ 2	PB Diagnostic Systems (Westwood, Massachussetts)	Antibodies to HIV-1 and HIV-2	20
Recombigen	Cambridge BioScience (Worcester, Massachussetts)	HIV antigen-antibody complexes	5
Serocard	Disease Detection International (Irvine, California)	Antibodies to HIV-1	7
Single use diagnostic system (SUDS)	Murex (Norcross, Georgia)	Antibodies to HIV-1	10
Testpak HIV	Abbott Laboratories (North Chicago)	HIV-1 and HIV-2 antibodies	10

DEVELOPING COUNTRIES

Several alternatives to conventional HIV screening strategies are being explored in an effort to minimize transfusion-related HIV transmission in developing countries. In contrast to the situation in industrialized countries, due to economic constraints, all blood in developing countries is *not* routinely screened. Aside from the high per test cost, the lack of sophisticated equipment and trained laboratory personnel are also limiting factors. Rapid, simple, and less costly HIV tests are needed in developing countries where conventional screening and confirmatory protocols—notably, ELISA and Western blot—are not feasible, economically and technically.

Ideal for these situations are diagnostic tests that are self-contained (requiring few, if any, additional reagents and no instrumentation), and whose results are easily (visibly) identified as positive, negative, or indeterminate. Kabeya Mulanga (Projet SIDA, Kinshasa, Zaire) and coworkers described one such practical, cost-saving HIV testing strategy based on their experience with five field-hospital laboratories in Zaire. Specimen samples were obtained from blood donors, pregnant women, persons engaging in high risk behavior, and persons with symptoms of AIDS. Dr. Mulanga's team evaluated the use of two commercially available rapid tests, one to be used as an initial screening test and the other as a supplemental confirmatory assay. (Negative or indeterminate results obtained from the second would be sent to a reference laboratory for Western blot analysis.) Serum samples (single, pooled sera, or small batches) are first screened with a "dot" immunobinding assay (HIVChek HIV-1, by DuPont), which yields results in 5–10 minutes. Positive or indeterminate samples are then assayed with an agglutination test (Serodia-HIV, by Fujirebio, Inc., Japan), which requires 1–2 hours. As part of the evaluation, sensitivity and specificity were

measured by comparing results to those obtained by Western blot. Specificity was 99% for HIVChek (3 false positives), 92% for Serodia (9 false positives); sensitivity was 94% for HIVChek (15 false negatives), 95% for Serodia (10 false negatives).

The proposed testing strategy, which was rated as highly accurate (by comparison with Western blot results), would allow immediate, confirmed test results for emergency screening situations. As outlined in Figure 4.3, the authors also found this proposed strategy to be cost effective compared to conventional testing—an important concern in the development of a sustainable national HIV testing program. A different strategy was proposed and tested by Dr. Frieda Behets (Projet SIDA, Kinshasa, Zaire) and colleagues: a combination of two rapid ELISAs in succession—HIVChek 1+2 (DuPont) and Testpak 1+2 (Abbott)—with a supplemental agglutination assay—Serodia (Fujirebio)—for confirmation. By examining three groups (1,992 patients with clinical suspicion of infection; 1,491 women from a maternity ward; and 1,513 consecutive blood donors), Dr. Behets' team found the rapid tests to have a sensitivity of 99% and specificity of 95% or greater, with the exception of Testpak 1+2, which only showed a specificity of 75% among the high-risk subjects. (Test results were measured against Western blot results for HIV-1 and HIV-2 individually.) The authors pointed out that the serial use of two rapid tests yields results similar to those achieved by commercial ELISAs.

Simple, rapid assays are being evaluated for their accuracy and relative ease of use in ongoing studies in several developing countries. Ketema Fassil (National Research Institute of Health, Addis Ababa, Ethiopia) reported that based on both ease of performance and sensitivity/specificity profiles, HIVChek (DuPont) surpassed Recombigen (an agglutination test, manufactured by Cambridge Biosciences). Shriram Joshi and colleagues (Institute of Immunohaematology, Bombay) found comparable rates of sensitivity and specificity between Serodia and Wellcozyme (a recombinant ELISA by Wellcome Diagnostics). While these rapid, simple assays may address an urgent need in the HIV screening programs in developing countries, the issue of HIV-2 screening has yet to be addressed. Diagnostic tests that simultaneously detect HIV-1 and HIV-2, although more costly, will need to be included in screening programs in developing countries, especially in Africa.

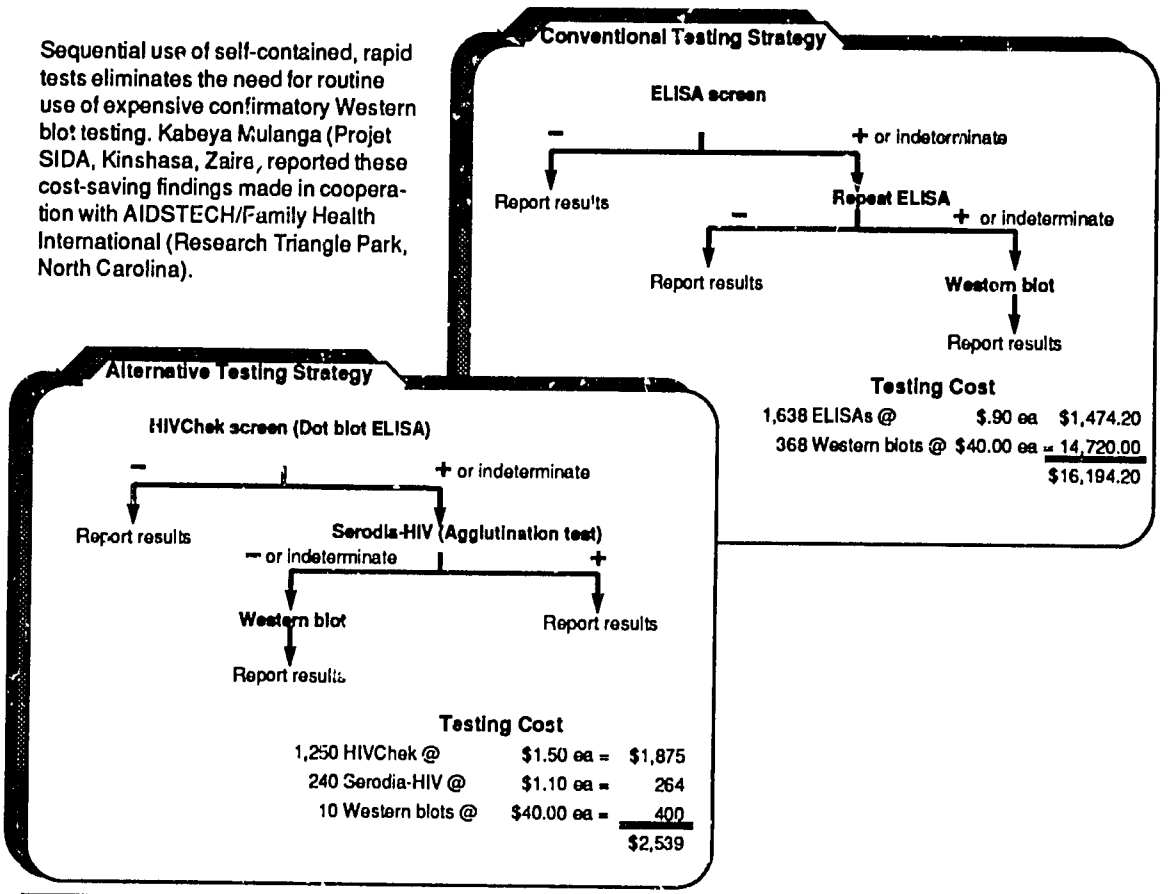
Dried Blood Spots. As a practical alternative to screening fresh or frozen serum, whole blood (obtained from fingerstick or heelstick) can be collected on filter paper, sent to a reference laboratory, and analyzed by ELISA, Western Blot, or immunofluorescence assay (IFA). Independent studies have demonstrated that HIV antibodies are detectable from dried blood spots. Dr. I. Bendet and coworkers (Brazilian Army Institute of Biology, Rio de Janeiro) reported that blood collected by fingerstick on filter paper could be stored for up to 2 months in an individual plastic bag at 4°C (refrigerated); tests of stored dried blood spots produced results comparable to those obtained by testing sera collected at the same time. Similarly, Nkandu Luo (University Teaching Hospital, Lusaka, Zambia) and colleagues, in collaboration with World Health Organization (Geneva), found that blood collected on filter paper and stored at room temperature gave identical results on ELISA and Western Blot as those obtained from sera. The authors concluded that dried blood spots collected on filter paper represents a practical solution to mass screening in Zambia.

Based on comparative analysis of dried blood spots from heelsticks of newborns and maternal serology, Mwandagalirwa Kashamuka (Projet SIDA, Kinshasa, Zaire) and colleagues, in collaboration with the CDC (Atlanta) and Institute of Tropical Medicine (Antwerp), reported that whole blood collected as dried blood spots on filter paper can be stored for at least 6 weeks in various tropical conditions (such as temperature and humidity) without affecting test reactivities.

Pooled Sera. Testing of pooled donor sera, as opposed to individual serum samples, from low-risk populations (such as blood donors) has been shown to yield highly reliable results.

Fig. 4.3. Alternative Testing Strategy for Developing Countries

Sequential use of self-contained, rapid tests eliminates the need for routine use of expensive confirmatory Western blot testing. Kabeya Mulanga (Projet SIDA, Kinshasa, Zaire, reported these cost-saving findings made in cooperation with AIDSTECH/Family Health International (Research Triangle Park, North Carolina).



Pooling blood for mass screening programs in developing countries could mean substantial economic savings as it would reduce the number of procedures required. (Only when a pooled sample gives a positive result does it become necessary to perform tests on the component samples to identify the positive one(s); conversely, pooled samples that yield negative results do not require any further testing.) Ofelia Monzon and coworkers (Department of Health Research Institute for Tropical Medicine, Philippines), in collaboration with Family Health International (North Carolina), reported their results on a comparison using varying pool sizes and diagnostic test types. Two ELISA formats (Abbott and Organon Teknika) and an agglutination assay (Serodia, Fujirebio) were used to screen 3,000 serum samples—individually and in pool sizes of 5, 10, and 20. The optimum pool size varied according to the method used: ELISA performed best with pools of 5 samples, whereas Serodia agglutination performed equally well at all pool sizes. The most cost-effective way to test for the presence of HIV without compromising sensitivity and specificity seems to be the agglutination test on pools of 20 samples. A joint study by the Ecuadorian Red Cross and Family Health International compared two commercially available ELISAs (Abbott and Organon Teknika) for their performance on a relatively small pool size (consisting of 3 individual samples). Galo Leoro-Monroy (Ecuadorian Red Cross, Quito) reported that the sensitivity of both tests was equal to that obtained with single serum samples; specificity, however, was slightly lower (92–96%).

CHILDREN BORN TO SEROPOSITIVE MOTHERS

HIV infection in infants and children is particularly difficult to identify due to the unknown source of circulating HIV antibodies: the mother or the infant. The presence or absence of

HIV-specific immunoglobulin G (IgG) antibodies cannot be used as a definitive diagnostic criterion because most antibodies from an HIV-infected mother (those of the IgG class) can cross the placental barrier and because infants do not start making their own (IgG) antibodies until about 12 months of age. True pediatric HIV infection cannot be reliably established using conventional (IgG) antibody tests until at least age 15 months. Transient seropositivity—also called seroreversion—in infants reflects the disappearance of maternally derived HIV antibodies and the absence of any infant-derived HIV antibodies. (Exposed to many potentially infectious agents as soon as they leave the womb, neonates essentially rely on carry-over protection, or passive immunity, from the mother's immune system to fight infection until their own immune system begins production of antibodies. In addition to prenatal transfer of antibodies through the placenta, antibodies can be transferred postnatally, through breast milk.)

Past epidemiological data have suggested that approximately 30% of all infants born to HIV-infected mothers are truly infected. Based on findings from several reports at the Conference this year, however, this figure may need to be revised as new data are collected—and as diagnostic techniques improve. According to studies in which the presence of HIV is established (using virus culture, viral antigen tests, or DNA-based tests), 40-50% may be a closer approximation of the true number of children infected perinatally.

One way in which researchers are attempting to establish a truer estimate of the proportion of HIV-infected children born to seropositive mothers is by investigating seroreverters. Although children who have lost HIV antibodies (seroreverters) are presumed to be free of HIV infection (because the transient seropositivity is attributed to the clearing of maternally derived HIV antibodies), some investigators are taking a harder look at this supposition. On finding HIV nucleic acid sequences in 29 of 35 seroreverters, one group from the Institut Pasteur suggested that HIV may be in a latent state in a significant proportion of newborns. Reporting similar results were Dr. Clara Gabiano (University of Turin, Italy) and collaborators who demonstrated that 19 of 32 seroreverters born to seropositive mothers had detectable HIV antigens (p24 and/or gp120) and 10 of these also had evidence of HIV DNA. Another 3 were found to be DNA-positive (by polymerase chain reaction, or PCR, technology; see below) but antigen-negative. These results also argue that a high proportion of seroreverters are actually infected. Elaine Abrams (New York City Perinatal HIV Transmission Collaborative Study Group), however, reported that the phenomenon of antibody-negative/virus-positive children is rare. Using PCR technology, her team studied 93 of 130 seroreverters born to seropositive mothers from three U.S. cities: of the 93 tested, none proved to be repeatedly PCR-positive (although 4 were initially PCR-positive).

Immune parameters other than antibodies may also prove useful for early detection of HIV infection in children. Dr. Remi Cheynier (Institut Pasteur, Paris) and associates, for example, were able to document a vigorous HIV-specific cytotoxic T cell (CTL) response in children as young as 3 months of age born to HIV-positive mothers. The authors were surprised to find such a strong and specific cellular immune response, comparable to that observed in HIV-infected adults. They thus suggested that for children of indeterminate status, identification of such a response could be used in conjunction with findings from standard antibody tests to aid in the diagnosis of true HIV infection.

IgA and IgM Antibody Detection. Because they are far less abundant than IgG antibodies, IgM and IgA serum antibodies are not typically the object of diagnostic testing. However, the predominance of maternal IgG antibodies in neonates has turned attention to IgA and IgM detection as a possible means of identifying true HIV infection in infants. Unlike IgG antibodies, IgA and IgM antibodies do not cross the placenta; thus detection of HIV-specific IgA and IgM serum antibodies in neonates would indicate new antibody production in response to primary HIV infection—and not the transferral of maternal antibodies.

The point at which production of IgG, IgA, and IgM antibodies begins in infants is unclear but, on average, appears to be around 6 months. Natasha Martin (University of California, San Francisco), however, reported that her team was able to detect IgA antibodies against HIV as early as 5 weeks after birth in children (retrospectively identified as HIV-infected) with a new diagnostic test they developed. This finding indicates that infected children mount an active immune response against the virus, even at this young age. Barbara Weiblen (Massachusetts State Laboratory, Newton) also presented information on a new test that detects IgA and IgM antibodies in infants. Of 20 specimens tested from infants (6–12 months of age), which were later shown to be true positives, the test was able to detect HIV-specific IgA antibodies in 14 specimens (4 of these 14 also had HIV-specific IgM antibodies). The 6 IgA-negative specimens were from 4 infants with advanced AIDS and/or IgG hypogammaglobulinemia (meaning they had very low titers of IgG antibodies). Of the HIV-infected infants older than 12 months, IgA antibodies were found in 30 of 30 specimens tested.

Polymerase Chain Reaction. A great deal of energy is being put into the development of tests that can detect viral DNA in infants born to seropositive mothers. Such tests, it is hoped, will provide a more sensitive and accurate measure of infectivity in this population than antibody tests. Diagnostic tests that detect HIV's DNA are based on a technique called polymerase chain reaction, or PCR (see "DNA-based tests," above; note that although HIV's genetic material is RNA, it is copied into DNA once inside host cells). Aside from its high sensitivity capabilities, PCR is considered particularly suited to newborns and infants because a very small blood sample is sufficient for the procedure.

Dr. Sonia Escaich (INSERM, Lyon, France) and coworkers used an RNA-based PCR (with *gag* sequences) to establish HIV infection in 20 newborns and children born to seropositive mothers. Of these 20, they found 9 harbored HIV RNA, including 6 infants who had clinical signs of HIV infection and who later progressed to AIDS, 1 of whom died at 6 months of age. Among the 9 PCR-positive cases, HIV could be cultured from 7.

Dr. Tamare Rakusan and coworkers (Children's National Medical Center, Washington, D.C.) reported that they correctly identified as HIV-positive 41 symptomatic and asymptomatic HIV-infected pediatric patients (older than 15 months) using PCR (with *gag*, *env*, and LTR sequences). In addition, they found 8 of 58 indeterminate (by other tests) pediatric patients to be HIV-positive by PCR, and none of the 50 PCR-negative children have shown clinical or antibody evidence of HIV infection thus far. The authors noted that while the use of several different HIV DNA sequences as probes should optimize sensitivity, in their experience *gag* sequences alone proved most sensitive (95-98%).

Dr. Roger Henrion (Hôpital Cochin, Paris) and coworkers are exploring the possibility that certain maternal PCR patterns (of detected HIV genes) are predictive of perinatal transmission. A follow-up study is underway on infants born to a group of high-risk seronegative pregnant women who were found to be PCR-positive. (These women, primarily IV drug users and sexual partners of seropositive individuals, were identified as PCR-positive using HIV regulatory gene sequences, *nef*, *vpr*, and *vpu*.)

A number of research groups recommended the use of PCR in combination with other diagnostic tests for HIV, such as coculturing of certain blood cells (blood mononuclear cells) with sample cells and HIV antigen tests.

Enzyme-Linked Immunospot (ELISPOT). A new immunological cell test for the detection of HIV-specific antibody-secreting B lymphocytes, called enzyme-linked immunospot or ELISPOT, was described at the Conference by Dr. Francis Lee (Emory University School of Medicine, Atlanta). Preliminary results suggest that this method may identify true HIV infection in infants since enumeration of HIV-specific antibody-secreting cells relative to the total number of the neonate's antibody-secreting cells can give a reliable indication of the source of these cells—and thus whether the presence of such cells represents passive immunity (from

the mother) or primary HIV infection. (*Note:* Some controversy surrounds the premise that B cells are able to cross the placenta.) By identifying HIV specific antibody-secreting B cells, ELISPOT detects cells secreting IgM and IgA as well as those secreting IgG.

Dr. Lee reported his team's experience using this new assay; in evaluating and comparing ELISPOT and PCR, the combination of ELISPOT with PCR proved more accurate for diagnosing primary HIV infection in neonates than either test alone. This combination approach (ELISPOT and PCR) was used to test 62 infants at age 3 days and the results obtained were later (at 18 months or older) matched against the subject's clinical and HIV-specific antibody status. At age 18 months, 14 of the 62 infants tested were found to be truly infected using standard antibody tests. All 14 were detected either by ELISPOT or PCR alone or by both. Furthermore, of 24 who had been found negative using the ELISPOT/PCR combination, all 24 seroreverted by 18 months. And of 24 infants who were found seronegative (and asymptomatic) at 15 months of age, 21 had been ELISPOT-negative and PCR-negative; the other 3 had discrepant result and are being followed up. Based on these results, Dr. Lee suggested that the combination ELISPOT/PCR be used for early (presumptive) HIV diagnoses in at-risk neonates.

Flow Cytometry. A quantitative technique used to analyze large numbers of cells in suspension—called flow cytometry—is able to identify and count individual cells as they traverse a focused laser beam in single file. For HIV detection, a fluorescent label is tagged to a p24 monoclonal antibody that binds to lymphocytes harboring p24 (see above, under "Antigen Tests"). Dr. Kenneth Rich (University of Illinois at Chicago) and colleagues reported that they were able to signal HIV infection in pediatric patients with the use of this rapid HIV antigen test. Using small-volume blood samples (2-4 ml) from 9 patients (aged 7 months to $5^1/_2$ years), HIV p24 antigen could be readily detected by flow cytometry. Of the 9 patients tested, 2 who had been classified as "indeterminate" were found to be p24-positive; all the known HIV-infected children were identified as such (5 of 5 asymptomatic HIV-infected children and 3 of 3 symptomatic HIV-infected children); and 1 patient known to be uninfected was p24-negative by flow cytometry. These findings suggest that p24 flow cytometry would be a rapid means of diagnosis in pediatric populations, and doubly attractive because of the small amount of blood needed to run the test.

QUALITY CONTROL

While numerous technological advancements have allowed for the unprecedented development of highly specific and sensitive HIV diagnostic tests, such tests are nevertheless subject to some degree of error. Factors that can affect test results include the stability of reagents in commercial test kits, sample contamination, and human error. Ongoing evaluation of laboratory testing procedures and performance—through internal and external resources—is among the efforts to minimize the error rate; to evaluate HIV testing performance, commercially available quality-control serum panels can be used. Three such products for monitoring HIV-1 antibody test performance include QC-HIV (heat-inactivated human sera), Sero-HIV (early seroconversion samples), Challenge HIV (weakly reactive samples), all manufactured and distributed by North American Biologicals, Inc., Miami. Performance can be best assessed by reproducibility of test results regardless of when the test is performed: for example, does a laboratory obtain the same (correct) result when multiple samples from one individual are included in one batch (intrashipment) or are seeded in separate batches to be tested at different times (intershipment)?

PROBLEMS & CAVEATS

External quality control schemes can pinpoint common intra- and inter-laboratory problems, such as difficulties with particular types of samples (for example, specimens with low

titers of antibodies); with particular commercial kits; with reagents of kit manufacturers; or with interpretation of test results. Discrepant findings and interpretations for a given batch, or lot, of samples can also result from one or more of the following:

- specimen misidentification
- contamination of negative serum by positive serum
- cases of seroreversion
- failure of standard methods to detect HIV antibodies (false negatives)
- detection of passive antibodies in samples from individuals receiving HIV-positive immunoglobulin therapy

The identification of potential interfering factors or contaminants is of great importance in developing accurate and reliable diagnostic tests. Such substances could conceivably yield false results, positive or negative. David Recker (National Institute of Allergy and Infectious Diseases, Bethesda, Maryland) and coworkers, in fact, identified a substance produced by normal (uninfected) macrophages that inhibits reverse transcriptase activity, as measured in a standard assay. Rabbit and human macrophage cultures, as well as freshly isolated human monocytes, were found to produce an inhibitor of reverse transcriptase, which was characterized as a nuclease (an enzyme that breaks apart nucleic acid molecules). Based on their findings, the authors pointed out that measurement of reverse transcriptase levels alone, as a detection scheme for HIV infection, appears to be inadequate.

Dr. Robert Garry (Tulane School of Medicine, New Orleans) and colleagues reported that patients with Sjogren's syndrome (an autoimmune disorder) give evidence of an antibody that reacts with HIV's major capsid, or core, protein (p24). Culturing extracts from patients' salivary glands with a T cell line yielded detectable HIV-like antigens in 2 of 6 cultures. Interestingly, preliminary virological and biochemical data indicate that a previously uncharacterized (type A) human retrovirus, antigenically related to HIV, was isolated from these cultures. This finding may ultimately shed light on a subset of "problem sera," which give a positive or indeterminate HIV result on antibody testing despite other evidence to the contrary.

EVALUATION PROGRAMS

In 1986 the Centers for Disease Control (Atlanta) implemented an HIV diagnostic test evaluation program in an effort to assess the quality of existing laboratory testing performance. Such a proficiency testing program is designed to evaluate both inter- and intra-laboratory operational performance. Approximately 1,400 U.S. and international laboratories participated in the program during 1988 and 1989. At the Conference, CDC scientist David Cross reported on the results of the CDC's Model Performance Evaluation Program (MPEP) in which laboratories were asked to analyze coded test samples (plasma) sent to them in August and November 1989. Participating laboratories, primarily hospitals, blood banks, health departments, and independent laboratories, were evaluated on their ability to correctly identify HIV antibody-positive, antibody-negative, and weakly reactive samples using enzyme immunoassay (ELISA) screening methods. Overall the participating laboratories had high marks with 99.4% of the HIV negative samples and 99.8% of the strongly positive samples correctly identified as such. Not surprisingly, testing performance for weakly reactive (positive) samples was lower; only 95.6% of samples were correctly identified as positive. Intra-shipment reproducibility (on multiple samples from one individual) ranged from 98.95% to 99.83% for weakly reactive, negative, and positive specimens.

114

CHAPTER 5

Page 115 **Background**
116 Major Obstacles in Development
117 Ethical and Liability Concerns
118 **Vaccine Strategies**
118 Epitope Identification
121 Eliciting Humoral Response
123 Eliciting Cellular Response
123 Enhancing Immunogenicity
125 **Types of Candidate HIV Vaccines**
125 Live (Attenuated) Virus Vaccines
125 Killed (Inactivated) Virus Vaccines
126 Subunit Vaccines
128 Adjuvant-Based Vaccines
129 Viruslike Particles
129 Idiotype-Based Vaccines
130 **Animal Models**
130 Chimpanzees
132 Macaques
134 Cats
134 Mice
134 SCID Mice
135 **Human Trials**
135 VaxSyn HIV-1
136 HIVAC-1e
136 HIV Immunogen
136 HGP-30
136 Other Candidate Vaccines

VACCINE DEVELOPMENT

BACKGROUND

An overall air of optimism permeated many of the vaccine sessions at the Conference, largely related to progress made in the last year with human trials of candidate vaccines in HIV-infected populations and with vaccine responses in animal models (see "Human Trials" and "Animal Models," below). Dr. Wayne Koff (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) noted that, due to these discoveries, the timetable for the successful development of several AIDS vaccines will likely be accelerated.

Numerous advances in immunology and vaccine development have taken place since the pioneering human vaccine trials conducted by the 18th-century scientists Edward Jenner and Louis Pasteur. Modern scientists and vaccine developers can call upon a sophisticated array of technological advances—notably, genetic engineering and biotechnology. Nevertheless, the basic principles that formed the foundation underlying construction and administration of the first successful human vaccines (against smallpox and rabies) remain the cornerstone of vaccine design today. The simple yet elegant principle was, and is, to challenge the immune system with a pathogen in a controlled manner, thereby inducing a specific immune response; thus primed, the body has been shown capable of mounting lasting (even life-long) immunity to that specific pathogen.

With the goal of evoking a strong and protective specific immune response, candidate vaccines for the prevention and/or control of HIV infection seek to mimic what the immune system naturally "sees" during HIV infection. Much of the vaccine research presented at the Conference this year focused on the antigenicity (the ability of a substance to elicit an antibody response) and immunogenicity (the ability to elicit an immune response) of the intact virus as well as discrete viral components (antigens). From work conducted thus far, it is readily apparent that determining which HIV antigens evoke specific immune responses remains one of the most pressing challenges in HIV vaccine research.

A second, not unrelated, major research discussion point at the Conference was that of antigen presentation—how antigens are presented to and recognized by the immune system. Particular conformations (spatial arrangements) of HIV antigens at the molecular level may be significant determinants for eliciting an effective immune response. It is not known whether there is a single "correct" conformation, or whether abundance is the key (that is, whether presenting more antigens elicits a stronger response than lesser quantities of the same antigens).

From a public health standpoint a protective pre-exposure AIDS vaccine would be most desirable, given its potential to control the spread of disease. However, based on accumulated data and experience with candidate HIV vaccines, Dr. Jay Berzofsky (National Cancer Institute, Bethesda, Maryland) pointed out that a post-exposure vaccine (which prevents clinical disease in infected persons, rather than preventing infection in unexposed individuals) may be the most obtainable goal. Nevertheless, researchers are still searching for a way to confer immunity to unexposed individuals; therefore, vaccine preparations are being designed that contain critical immune-stimulating HIV components but render HIV devoid of its potential to replicate. Such candidate vaccines are particularly attractive because they bear no risk of causing HIV infection.

MAJOR OBSTACLES IN DEVELOPMENT

As revealed by extensive studies seeking to determine HIV's pathogenesis (disease-causing mechanisms), AIDS vaccine researchers are keenly aware of many of HIV's peculiarities. Not surprisingly, these peculiarities have turned out to be major obstacles in vaccine development.

- *HIV's ability to rapidly alter its surface antigens (referred to as heterogeneity or antigenic drift).* Because of this property, HIV is particularly successful in escaping clearance by the immune system and in establishing persistent infection. For vaccine developers, HIV's heterogeneity means that a vaccine preparation must somehow enable the immune system to recognize different strains of HIV, each of which possesses slightly different antigens; the successful HIV vaccine, then, must be able to protect against many strains of HIV. Toward this end, *in vitro* (test tube) as well as *in vivo* (animal or human) experiments test candidate vaccines for their efficacy against strains from different geographic locations; North American, Western European, and African HIV isolates are typically employed in such testing.
- *HIV's ability to establish latent infection, whereby the virus "hides" in resting cells by insinuating itself into a host cell's DNA (without expressing its genes).* In addition to infecting and killing susceptible host cells (while simultaneously co-opting the cells' protein-synthesizing machinery to generate progeny virions), HIV can also infect cells, integrate its genetic code into the host cell's DNA and remain dormant in that state until some later time when the cell becomes activated. Without having its genes transcribed and translated into proteins (antigens), HIV can effectively evade detection by the surveillance arm of the immune system. In contrast to latent infection, productive HIV infection results in viral antigen expression on the host cell's surface.
- *HIV's proficiency at covert cell-to-cell infection (without exposing itself to the patrolling immune system).* A well-recognized cytopathic (cell-damaging) effect of HIV infection is that of giant cell, or syncytia, formation in which a cell harboring HIV comes into contact with an uninfected cell, affording HIV the opportunity to infect without transforming itself into a cell-free virion. By not exposing itself to the immune surveillance system outside its host cell, HIV can covertly enter neighboring susceptible cells.

- *HIV's tropism, or preference, for certain immune system cells.* By choosing as its host cells the very cells responsible for defending the body against invading pathogens, HIV subverts the immune system and ultimately leaves the body without a ready or reliable defense. For the development of a post-exposure vaccine (for individuals already infected with HIV), this poses significant problems as the immune system of even asymptomatic HIV-infected individuals is already compromised to some degree. Thus the vaccine that attempts to muster appropriate immune components may be calling to assembly diminished or disabled troops.

Another frustrating problem encountered in designing and developing a successful AIDS vaccine is that, to date, it is not known what exactly constitutes an effective immune response against HIV. Numerous research groups are attempting to address this fundamental question; *in vitro* and/or *in vivo* models are being sought in efforts to assist speedy vaccine development. As is discussed below (see "Vaccine Strategies"), most vaccine researchers believe that the type of immune response necessary to fight or control HIV will include cellular responses as well as an antibody response.

"Traditional" vaccine preparations against other pathogenic viruses use virus that is killed (inactivated) or rendered less virulent (attenuated or weakened). While successful in protecting against some viral diseases, applying this method to HIV is generally considered potentially dangerous and too risky to test in an unexposed population. The main fear is that even using methods that have previously succeeded in either killing or weakening other viruses, these treatments may not have the same effect on HIV; with even a remote possibility that HIV could still be infectious, it hardly seems prudent to test a killed or live (attenuated) HIV vaccine in humans. Scientists are however exploring different ways in which to fully inactivate or weaken HIV; see "Types of Candidate Vaccines," below, for a detailed discussion.

ETHICAL AND LIABILITY CONCERNS

Design of clinical trials for candidate HIV vaccines includes consideration of recruitment issues as well as safety and efficacy analyses. Decision points in the phased clinical development of candidate vaccines must also be determined; for example, at what point would a candidate vaccine be considered effective enough to expand its testing from a control (low-risk) population to a high-risk population? Indeed, conducting clinical trials of candidate AIDS vaccines poses significant moral, scientific, and ethical challenges. The principle of "the greater good" duels with the duty to protect the individual; meanwhile more mundane concerns also require resolution, such as the sheer logistics of informed consent for trial participants and volunteer confidentiality/anonymity.

A presentation by Wendy Mariner (Boston University) pointed out the major ethical problems inherent in any candidate HIV vaccine trial in humans:

- deciding which vaccines to test (should expert panels be established?)
- choosing measures of vaccine efficacy
- identifying subject populations
- weighing potential benefits against harms
- conducting trials in developing countries
- mandating equitable distribution of any resulting successful vaccine

Liability for personal injury, including that resulting from participation in a vaccine trial (as well as that resulting from use of an approved vaccine), is a major issue for HIV vaccine manufacturers. In the United States, and elsewhere, existing legal principles relating to negligence hold vaccine manufacturers liable. Negligence in design, manufacture, instructions, or warnings are considered in cases of personal injury. Under discussion now is some consideration of whether governments will grant to companies developing HIV vac-

cines immunity from lawsuits arising from their HIV vaccine product. Compensation to individuals who incur personal injury directly related to a vaccine would then come from the government, not the manufacturer.

VACCINE STRATEGIES

The underlying strategy common to all candidate HIV vaccines is to mobilize the immune system to mount an immune response that will effectively thwart HIV's attack on the body. Prerequisite to the design of almost any vaccine against a pathogenic microorganism is the identification of discrete portions of the pathogen's antigens—antigenic sites or epitopes—which in and of themselves are capable of eliciting specific immune responses. Definition of such epitopes (in terms of amino acid sequence and/or conformation) and characterization of the responses they elicit is crucial to the design of both whole virus and subunit virus vaccines. (Subunit vaccines employ discrete antigens—or fragments—derived synthetically or by recombinant DNA techniques.)

A second essential in vaccine development is determining the most effective way of presenting antigens to the immune system. Although the immune system recognizes and responds to specific sites on antigens (epitopes), many scientists believe that antigens presented in their natural conformation—as part of intact viral particles—elicit the strongest, most protective immune response. Examples of natural life-long immunity (versus immunity acquired via artificial exposure through vaccine challenge) against various infectious agents certainly support this belief. However, because of the fear that methods previously used to inactivate or weaken pathogenic viruses may not work with HIV, scientists are reluctant to introduce intact HIV into unexposed individuals.

Although a small number of research groups are exploring the possibility of using killed or inactivated but otherwise intact HIV as a vaccine, the majority of candidate HIV vaccines consist of discrete HIV antigens, some of which are recombinantly or synthetically derived. Some of these subunit vaccines are "cocktails" of several antigens. Delivery systems for vaccine components (whole virus, recombinant antigens, or synthetic peptides) can actually enhance the immunogenicity, or immune stimulating ability, of a vaccine's components. Suspending vaccine components in mineral oil, alum, or lipids, for example, generally yields a heightened immune response (see "Enhancing Immunogenicity," below).

A normal host response to any invading pathogen includes both a humoral (antibody) response and a cellular response (specifically, the activation of T cells). Historically, a vaccine has been considered successful based on its ability to elicit an antibody response and to establish circulating "memory" (antibody-producing) cells; most AIDS vaccine researchers indicate that a successful HIV vaccine will have to stimulate both branches of the immune system.

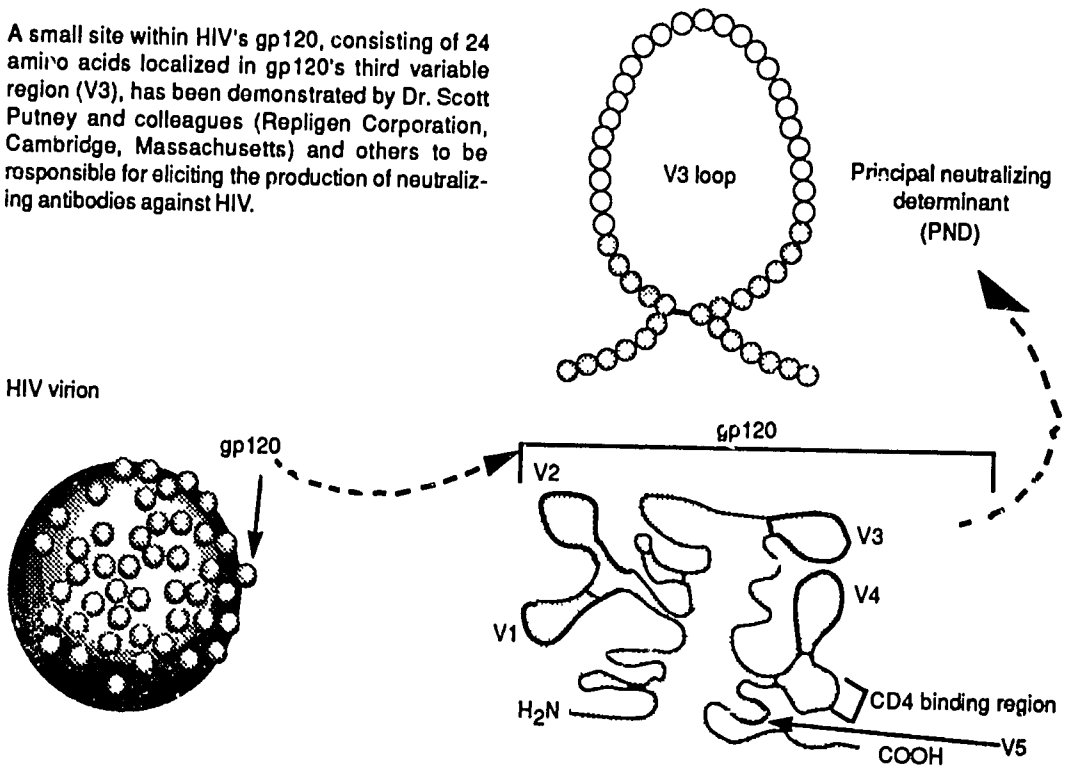
Note that most HIV vaccine research has been concentrated on HIV-1—the development of vaccine preparations containing HIV-1 viral components or inactivated or killed HIV-1. HIV-2 also causes AIDS; however, the worldwide incidence of HIV-2 infection is significantly less than that of HIV-1. One group of investigators is testing an HIV-2 vaccine in chimpanzees and rhesus monkeys (see "Animal Models," below).

EPITOPE IDENTIFICATION

Identification of HIV epitopes (discrete portions of antigens, also called antigenic sites or determinants) that elicit either HIV-specific antibodies or HIV-specific cellular responses (or both) has proved to be a major task. Furthermore, various HIV isolates must be tested to see if the epitope is common to all (conserved)—or is found only on one or a few isolates. As discussed above, different HIV isolates may possess slightly different antigens, which

Figure 5.1. HIV's Principal Neutralizing Determinant (PND)

A small site within HIV's gp120, consisting of 24 amino acids localized in gp120's third variable region (V3), has been demonstrated by Dr. Scott Putney and colleagues (Repligen Corporation, Cambridge, Massachusetts) and others to be responsible for eliciting the production of neutralizing antibodies against HIV.



means that antibodies primed to target HIV antigens from one HIV isolate may not react with HIV antigens from another isolate. And cytotoxic T cells (CTLs) primed to target HIV-infected cells that display antigens from one HIV strain may not recognize HIV-infected cells infected with a different strain.

Antibody reactivities to different HIV isolates have received considerable scrutiny; indeed, the inability of antibodies from a single serum sample to recognize diverse HIV isolates represents a major roadblock in vaccine development. Even serum samples taken from one individual at different times show changes in antibody reactivity. Luba Vujcic and colleagues (Food & Drug Administration, Bethesda, Maryland), for example, found that antigenic variation of defined HIV epitopes occurs periodically *in vivo* during chronic infection. Examination of sequential serum samples from five HIV-infected homosexual men collected over a 1–4 year period revealed that changes in properties of serum antibodies (such as ability to neutralize virus) were associated with changes in a region of HIV's gp120 envelope protein. Carla Kuiken (Academic Medical Center, Amsterdam) and coworkers also noted antigenic changes (again mapped to a region within HIV's gp120) in sequential serum samples obtained from two HIV seropositive men.

Nevertheless, based on antibody profiles of HIV-infected individuals, *in vitro* immunological assays, and the systematic (molecular) dissection of HIV's antigens, vaccine researchers have identified several potentially useful epitopes that elicit antibodies and T cells.

Several presenters at the Conference reported their progress in producing mouse or human monoclonal antibodies developed to recognize and bind to specific regions of HIV. (Monoclonal antibodies—antibody molecules "raised" to recognize and react with a specific antigen—are the fusion product of a single antibody-producing B cell and cells that possess the ability to divide virtually indefinitely, such as cancer cells.) Epitope "mapping"

studies using monoclonal antibodies, it is hoped, will aid in defining immunogenic regions of HIV. Furthermore, characterization of the HIV neutralizing capabilities of human monoclonal antibodies should provide clues to epitopes that are immunologically relevant in natural HIV infection in humans. (Note that monoclonal antibodies are also being designed to act as therapeutic agents; see Chapter 6: ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS.)

Principal Neutralizing Epitope. Residing within HIV's envelope protein, gp120, is a particularly immunogenic short (24) amino acid sequence: it has been shown to be capable of "neutralizing" viral infectivity by a number of research groups. This region of gp120, called the principal neutralizing determinant or PND, is located within a larger, highly variable region of gp120 referred to as the V3 loop (so-called because the amino acids at this site are physically arranged in a loop in the third variable region of gp120).

A model of HIV's PND proposed, in 1987, by scientists at Repligen Corporation (Cambridge, Massachusetts) in collaboration with Merck, Sharp & Dohme Laboratories (West Point, Pennsylvania) is depicted in Figure 5.1. The PND is centrally located within a loop, which protrudes on the surface of the virus and hence is accessible to immune system components and may partially explain the exceptionally strong neutralizing antibody response that the PND elicits.

At the Conference, Dr. Scott Putney (Repligen Corporation) reported that the PND may contain a stretch of conserved amino acids and a conserved structure—even though this region has been previously characterized as highly variable. This finding was based on analyses of 245 North American and European HIV isolates. In practical terms, this finding means that a subregion of the PND may be able to elicit neutralizing antibodies that react with HIV antigens from different isolates. Dr. Putney's team is currently working on a vaccine that may contain as few as 8 amino acids that lie within the PND. (See also "Neutralizing Antibodies," below.)

Work conducted by Professor Jaap Goudsmit (Academic Medical Center, Amsterdam) and colleagues also revealed that another region within the PND (distinct from that being studied by Dr. Putney's team) is conserved among different HIV isolates. Because it is well documented that envelope proteins of other enveloped viruses evoke neutralizing antibodies, researchers are continuing to search for other neutralization epitopes on the viral envelope. Giovanna Viale (Universita di Milano) and coworkers reported that antibodies to the transmembrane protein of the HIV envelope (gp41) recovered from HIV-positive sera exhibited, in some cases, neutralizing activity. Further work is necessary to determine whether gp41 contains a major neutralizing epitope.

CTL Epitopes. Some success has been reported in the search for epitopes recognized by cytotoxic T lymphocytes (CTLs). HIV-specific CTLs have been identified in human sera and are capable of inhibiting replication and participating in killing of virally infected cells. If the epitopes that elicit these CTLs can be identified, vaccine developers may be able to incorporate them into candidate vaccines. Dr. Giles Dadaglio (Institut Pasteur, Paris) and coworkers reported identification of six highly conserved epitopes within HIV's gp120 that were recognized by HIV-specific CTLs from HIV-positive patients. However, this reaction was restricted to persons whose CTLs bear the HLA-A2 cell surface marker, as shown by the inability of CTLs with the HLA-A3 marker to recognize these epitopes. (HLA or MHC proteins identify cells as "self" or "non-self"; cells with non-self molecules on their surface are recognized as foreign by immune cells. Multiple HLA types have been distinguished in humans. According to the authors, 20% to 30% of the European population are positive for HLA-A2.) Dr. Dadaglio's team also tested peptides corresponding to gp41 envelope protein: no epitopes were recognized by the CTLs elicited by gp120 epitopes. Dr. Ammar Achoar (Université Pierre et Marie Curie, Paris) and colleagues reported that a previously identified CTL epitope within the HIV gp160 protein (peptide 18) elicits a response in association with multiple HLA types: HLA-A2, HLA-A3, and HLA-A11.

Dr. Paul Johnson (Massachusetts General Hospital, Boston) and colleagues reported their work on characterization of epitopes of the highly conserved HIV *gag* (core) proteins, p55, p24, and p17, which elicit antigen-specific CTLs. CTLs from HIV seropositive subjects were tested for cytotoxic activity against various peptides corresponding to (covering) the entire *gag* protein; *gag*-specific CTLs were shown to recognize epitopes in p24 and p17. Using mice immunized with vaccinia virus (as a vector for *nef*, *gag*, and *env* proteins), Dr. Frederique Michel (Institut Pasteur, Paris) and associates found several epitopes from these gene products that appear to stimulate a CTL response. Furthermore, these epitopes stimulated proliferation of a subset of T cells ($CD4^+$ and $CD8^+$). Additional study should reveal whether these epitopes also exist as such for humans or other primates.

ADCC Epitopes. Epitopes that elicit antibody-dependent cellular cytotoxicity (ADCC) have also been mapped. Because antibodies are necessary for cell killing by natural killer cells, this process is referred to as antibody-dependent cellular cytotoxicity. Dr. Lauren Wood (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) reported that peptide sequences from the gp41 envelope region are recognized by antibodies from HIV-positive sera and that cells bearing these sequences are lysed (killed) by natural killer cells. Two synthetic (linear) epitopes from the gp41 protein were identified as being responsible for the recognition and lytic process. It has not been determined whether these epitopes (in their native, or natural, conformation) mediate HIV-infected cell killing *in vivo*.

Carbohydrate Epitope. Findings based on monoclonal antibodies that recognize abnormal cell surface carbohydrates, reported by Dr. John-Erik Hansen (Hvidovre Hospital, Denmark), suggest that carbohydrate portions of the HIV envelope glycoproteins may be immunogenic, capable of inducing neutralizing antibody production. Because the carbohydrate portion of HIV's envelope glycoproteins is not virally encoded—but is derived from the host cell membrane—antibodies directed against the carbohydrate portions should be able to recognize virtually all HIV strains since antigenic drift would not be at issue. Dr. Hansen's group set out to screen a number of monoclonal antibodies (previously developed to recognize carbohydrates on the surface of cancerous cells) for their ability to recognize and bind to HIV's envelope glycoproteins; four monoclonal antibodies were found to do so, and were also noted to inhibit infection of human T cells in culture.

ELICITING HUMORAL RESPONSE

A humoral (or antibody) response typically follows exposure to any infectious agent. Antibodies will bind to the "intruder," marking it for destruction by other elements of the immune system. Indeed, the presence of specific antibodies in a patient's serum indicates that the immune system is functioning properly, attempting to clear the pathogen. In HIV infection, however, a significant proportion of HIV-specific antibodies are apparently ineffectual—that is, they are unable to "neutralize" HIV.

Neutralizing Antibodies. All vaccine preparations are tested for their ability to elicit neutralizing antibodies. *In vitro* neutralization assays are used to test for antibodies that recognize and bind to specific antigenic sites of a pathogen, and thereby "neutralize" the pathogen, halting multiplication of the pathogen or inhibiting specific cytopathic properties—such as induction of giant cell, or syncytia, formation. One measure of the potential efficacy of the vaccine preparation is the relative amount of neutralizing antibodies present in a patient's blood sample following vaccine challenge.

As discussed above, a region of HIV's gp120 protein called the principal neutralizing domain (PND) has been shown to elicit neutralizing antibodies. Early experiments demonstrated that antibodies collected from sera of goats immunized with PND could neutralize cell-free and cell-associated HIV and could block fusion of virally infected cells with unin-

fected cells. However, such neutralizing antibodies are type-specific—that is they only bind to and neutralize the HIV isolate against which they were raised. New data reported by Dr. Scott Putney (Repligen Corporation, Cambridge, Massachusetts) and colleagues may show that it is possible to induce group-specific neutralizing antibodies using PND. Dr. Putney's team's finding of conserved, or shared, elements of the PND offers promise that a vaccine containing these elements may be able to elicit neutralizing antibodies effective against a spectrum of HIV isolates.

Dr. Kathelyn Steimer (Chiron Corporation, Emeryville, California) reported that conformation of HIV epitopes influences the cross-neutralization capabilities of neutralizing antibodies. Using a recombinant (linear) gp120 protein derived from a single HIV isolate, Dr. Steimer's team was able to demonstrate that antibodies from human sera recognize linear epitopes, but these antibodies are only effective in neutralizing type-specific HIV isolates. However, human sera depleted of such antibodies contain neutralizing antibodies that recognize native (non-linear) gp120, and these antibodies were very effective in cross-neutralization. These results suggest that neutralizing antibodies directed against conformation-dependent epitopes of gp120 exist and that these possess group-specific neutralization capabilities. Other research, including that presented by Professor Jaap Goudsmit (Academic Medical Center, Amsterdam) and coworkers, also showed that HIV-specific neutralizing antibodies appear to be conformation-dependent.

In examining human sera from geographically diverse regions (United States, Zaire, Haiti, and Brazil), Emily Carrow (Food & Drug Administration, Bethesda, Maryland) and coworkers found that one strain of HIV (MN) consistently elicited higher titers of neutralizing antibodies than did another strain (IIIB). By comparing the prevalence of neutralizing antibodies in the serum samples to prevalence of antibodies that bound to synthetic peptides corresponding to the V3 region of gp120 of both strains, it was evident that this epitope only accounts for some—but not all—of the virus neutralization. Indeed, this group showed in a separate presentation, led by Dr. Gerald Quinnan, that a neutralization epitope could be mapped to a region of gp120 that lies outside the V3 loop.

Enhancing Antibodies. During the past few years, a number of investigators have demonstrated that not all antibodies produced in response to HIV infection are protective. (See Chapter 2: PATHOGENESIS & IMMUNOLOGY for a detailed discussion on enhancing antibodies in HIV infection.) In summarizing accumulated data on these antibodies—which actually enhance (the spread of) HIV infection, rather than neutralize virus—Dr. Edward Robinson (Vanderbilt University, Nashville, Tennessee) called attention to the fact that enhancing antibodies have been identified in more than 90% of HIV-infected individuals. Dr. Jay Levy (University of California, San Francisco) pointed out that long-term survivors of HIV infection characteristically show an absence of enhancing antibodies.

Dr. Robinson's group has constructed human monoclonal antibodies to HIV that enhance infection *in vitro* and are incapable of neutralization. Characterization of these monoclonal antibodies will allow identification of specific regions of HIV to which enhancing antibodies bind. So far, four monoclonal antibodies have been found to recognize and bind to a region within gp41, which is highly conserved among HIV-1 isolates, as well as HIV-2 and SIV (simian immunodeficiency virus). Dr. Robinson referred to this identified region as the primary enhancing epitope; a secondary enhancing epitope has also been identified. Although it is unknown whether all of the current candidate vaccines (being tested in animal and human trials) stimulate production of enhancing antibodies (and whether the production of such antibodies is actually detrimental *in vivo*), it seems prudent to delete all known enhancing epitopes from candidate vaccines.

ELICITING CELLULAR RESPONSE

Accompanying the humoral (antibody) response to any infection is a cellular immune response, which involves helper T lymphocytes, cytotoxic T lymphocytes (CTLs), and natural killer cells. As has recently become appreciated, activation of T cells is not only necessary for cellular defense tactics (killing by CTLs, for example), but also for "helping" antibody responses. Studies demonstrating the role of cytokines secreted by T cells in specific immune responses also underscore the importance of cellular immunity in infection. Although antigen recognition by T cells is thought to be more complex than that by antibodies, the intricacies of T-cell recognition and subsequent cellular immune responses are progressively being revealed. (T-cell recognition of antigen involves interaction with the major histocompatibility, or MHC, protein molecules on the surface of certain "antigen presenting" cells.) Vaccine developers are intent on determining whether cellular responses can be induced to confer some degree of protective immunity in HIV infection.

CTL Response. The activity of CTLs, which specifically recognize HIV antigens and kill HIV-infected cells, have been documented in humans, primates, and mice by several investigators. Previously, Dr. Fernando Plata (Institut Pasteur, Paris) and others have shown that, based on examination of serum samples of HIV-infected patients, declines in HIV-specific cell killing by $CD8^+$ CTLs correlate with HIV-related disease progression *in vivo*. At the Conference, Dr. Plata's team presented data that compared CTL responses of seronegative individuals with those of HIV-infected patients: seronegative subjects (whose CTLs were primed to target cells expressing HIV genes) were found to have an increased CTL response. This finding suggests that it is possible for HIV vaccines to stimulate a CTL response, which, it is hoped, will confer some degree of "resistance" to infection, if not protection.

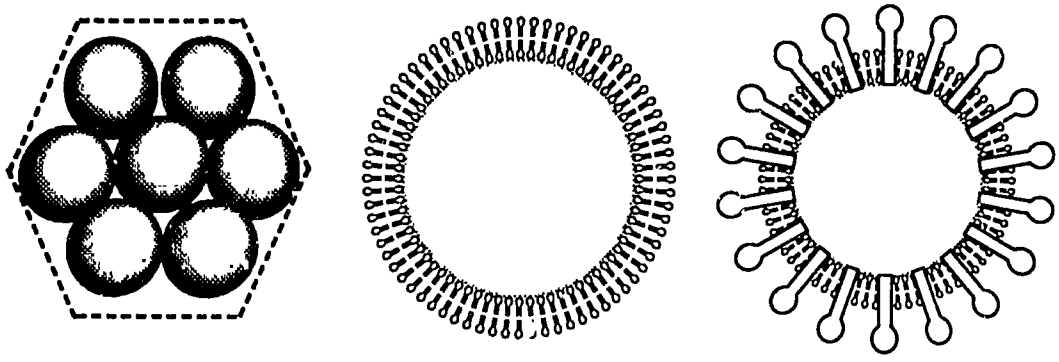
ADCC Response. Although antibody-dependent cellular cytotoxicity (ADCC) responses against HIV have been noted in HIV-infected individuals, it is not clear if eliciting a strong ADCC response actually serves to control HIV infection *in vivo*. For this reason, the value of stimulating an ADCC response is in question as a vaccine strategy. In a study of 24 HIV-infected hemophiliac patients, Dr. Anne Sinclair at Medical Research Council Clinical Research Center, Harrow, U.K., and coworkers were unable to detect any appreciable difference in ADCC lysis of HIV-infected cells between asymptomatic and deteriorating patients.

T-cell Activation. As shown by previous studies, T-cell activation is inhibited by HIV gp120 binding to CD4 molecules on the surface of helper T cells. Data reported by Dr. Manca Fabrizio (S. Martino Hospital, Genoa) and colleagues gave evidence that the physical structure of gp120 determines its immunogenicity as well as its inhibitory lymphoproliferative effects. Dr. Fabrizio's team reported at the Conference that heat denatured (structurally altered) gp120 does not bind to CD4 and therefore could not inhibit gp120 antigen-dependent T cell activation. When gp120 was added directly to cultures of T cells and antigen-presenting cells, proliferation was significantly lower with native (unaltered) gp120 than with denatured gp120. While heat denaturation of gp120 disallows binding with CD4, it spares the immunogenicity of the protein—that is, the protein can still stimulate an immune response—making this a potentially useful strategy for HIV vaccine design.

ENHANCING IMMUNOGENICITY

Not only are vaccine researchers working to pinpoint precise regions and conformation of viral antigens involved in eliciting a protective immune response, they are also working on ways in which to optimize presentation of these antigenic regions to the immune system. The immunogenicity, or immune-stimulating properties, of antigens (such as the ability to elicit neutralizing antibody production) can be enhanced by combining them with substances called adjuvants (literally "helpers"). Polysaccharides, alum, and mineral oil are examples of adjuvants; when antigens are combined with such substances, an immune

Fig. 5.2. Immunogenic Molecular Structures



A **Immune-stimulating Complexes (ISCOMs)** Surface view of an ISCOM particle; antigens assemble in a regular pattern on a cage-like matrix of the adjuvant glycoside Quil A.

B **Liposome** Cross-sectional view showing the cell-membranelike lipid bilayer. Antigens with adjuvants can be encapsulated in the hollow cavity.

C **Immunosome** Cross-sectional view of an immunosome—a liposome containing HIV gp120 embedded in the lipid bilayer.

response greater than that observed for the antigen alone is evoked. Substances from bacteria, such as muramyldipeptide (MDP) and lipid products, also appear to act as adjuvants and are being tested for their safety in humans.

Other methods for enhancing immunogenicity include the use of hollow lipid spheres, called liposomes, and immune-stimulating complexes (ISCOMs), particles that contain symmetrically arranged, repeated units of an antigen sitting on a cagelike matrix of adjuvant. A third structure, the immunosome—a liposome with antigens embedded in its lipid bilayer—is being tested for its immune-stimulating properties. (See Figure 5.2.) Dr. Shiu-Lok Hu (Oncogen Corporation, Seattle), in collaboration with National Veterinary Institute, Uppsala, Sweden, reported their findings on the relative immunogenicity of two candidate HIV gp160 vaccines formulated in various adjuvants. Rabbits immunized with gp160 formulated with Freund's adjuvant (a common immunological adjuvant containing mineral oil, an emulsifier, with or without killed mycobacteria), or with monophosphoryl lipid A (a bacterial lipid derivative) with cell wall skeleton, or as ISCOMs were found to elicit a higher titer of HIV neutralizing antibodies than gp160 formulated in alum.

The timing of antigen presentation to the immune system may prove to be a determinant in the level of immunogenicity of vaccine components. Priming the immune system and then boosting the immune response by re-challenging the immune system has been shown to be particularly effective in conferring protective immunity in other diseases.

Dr. Elizabeth Cooney (University of Washington, Seattle) and coworkers set out to study whether humoral and cellular responses to the HIV envelope could be augmented by such a boosting effect in human subjects. The candidate HIV vaccine HIVAC-1e (composed of vaccinia virus expressing the HIV *env* gene and manufactured by Oncogen Corporation, Seattle) was administered to 11 subjects in two doses and followed 1 year later with two doses of another candidate HIV vaccine VaxSyn HIV-1 (a recombinantly produced gp160 protein manufactured by MicroGeneSys, Inc., West Haven, Connecticut). Following administration of recombinant gp160 (VaxSyn HIV-1), all 11 subjects had heightened T cell responses to HIV, and 9 of 10 developed antibodies to HIV, suggesting that anamnestic responses may be significant and contribute to protective immunity. (Note, the 11th subject had developed antibodies prior to administration of VaxSyn HIV-1.) This regimen proved to elicit overall greater immune responses that that obtained with either vaccine alone.

TYPES OF CANDIDATE HIV VACCINES

Historically, a number of successful vaccines for viral diseases have been based on the use of live, attenuated (weakened) virus and killed (inactivated) virus. However, both approaches carry significant risk in the context of HIV disease, prompting many vaccine researchers to turn to the development of subunit vaccines, idiotype-based vaccines, and "pseudo-virion" vaccines.

According to National Institute of Allergy & Infectious Diseases (Bethesda, Maryland), more than 30 vaccine approaches are currently in preclinical development worldwide. Discussed here is updated information on the various types of candidate AIDS vaccines (some in the earliest stages of development) based on research presented at the Conference. Table 5.1 catalogues the broad categories of candidate vaccines, along with their respective components. See "Animal Models" and "Human Trials," below for details on specific vaccine progress.

LIVE (ATTENUATED) VIRUS VACCINES

Certain pathogenic mechanisms of HIV make a live virus vaccine approach extremely risky; vaccination with an attenuated retrovirus still leaves a live—potentially infectious—pathogen in the body. The risk of introducing HIV with all its parts intact—including viral RNA, its genetic material—is considered unacceptable by most scientists. The concern is that this approach could backfire: viral RNA could find its way into host cells, and while not necessarily retaining the ability to cause AIDS, could prove hazardous just on the basis of having this template stored in a host cell. If the cell were later infected by another retrovirus, the HIV RNA template could be reverse transcribed into DNA,possibly recombine with the other retroviarl genome, and produce an HIV strain or an HIV-like virus with an inestimable potential virulence.

Drs. Kesh Prakesh and Stanley Plotkin (Children's Hospital of Philadelphia) presented provocative data on "deletion mutants" of HIV and SIV (simian immunodeficiency virus) and their potential use in live-virus HIV vaccines. By removing the portion of the *pol* gene that encodes the integrase (or endonuclease) viral enzyme, SIV and HIV are unable to integrate their DNA into the host cell's genome. The replicative potential of such (integrase) deletion mutants, however, is preserved. Data presented (on reverse transcriptase activity) demonstrated that both a parental SIV strain and a deletion mutant derivative were equally capable of directing efficient production of virus in a susceptible T cell line. The ability of the deletion mutant to produce progeny virus means that an immune response will be stimulated.

DNA analyses of cells in which the genome of the parent SIV strain and deletion mutant were introduced revealed that integration of the SIV deletion mutant had not occurred and that unintegrated DNA from the deletion mutant was, as expected, of shorter length than the parental strain. Together, these results clearly show that integrase-deletion mutants of SIV lose the ability to integrate their DNA (and therefore are unable to establish latent infection) without losing the capacity to replicate. Preliminary results with the MN strain of HIV show that deletion mutants are also capable of producing virus (in a human sarcoma cell line). Further work is necessary to determine whether HIV deletion mutants are truly unable to establish persistent infection *in vivo*—in animal models—while retaining the capacity to produce virus and therefore an immune response.

KILLED (INACTIVATED) VIRUS VACCINES

A killed or inactivated, whole virus HIV vaccine is being developed by a research team led by Dr. Jonas Salk at the Salk Institute (La Jolla, California). Although the virus's genetic material is destroyed by ultraviolet irradiation, the structural integrity and presumably

immunogenicity of whole virus is likely preserved. Dr. Alexandra Levine (University of Southern California School of Medicine, Los Angeles) presented an update on the status of the killed virus, envelope-depleted HIV vaccine (called HIV Immunogen, manufactured by Immune Products, Ltd., Horsham, Pennsylvania). The vaccine is being administered to HIV-infected individuals as a post-exposure vaccine or as immunotherapy; see "Human Trials," below, for details.

Unexpected results using killed, inactivated SIV vaccine in simians reported last year by Dr. Michael Murphy-Corb (Yerkes Regional Primate Center, Georgia) and by Dr. Ronald Desrosiers (New England Regional Primate Center, Massachusetts) this year have caused vaccine researchers to re-analyze their skepticism toward a killed-virus vaccine for HIV infection. The risk of introducing HIV RNA into an unexposed individual is certainly reason enough for caution; nevertheless, reports of a protective immune response in monkeys inoculated with killed SIV and then re-challenged with a different strain of SIV give room for cautious respect for this approach. (The SIV vaccine model is discussed below in "Animal Models.")

SUBUNIT VACCINES

Subunit vaccines, in which only nonreplicating antigens or antigenic fragments of HIV are used, are similar to traditional vaccines in that they are administered as a means of stimulating an immune response against a target pathogen. Viral antigens can be either recombinantly produced via DNA technology or chemically synthesized (synthetic peptides). Moderate success has been achieved with the first human subunit vaccine, which was developed for hepatitis B.

Recombinant Vaccines. Genetic engineering techniques have enabled vaccine researchers to either produce "recombinant" HIV antigens (by inserting the corresponding genes into vectors and collecting and purifying the HIV gene products) or to display HIV antigens on the surface of viral vector12s—the aim of both being to elicit an immune response against these antigens, and, it is hoped, against the virus when challenged. HIV genes encoding antigens that have been demonstrated to be immunogenic are currently being used in the subunit vaccines in development. Proteins (serving as antigens) derived using recombinant DNA techniques include:

- gp160 (derived from vaccinia virus)
- gp160; p24 (derived from baculovirus system)
- gp160; gp120 (derived from mammalian cell system)
- gp120; p55 (derived from yeast system)

Recombinant HIV viral vectors—that is, viruses into which HIV genes have been introduced—being used as vaccine approaches include:

- vaccinia virus (vaccinia/env; vaccinia/gag; vaccinia/env•gag•pol)
- adenovirus (adenovirus/env)
- poliovirus (poliovirus/env)
- BCG (BCG/env)

Synthetic Peptide Vaccines. Strings of amino acids, or peptides, that correspond to discrete HIV proteins or portions of proteins can be synthesized and used to elicit an immune response. Based on Conference presentations, peptides corresponding to regions of the HIV envelope, gp120 or gp41, are receiving much attention. One group reported success in creating two chimeric (fusion) peptides each containing a neutralizing antibody epitope from the envelope region and a highly conserved T cell epitope from HIV p24 (the latter acting as a "carrier"). Dr. Dwo Sia and colleagues (Connaught Centre for Biotechnology Research, Ontario) reported that these chimeric synthetic peptides, when inoculated into

Table 5.1. Types of HIV Vaccines

(Note that while virtually all vaccine preparations are formulated in adjuvant, the microstructures liposomes and ISCOMs, have come to be categorized as "adjuvant-based" vaccines.)

Vaccine Type	Vaccine Components
Adjuvant-based (liposomes and ISCOMs)	Viral antigens are combined with adjuvants (substances known to enhance immune responses) to optimize antigen presentation. Liposomes—microspheres whose composition closely matches that of cell membranes—can enhance the immunogenicity of the antigens they carry. ISCOMs (immune-stimulating complexes) are microstructures consisting of repeated units of a single antigen; such redundancy is thought to maximize presentation of antigen to the immune system.
Idiotype-based	Antibodies (idiotypes) that bear a likeness to an HIV antigen can themselves elicit the production of antibodies (anti-idiotypes) when introduced into humans. These anti-idiotypes will recognize and bind to the idiotype antibodies, but more importantly, they will bind to HIV. Virtually all idiotype-based vaccines are employing CD4-based antibodies as idiotypes.
Killed (inactivated) virus	The genetic material of HIV is destroyed with chemicals (e.g., formalin) or radiation while the structure of the whole virus is left intact. Both irradiated HIV vaccines and chemically-inactivated HIV vaccines are in development.
Live (attenuated) virus	Although considered risky, research into the use of live, but weakened, HIV is being conducted at the *in vitro* level and in animal models. Such research is addressing the issue of how to accomplish attenuation; one group is examining HIV "deletion mutants," whereby bits of the genetic material are excised from the total genomic content of HIV.
Recombinant (subunit)	HIV antigens are produced by inserting the corresponding gene(s) into a convenient system (e.g., yeast cells,) which are then collected and purified; alternatively, vector systems can be used whereby a vector (e.g., vaccinia virus) displays HIV antigens on its surface.
Synthetic peptide (subunit)	Protein components (peptides) that correspond to HIV antigens are biochemically synthesized in the laboratory.

mice in a priming-and-boosting regimen, elicited a strong antibody response against the epitopes from the envelope region. This finding was particularly noteworthy because when mice were inoculated with either envelope epitope alone, no antibody response was observed. Antibodies elicited by the chimeric peptides were capable of recognizing both the synthetic (envelope) epitopes and the corresponding native envelope proteins, gp41 and gp120. Preliminary studies showed that antibodies reactive against native gp41 elicited by the chimeric peptide containing gp41 sequences inhibited HIV infection of human CD4 lymphocytes *in vitro*.

A synthetic peptide–based p17 vaccine (HGP-30, manufactured by Viral Technologies, Inc., Washington, D.C.), which contains human T and B cell epitopes, is being tested in a seronegative population and in an HIV-infected population. Dr. Allan Goldstein (George Washington University Medical Center, Washington, D.C.) reported preliminary findings from these clinical trials; see "Human Trials," below.

Dr. Thomas Buchanan (University of Washington, Seattle) reported *in vitro* immunoreactivity results obtained with synthetic peptides corresponding to several regions in gp160—the V3 region, CD4-binding region, and gp41 immunodominant region. Antibody binding was evaluated using serum/plasma from

- 11 human volunteers immunized with HIVAc-1e (a recombinant vaccine: vaccinia/env) and boosted with VaxSyn HIV-1 (a recombinant vaccine: gp160 from baculovirus)
- 4 recent seroconverters
- 15 symptomatic HIV-infected patients

No antibodies directed against the CD4-binding region peptides were detected. Antibodies recognizing the gp41 region were detected in all samples from the seroconverters and from symptomatic HIV-infected patients, and only 1 of 11 vaccinees. Antibodies against the V3 region were detected in 14 of 15 symptomatic patients, 4 of 4 seroconverters, and 1 of 11 vaccinees. Further analysis of the reactivities of V3 region antibodies from the 14 symptomatic patient responders revealed characteristics of a type-specific response: for example, only 2 of 14 had antibodies that reacted with the HIV IIIB strain but 14 of 14 had antibodies that reacted with the HIV MN strain. Dr. Buchanan offered that the relatively poor response of the vaccinees could be explained as being dependent on the conformation of the epitope given that sera from 8 of 11 vaccinees did contain antibodies reactive in whole virus lysate ELISA.

ADJUVANT-BASED VACCINES

As discussed above, vaccine components can be combined with substances that enhance their immunogenicity—such as eliciting an intensified or prolonged antibody response. Although their action is not understood, these substances, called adjuvants, are used in virtually all vaccines. For purposes of categorization, vaccines using microstructures that act as adjuvants are grouped as a vaccine type, which so far consists of liposomes, immunosomes, and ISCOMs. See Figure 5.2 for a schematic representation of these microstructures.

ISCOMs. Immune-stimulating complexes, or ISCOMs, are symmetrically arranged, repeated units of a single antigen sitting on a cagelike matrix of adjuvant (cholesterol and Quil A). The antigenic redundancy is thought to maximize the presentation of antigen (to immune system components) and thereby stimulate a higher grade immune response. Several ISCOM-based SIV or HIV candidate vaccines are being tested; the responses elicited are being compared with those elicited by counterparts to these vaccines, formulated in different adjuvants. Professor Gunnel Biberfeld (National Bacteriological Laboratory, Stockholm), for example, reported preliminary results from his team's work with two vaccines in rhesus monkeys, using HIV-2 envelope and core antigens formulated in (Freund's incomplete) adjuvant and using the same antigens in ISCOM particles. Although only one of two monkeys immunized with the ISCOM version was protected following challenge with live virus, all four monkeys inoculated with Freund's adjuvant version showed no evidence of infection after live virus challenge.

Research published earlier this year and discussed at the Conference by Dr. Jay Berzofsky (National Cancer Institute, Bethesda, Maryland) demonstrated that ISCOMs composed of purified HIV gp160 elicit neutralizing antibodies against HIV as well as HIV-specific $CD8^+$ CTLs (the major class of CTLs) in immunized mice. The same preparation of purified gp160 in other adjuvants or in saline solution, however, did not elicit CTL activity. (Prior to this finding, subunit vaccine preparations have only been able to stimulate production of $CD4^+$ CTLs.) This first demonstration of the use of ISCOMs as a vaccine strategy to introduce viral proteins into cells for induction of CTL responses has been heralded by several groups of investigators.

Liposomes. Lipid vesicles, or liposomes, are bilayered lipid (fat) or phospholipid hollow spheres that are easily constructed in the laboratory. Antigens and adjuvant can be encapsulated in these vesicles or they can be incorporated into the lipid bilayer, the latter resulting in a membranelike microsphere with antigen exposed on the outer surface (these have been recently called immunosomes).

VIRUSLIKE PARTICLES

Particles that resemble viruses in terms of size and shape (as viewed by electron microscopy), but are actually composed of protein only, are being exploited as a novel vaccine delivery system. The discovery that certain proteins naturally self-assemble into tiny particles has led to the construction of fusion viruslike particles (VLPs); the gene encoding a protein of interest is combined with the gene encoding self-assembling proteins. If the gene encoding the yeast protein Ty (which is able to form such VLPs) is fused to a gene encoding an HIV protein, the yeast cell makes a hybrid protein that self-assembles into a VLP, carrying on its surface multiple copies of the HIV protein encoded by the HIV gene. Researchers at British Biotechnology Limited (Oxford), in collaboration with Oxford University, have constructed several Ty/HIV VLPs that react with antibodies to HIV and elicit production of antibodies to HIV in rabbits. A Ty/HIV-p24 hybrid VLP is currently being tested in uninfected human volunteers in a clinical trial in London.

An extremely valuable offshoot of this research is that Ty/HIV hybrid VLPs yield a large quantity of purified fusion protein; the Ty portion of this protein can easily be removed, providing a convenient method to produce large quantities of purified HIV antigens. Specific HIV antigens, and even immunogenic fragments of these antigens, can thus be mass produced for use in this or other vaccine approaches.

Nonreplicating HIV-like particles can also be generated using recombinant vaccinia viruses and baculoviruses. Dr. Dennis Panicali (Applied bioTechnology, Cambridge, Massachusetts) and colleagues reported their work with VLPs (also dubbed "pseudovirions") that include either HIV or SIV *env*-, *gag*-, and *pol*-encoded proteins. Electron microscopy and electrophoresis or Western blot analysis revealed that HIV-like particles had both the gp41 and gp120 envelope glycoproteins on their surface. Dr. Omar Haffer and colleagues (Oncogen Corporation, Seattle) and, independently, Dr. Ralf Wagner and associates (Max von Pettenkofer-Institut, Munich) also reported stable expression of HIV core and envelope proteins as immunogenic VLPs. Dr. James Wilhelm and colleagues (Wyeth-Ayerst Research Laboratories, Philadelphia) used a recombinant adenovirus system from which HIV core proteins assembled into VLPs. These research reports hint that a high level of immunogenicity may be achievable with non-infectious VLPs; their use as possible vaccines is especially attractive since there is no risk of inadvertent exposure to infectious virus.

IDIOTYPE-BASED VACCINES

By taking advantage of the immune system's ability to generate antibodies to virtually every foreign (non-self) molecule, vaccine researchers may be able to coerce the immune system into generating antibodies in response to the introduction of foreign antibodies. As described by Dr. Michael Fung and colleagues (Tanox Biosystems Inc., Houston), antibodies can be designed to "serve as surrogate antigens to induce HIV-neutralizing responses." Antibodies that act as surrogate antigens are called idiotypes, and the specific antibodies they elicit are called anti-idiotypes.

Numerous research teams are exploring the use of monoclonal antibodies (antibody molecules "raised" to recognize and react with a specific antigen) constructed to bear a like-

ness to immunologically relevant HIV antigens. When administered as a vaccine, these monoclonal constructs will trigger antibodies that will recognize the constructs as well as the true HIV antigens they mimic. (See Figure 5.3.)

Dr. Fung's team previously reported that a monoclonal antibody that mimics the gp120 binding region of the CD4 molecule is capable of neutralizing HIV *in vitro*. When introduced into mice, this monoclonal antibody (CD4-based idiotype) elicits anti-idiotypes whose shape mimics that of the CD4 binding region of gp120 and therefore should be able to bind to CD4. At the Conference, Dr. Fung and colleagues presented data demonstrating that these anti-idiotypes, when injected into rabbits, could induce immune responses directed against HIV. When rabbits were immunized with an anti-idiotype, the antibodies generated were capable of recognizing and binding to CD4 cells infected by one of two strains of HIV, as shown in *in vitro* experiments. More significantly, the antibodies could neutralize the infectivity of both strains.

Preparation of idiotype-based antibodies (anti-idiotypes) was also independently reported by Dr. Kohji Ohki (Hokkaido University, Sapporo, Japan) and colleagues; Dr. David Buck (Becton Dickinson Monoclonal Center, Mountain View, California) and colleagues; Dr. Yosuke Maeda and coworkers (Kumamoto University Medical School, Japan); Dr. Christopher Sutor and colleagues (Medical School of Hannover, FRG); and Dr. Micaela Pelagi (Universita di Milano) and associates. While the technical aspects of constructing idiotype-based antibodies appear to have advanced significantly during the past year, characterization of the anti-idiotype response(s) continues. Whether idiotypes—anti-CD4 monoclonal antibodies, for the most part—can be successfully used as an HIV vaccine remains in question. Researchers need to demonstrate that anti-idiotypes capable of neutralizing HIV can indeed be generated following immunization with idiotypes.

ANIMAL MODELS

Several animal species have been shown to be susceptible to HIV infection or to an HIV-like disease caused by a similar retrovirus (see "Animal Models" in Chapter 1: VIROLOGY). In an effort to define a protective immune response, these animals are being systematically tested with various candidate vaccines. Detailed characterization of vaccine-induced responses in appropriate animal systems will allow evaluation of vaccine strategies (whole virus versus subunit vaccines, for example), including vaccination dosing and scheduling. Currently several animal models serve as a basis for HIV vaccine research:

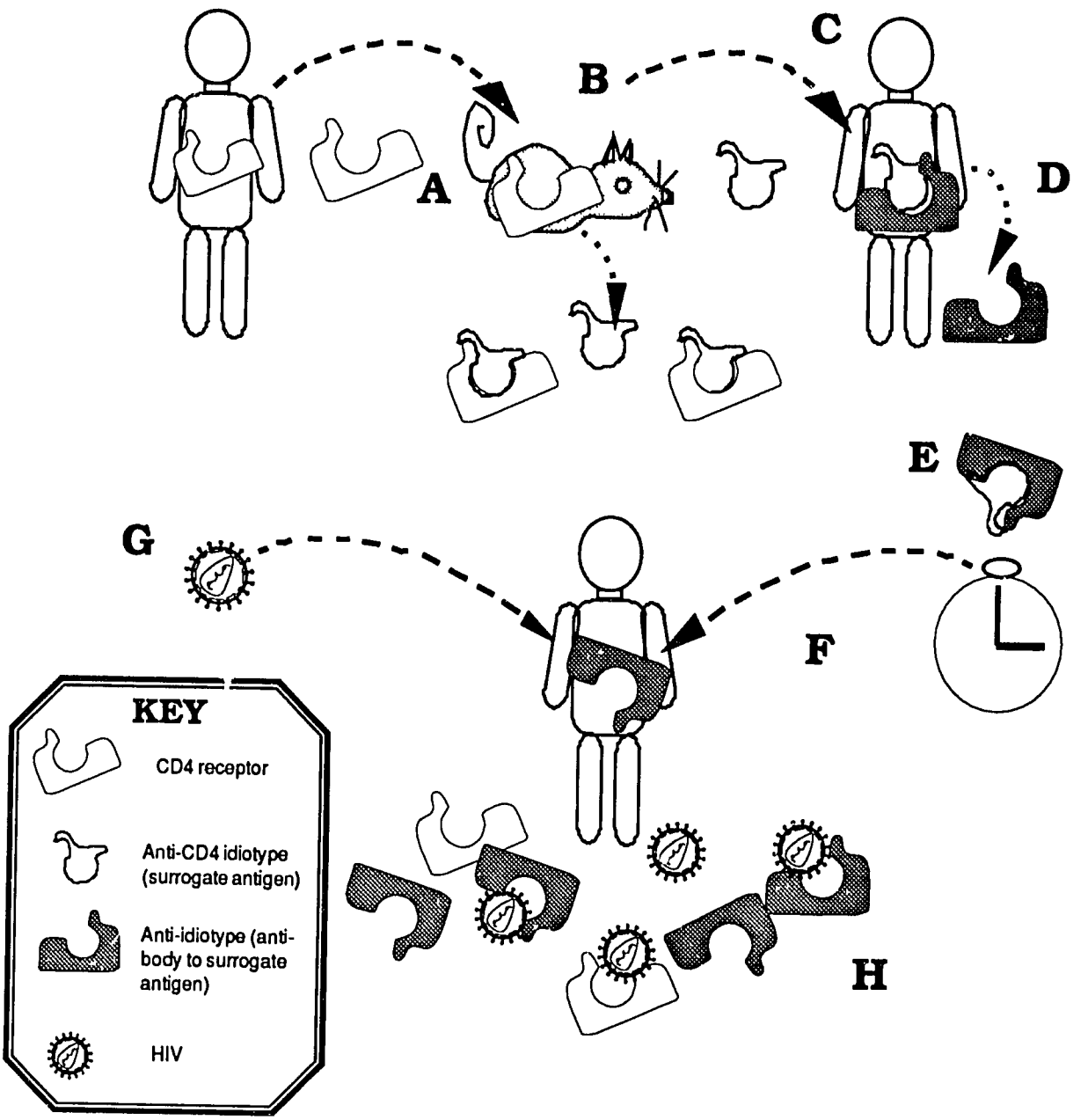
- chimpanzees infected with HIV-1 or HIV-2
- macaques infected with SIV (simian immunodeficiency virus, various subtypes) or HIV-2
- cats infected with FIV (feline immunodeficiency virus) or FeLV (feline leukemia virus, an oncovirus)
- mice infected with Rauscher murine leukemia virus (a retrovirus)
- SCID mice (with transplanted lymphocytes from human vaccinees) infected with HIV

Nonhuman primates continue to be vigorously tested, and recent findings of acquired protection against live virus challenge in both macaques and chimpanzees have spurred much optimism that a vaccine for HIV infection in humans can be developed. Vaccine protection in cats infected with FIV has also recently been reported.

CHIMPANZEES

Although chimpanzees are susceptible to HIV infection (following experimental inoculation of HIV, they develop antibodies to HIV and virus or viral antigens can be detected), only some become sick and only for a short while; none manifest ARC or AIDS symptoms. At the

Figure 5.3. Principle of Idiotype-Based Vaccines



If human CD4 receptor molecules **(A)** are injected into laboratory animals, antibodies to the CD4 molecule, or idiotypes, are produced **(B)**. Indeed, such antibodies (idiotypes) have been raised in mice and rabbits and have subsequently been shown to bind to specific regions of the CD4 molecule *in vitro*. If the mouse or rabbit idiotypes are then injected into humans **(C)**, antibodies to these idiotypes, or anti-idiotypes that mimic CD4, will be produced **(D)**. Because they mirror CD4, these anti-idiotypes will bind **(E)** to the anti-CD4 molecules (idiotypes) and—more importantly—to HIV. Although the introduced idiotypes and the induced anti-idiotypes will be cleared from the body, a template for the anti-idiotype **(F)** will have been created and stockpiled to await future demand. Production of CD4 replicas (the anti-idiotypes) from this template will be triggered by memory cell recognition on exposure to HIV **(G)** because the HIV binding site is presumably identical to that of the idiotype. When numerous CD4 anti-idiotypes are put into circulation, they will compete with CD4 for HIV binding sites **(H)**, theoretically preventing HIV from entering susceptible cells (those displaying CD4 molecules) and establishing infection.

Conference, Dr. Jorg Eichberg (Southwest Foundation for Biomedical Research, San Antonio, Texas) summarized the ongoing vaccine efficacy trials using the chimpanzee model. Currently 13 types of vaccines are being tested, including killed (inactivated) HIV, (anti-CD4) idiotype-based vaccines, and passive immunization with HIV immunoglobulins (antibodies to HIV from sera of HIV-infected individuals). Although an antibody response is observed with virtually all candidate vaccines, neutralizing antibody titers are extremely low. With respect to cellular immune responses, helper and cytotoxic T cells have been detected in most immunized animals. Other vaccine researchers using the chimpanzee model report similar findings, and note that T-cell responses include long-lasting memory and substantial proliferation.

Earlier this year several independent vaccine research teams announced that their respective candidate vaccines conferred protection to immunized chimpanzees against subsequent challenge with live virus. (Some researchers caution, however, that it may be too early to say with certainty that these vaccines conferred full protection; generally, immunized animals are monitored for 1 year to determine if they in fact have been completely protected against infection.) Dr. Josef Manhalter (ImmunoAG, Vienna) and colleagues, Dr. Jean-Claude Gluckman (Pitie-Salpêtrière Medical School, Paris) and colleagues, and Dr. Phillip Berman (Genentech, Inc., South San Francisco) and colleagues independently reported achieving protective immunity in chimpanzees.

Using a recombinant gp160 vaccine, Dr. Manhalter's group noted that two immunized chimpanzees had gp160-specific T-cell responses but very low neutralizing antibody titers following immunization. However, following live HIV challenge, one chimpanzee had no detectable anti-HIV antibodies suggesting that viral infection was averted or replication was controlled. The other immunized animal and a control animal developed antibodies to the core antigen. Since the Conference, this candidate vaccine has been approved for testing (safety trials) in humans by the U.S. Food & Drug Administration.

Dr. Gluckman's team reported findings from serological analyses of chimpanzees immunized with different combinations of HIV-antigen preparations, including killed HIV (inactivated in formalin and beta-propiolactone), recombinant vaccinia/gp160, p25, or p27 viruses, and synthetic peptides corresponding to the gp120 V3 region. Earlier this year these researchers reported that two chimpanzees immunized with one of their "cocktail" preparations were protected from challenge infection. At the Conference, focus was on the types of vaccine-induced responses: antibody-dependent cellular cytotoxicity (ADCC) was evident in some immunized animals but did not appear to be sufficient to confer protective immunity. Furthermore, antibodies participating in ADCC could be distinguished from neutralizing antibodies. Continued monitoring of the infection-free immunized animals will hopefully provide information on the nature and elements of a protective immune response.

Dr. Phillip Berman's team found that their recombinant gp120 vaccine preparation (produced in Chinese hamster ovary cells and formulated in aluminum hydroxide adjuvant) could confer protection for more than 6 months, but a comparable gp160 vaccine could not. Compared with control chimpanzees and gp160-immunized chimpanzees, the two gp120-immunized chimpanzees showed a progressive decline in gp120 antibody titers over time and eventually reached baseline levels; an anamnestic immune response was observed in the other animals (antibody titers declining for 7-8 weeks, but increasing thereafter to levels higher than that achieved immediately following inoculation). Although still being followed, no signs of HIV infection have been observed in the gp120-immunized animals.

MACAQUES

Experimental infection of macaques results in depletion of $CD4^+$ lymphocytes, and to a lesser degree, macrophages, as in HIV infection in humans. A disease course remarkably

similar to that seen in HIV-infected humans is seen in macaques; simian AIDS culminates in death approximately 6–12 months after onset of disease symptoms. In summarizing the current status of the SIV macaques model with respect to vaccine research, Dr. Murray Gardner (University of California School of Medicine, Davis) pointed out that in the last year in three U.S. primate centers, rhesus macaques have been shown to be protected against low-dose live SIV virus challenge following vaccination with killed whole virus vaccine preparations using muramyldipeptide (MDP) as adjuvant. Characterization of the vaccine-induced responses are continuing, with hopes of determining the elements of a protective immune response. Dr. Gardner reported that recent work at University of California, Davis, revealed that antibodies inhibiting syncytia formation are present in higher titers in protected animals than in animals who succumbed to infection following challenge and control animals (not immunized before challenge). Although protection against heterologous SIV strains (strains other than that used in the vaccine preparation) will need to be achieved, only protection against homologous strains has been conferred to monkeys so far.

Even though full protection (against low-dose challenge with a homologous strain) is not conferred in 100% of the animals vaccinated, other benefits have been noted. For example, vaccinated monkeys who become infected following challenge appear to live longer than infected, unvaccinated controls. And studies at University of California, Davis, with a live (attenuated) SIV vaccine show that, while not protected against live virus challenge, the monkeys do live significantly longer than controls. Preventing early death from SIV-induced simian AIDS with a vaccine at least paves the path for a similar vaccine in humans.

Other SIV vaccine approaches presented at the Conference included an SIV envelope peptide/beta-galactosidase fusion-protein vaccine (Dr. Avigdor Shafferman, Israel Institute of Biological Research, Ness-Ziona, and colleagues) and a recombinant SIV/adenovirus vaccine (Dr. Sheau-Mei Cheng, Wyeth-Ayerst Research, Radnor, Pennsylvania, and colleagues). Dr. Shafferman reported that macaques may be completely or partially protected against SIV challenge with a SIV envelope peptide/beta-galactosidase fusion protein vaccine; the vaccine preparation consisted of peptides corresponding to SIVmac gp150/gp120 and gp41/gp32 produced as a beta-galactosidase fusion protein. Following immunization, antibodies reacting with the synthetic SIV envelope peptides and also reacting with native SIVmne gp150 and gp32 were recovered in 3 of 3 macaques. Challenge with high dose SIVmne led to an early rise in SIV-specific-antibody titers followed by a steady decline. At 24 weeks post challenge, SIV DNA sequences were not detectable via PCR in two of the three animals, one of which was also persistently virus-culture negative. Studies at longer post-challenge intervals will provide a clearer picture of the degree of immunity conferred with this peptide fusion-based vaccine.

Two different HIV-2 vaccine approaches were also tested in the macaque model. One, a killed-virus vaccine using HIV-2, has also been shown to confer protective immunity in two rhesus macaques. Professor Gunnel Biberfeld (National Bacteriological Laboratory, Stockholm) reported his team's results in two cynomolgus monkeys using killed HIV-2 formulated in incomplete Freund's adjuvant. No viral antigens were detected following live-virus challenge and no virus has been recovered from these immunized animals. Dr. Guillaume Vogt (SSA/DPS/SPE/CEA, Fontenay aux Roses, France) and coworkers reported preliminary work on a cocktail vaccine consisting of a mixture of recombinant HIV-2/vaccinia viruses in which the HIV-2 genes *gag*, *pol*, *nef*, *vif*, and *env* are incorporated into vaccinia. This mixture was used as a primer, and a vaccine containing the purified protein counterparts of the individual genes was used as a booster. Characterization of the vaccine-induced responses included high titers of antibodies following boosting with purified proteins. Live-virus challenge had not been performed at the time of the report.

Molecular research on monoclonal antibodies recognizing gp120 or gp41/32 of SIVmac251, presented by Dr. Karen Kent (National Institute for Biological Standards & Control, Potters Bar, U.K.) and colleagues, revealed a conserved epitope shared by three SIV strains and one HIV-2 strain. Also identified was an epitope unique to SIVmac251. Definition of antigenic similarities and differences between SIV and HIV should factor into the possible adaptation of SIV vaccines to the human "model."

CATS

Due in part to the lack of availability and cost of simians, several vaccine research teams have turned to the domestic cat as a model for developing a vaccine capable of conferring protection against retroviral infection. The feline immunodeficiency virus (FIV), now known to have an overall worldwide prevalence rate of about 1%, induces disease symptoms comparable to those seen in AIDS patients (lymphadenopathy, opportunistic infections, and neurological symptoms, for example) about 2 years after infection. FIV is also quite similar to HIV in its virologic and immunological aspects of infection. Dr. Murray Gardner (University of California, Davis) reported recent results with a "cell vaccine" developed in Dr. Janet Yamamoto's laboratory at the university. The vaccine preparation consisted of feline lymphocytes (from a healthy cat) that were infected with FIV *in vitro* and then inactivated with paraformaldehyde formulated in MDP adjuvant. Cats serially immunized with this cell vaccine developed antibodies to core and envelope proteins. At day 49 after challenge with low-dose infectious FIV, 8 of 9 vaccinated cats appeared to be protected (as determined by lack of viremia); 10 of 10 control animals became infected. Cats challenged with a much larger dose of live FIV were not protected against infection, but they did live significantly longer than infected controls.

MICE

Research with a live retrovirus vaccine is being conducted in a common laboratory strain of mice (BALB/c). Dr. Ruth Ruprecht (Dana Farber Cancer Institute, Boston) reported her team's work using a live, pathogenic retrovirus (Rauscher murine leukemia virus) as a vaccine challenge in these mice. The reverse transcriptase inhibitor AZT and alpha interferon were administered at the time of inoculation and, when examined after a 3-week course of therapy, the mice were observed to be free of viremia and disease. Re-challenge with live retrovirus (and no therapy) on day 25 resulted in resistance in more than 90% of the mice. T cells from these mice were then transferred into unexposed (naive) mice. Following retrovirus challenge in these recipient mice, no evidence was found of viremia, splenomegaly, or other signs of disease. DNA analysis (by polymerase chain reaction), however, revealed low levels of retroviral sequences. Dr. Ruprecht concluded that the data suggest that a strong T cell immunity can prevent disease and maintain latency.

SCID MICE

Dr. Donald Mosier (Medical Biology Institute, La Jolla, California) reported findings on mice with severe combined immunodeficiency disease (SCID) in which blood cells from vaccinated humans were injected. Laboratory mice with the SCID mutation can be created in inbred mouse strains; these mice have no T or B lymphocytes and therefore cannot mount an immune response. Transferral of adult human blood lymphocytes leads to a reconstituted, functional immune system.

The SCID mice were injected with T cells recognizing gp160; these cells were recovered from sera of humans receiving either a recombinantly produced gp160 vaccine (VaxSyn HIV-1) alone or VaxSyn HIV-1, but primed with a recombinant vaccinia virus/HIV gp160

vaccine (HIVAC-1e). Preliminary results have demonstrated that 5 of 6 SCID mice receiving cells from the latter group (those receiving a combination of HIV candidate vaccines) were protected against HIV infection when challenged with infectious virus. Dr. Mosier commented that these results are encouraging for the use of SCID mice as an animal model in the evaluation of candidate vaccines; answers regarding the effectiveness of various vaccines can be obtained within months, instead of the years required by primate models.

HUMAN TRIALS

The sheer size of the existing—and still-increasing—HIV-infected population and the associated lengthy incubation period mandate development of a post-exposure vaccine as well as a pre-exposure vaccine as quickly as possible. Both types of vaccines are being evaluated in clinical trials with human volunteers. While safety and characterization of vaccine-induced immune responses may be determined with relative speed, determining a vaccine's effectiveness in preventing infection or delaying progression to AIDS requires a significant period of study.

VAXSYN HIV-1

A subunit vaccine based on recombinantly produced HIV gp160 derived from baculovirus and formulated in alum (manufactured by MicroGeneSys, Inc., West Haven, Connecticut) is currently in phase I testing as a protective vaccine (administered to uninfected, low-risk individuals) and as a post-exposure vaccine for asymptomatic HIV-infected individuals. At the Conference, Dr. Gale Smith (MicroGeneSys, Inc.) reported data from an ongoing multicenter trial aimed at evaluating the safety and efficacy of various doses of VaxSyn HIV-1 in more than 200 subjects. No significant toxicities have been reported in any of the trial participants; ongoing studies seek to establish dose ranges and dose schedules. Also under way is a trial employing another candidate recombinant gp160 vaccine (HIVAC-1e, developed by Oncogen Corporation, Seattle) to "prime" the immune system before immunization with VaxSyn HIV-1.

Vaccine-induced immune responses in a subset of volunteers who received higher doses and multiple injections included production of antibodies that, when tested *in vitro*, could block fusion of HIV-infected cells with uninfected cells. Analysis of cytotoxic T lymphocyte (CTL) activity in sera from 8 vaccinees, reported by Dr. Robert Siliciano (Johns Hopkins University School of Medicine, Baltimore), revealed that 3 vaccinees had detectable gp160-specific CTL responses. CTL clones derived from sera of these trial participants recognized gp160 from divergent HIV isolates. Furthermore, most of these clones could lyse target cells displaying the HIV gp160 protein as well as HIV-infected lymphocytes bearing identical (autologous) cell markers in culture.

Dr. Robert Redfield (Walter Reed Army Institute of Research, Washington, D.C.) and colleagues reported that in 12 of 19 HIV-infected (early infection) patients participating in a separate arm of clinical testing with VaxSyn HIV-1, specific humoral and cellular responses were found to be augmented. Interestingly, vaccine-induced and natural-infection–induced responses were quantitatively and qualitatively different: higher titers of antibodies to specific regions within gp120 and gp41, which are not typically seen in HIV-infected patients, for example, were detected in vaccine recipients. Certain anti-HIV T cell responses were also observed to be somewhat different from those in HIV-infected patients. Of 11 patients who have completed the first phase of the study, 8 showed augmented anti-HIV responses. The vaccine responders had a slower rate of decline in CD4 cell count during the 8-month study period, in contrast to nonresponders and matched, untreated individuals.

HIVAC-1e

Results from a joint clinical trial with HIVAC-1e (a recombinant vaccinia/HIV gp160 virus vaccine manufactured by Oncogen Corporation, Seattle) and VaxSyn HIV-1 were reported by Dr. Lawrence Corey (University of Washington, Seattle). In this trial, 11 seronegative volunteers were first primed with HIVAC-1e and then received a booster of VaxSyn HIV-1 one year later. Dr. Corey's team found that, in comparison to 20 volunteers in a separate trial receiving VaxSyn HIV-1 alone, this combination approach led to an overall better antibody response. [*Editors' note:* Because of overall poor vaccine responses, clinical development of this vaccine was discontinued at the end of 1990. A second-generation vaccine is under development.]

HIV IMMUNOGEN

A killed (inactivated) envelope-depleted HIV virus vaccine in which the viral genetic material has been destroyed by UV irradiation and formulated in Freund's incomplete adjuvant is being tested either as a post-exposure vaccine or as immunotherapy in HIV-infected individuals. This vaccine, HIV Immunogen, is manufactured by Immune Products, Ltd., Horsham, Pennsylvania (a joint venture between Immune Response Corporation, San Diego, and Rorer-Rhone Poulec, Fort Washington, Pennsylvania). Dr. Alexandra Levine (University of Southern California School of Medicine, Los Angeles) presented data on 19 seropositive men with clinical symptoms who were inoculated with the killed-virus vaccine preparation in 1988 and on 50 infected, asymptomatic individuals who have more recently been inoculated with HIV Immunogen. No harmful side effects have been noted. Although no evidence of increased HIV-specific antibody titers have been observed in any of the trial participants, at a median of 24-30 months follow-up, no deaths have occurred. Three subjects have developed Kaposi's sarcoma, one has developed pneumonia, and one has developed retinitis. A cell-mediated immune response, measured by a skin test (for antigen reactivity), has been observed in approximately 60% of 82 subjects. Placebo-controlled trials are slated to begin this year.

HGP-30

Dr. Allan Goldstein (George Washington University Medical Center, Washington, D.C.) gave preliminary results obtained in HIV-infected individuals and seronegative individuals receiving a synthetic peptide-based p17 vaccine (HGP-30, manufactured by Viral Technologies, Inc., Washington, D.C.) in separate clinical trials being conducted in San Francisco and London, respectively. Among HIV-infected trial participants, 43% exhibited a lymphoproliferative response following vaccination, and *in vitro* tests showed a cytotoxic response to target cells coated with HGP-30 and bearing identical (autologous) cell markers. HGP-30-specific antibodies were present in 4 of 6 low-dose and 3 of 6 medium-dose vaccinees. Among the seronegative group, $CD8^+$ CTLs targeted against HGP-30 were found to persist for up to 1 year following vaccination. No vaccine-related toxicities have been noted.

OTHER CANDIDATE VACCINES

Dr. Claude Desgranges (INSERM, Lyon) and colleagues reported results from sera analyses of 30 seronegative volunteers immunized with a recombinant vaccinia/gp160 vaccine protocol, which was developed by Dr. Daniel Zagury in 1986 and consists of a priming dose of recombinant vaccinia/gp160 followed by a boosting dose of cells that are taken from the vaccinee, infected with recombinant vaccinia/gp160, and fixed with paraformaldehyde.

Although only 6 of the 30 vaccine recipients had stable titers of neutralizing antibodies, the antibodies reacted with diverse strains of HIV. Furthermore, enhancing antibodies were not detectable in these sera by assays for complement-mediated antibody-dependent enhancement of infection.

In a clinical trial using a similar schedule (but as a post-exposure vaccine) with 19 ARC/AIDS patients, Dr. Odile Picard (Universite Pierre et Marie Curie, Paris) and coworkers noted that progression to disease appears to be delayed among this group: No opportunistic infections have been observed in any patients since administration of the post-exposure vaccine. In comparison with control subjects, stabilized or increased CD4 cell counts were found and no significant side effects have been noted. (This schedule consisted of a set of peptides corresponding to *env*, *gag*, and *pol* proteins and cells that were taken from the vaccinee, infected with recombinant vaccinia virus that expressed HIV *env*, *gag*, and *pol* genes, and fixed with paraformaldehyde.)

Editors' note: In addition to the candidate vaccines discussed here, clinical testing was begun on a novel viruslike-particle–based p24 vaccine (manufactured by British Biotechnology, Ltd., Oxford) in London in 20 seronegative, low-risk volunteers in September (see Ty/HIV-p24 hybrid VLP in "Viruslike Particles," above). U.S. clinical trials for a revamped version of a recombinantly produced gp120 subunit vaccine (manufactured by Biocine, a joint venture between Ciba-Geigy and Chiron Corporation) are scheduled to begin in late 1990.

CHAPTER 6

Page 139 **BACKGROUND**
140 Elements of Drug Development
146 Combination Therapy
147 **ANTIVIRAL AGENTS**
147 Agents that Block Virus-Cell Interaction
151 Agents that Target Replication
154 Agents that Target Viral RNA
158 Protease Inhibitors
159 Blockers of Viral Assembly and Budding
159 **IMMUNOTHERAPEUTIC AGENTS**
159 Passive Immunotherapy
160 Cell Therapy
160 Active Immunotherapy
167 **COMBINATION THERAPY**
167 Antiviral Agents
167 Antiviral and Immunotherapeutic Agents

ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS

BACKGROUND

The research and development of drugs or other agents with the potential to treat HIV infection has two major focuses: interrupting the viral life cycle to suppress viral replication, and preventing or minimizing the damage HIV causes. Perhaps the most intense effort in drug development today is in the designing of compounds that target HIV enzymes, which act at specific stages of the viral life cycle. By drawing on knowledge of the precise molecular structure and biological activity of HIV's enzymes, new compounds can be designed to paralyze these enzymes—and thereby halt new virus production. (So far, protease is the most fully characterized HIV enzyme, its three-dimensional structure was revealed in 1989.) On another front, in the wake of recent technological advances in molecular biology, nucleic acid molecules are being designed that will hone in on specific HIV gene sequences, bind to these sequences, and thereby deactivate the virus. A subset of these molecules may prove capable of snipping apart specific HIV gene sequences (see "Antisense Molecules and "Ribozymes").

Armed with a growing knowledge of HIV's molecular structure and the peculiarities of its life cycle, scientists are attempting to develop highly specific anti-HIV drugs that will have little or no adverse effects on normal cellular functions. New information on the virology and molecular biology of HIV continues to fuel the race to design such agents. And while the primary target of drug development is the underlying cause of disease—the virus itself—efforts are also being directed toward restoring the patient's immune system. Table 6.1 provides a comprehensive catalog of the antiviral and immunotherapeutic agents presented at the VI International Conference on AIDS.

ELEMENTS OF DRUG DEVELOPMENT

Mechanism of Action. A primary consideration in the development of any drug is the way in which it achieves its effects—its mechanism of action. Many scientists believe that the mechanism of action most likely to succeed in combating HIV is disruption of the viral life cycle. (See Chapter 1: VIROLOGY for a detailed discussion of the HIV life cycle.) The complex processes that HIV employs to self-replicate and produce new virus offer several attractive opportunities for therapeutic intervention. Disruption of the HIV life cycle at any phase (reverse transcription or integration, for example) would necessarily halt the production of new functional, infectious virus particles. Figure 6.1 illustrates the sites of action in the HIV life cycle of various classes of antiviral agents.

Complementing this direct approach is a second mechanism of action, one that seeks to boost the compromised immune system. These agents—immunomodulators or biologic response modifiers—may augment the effects of antiviral drugs and may also help stave off opportunistic infections.

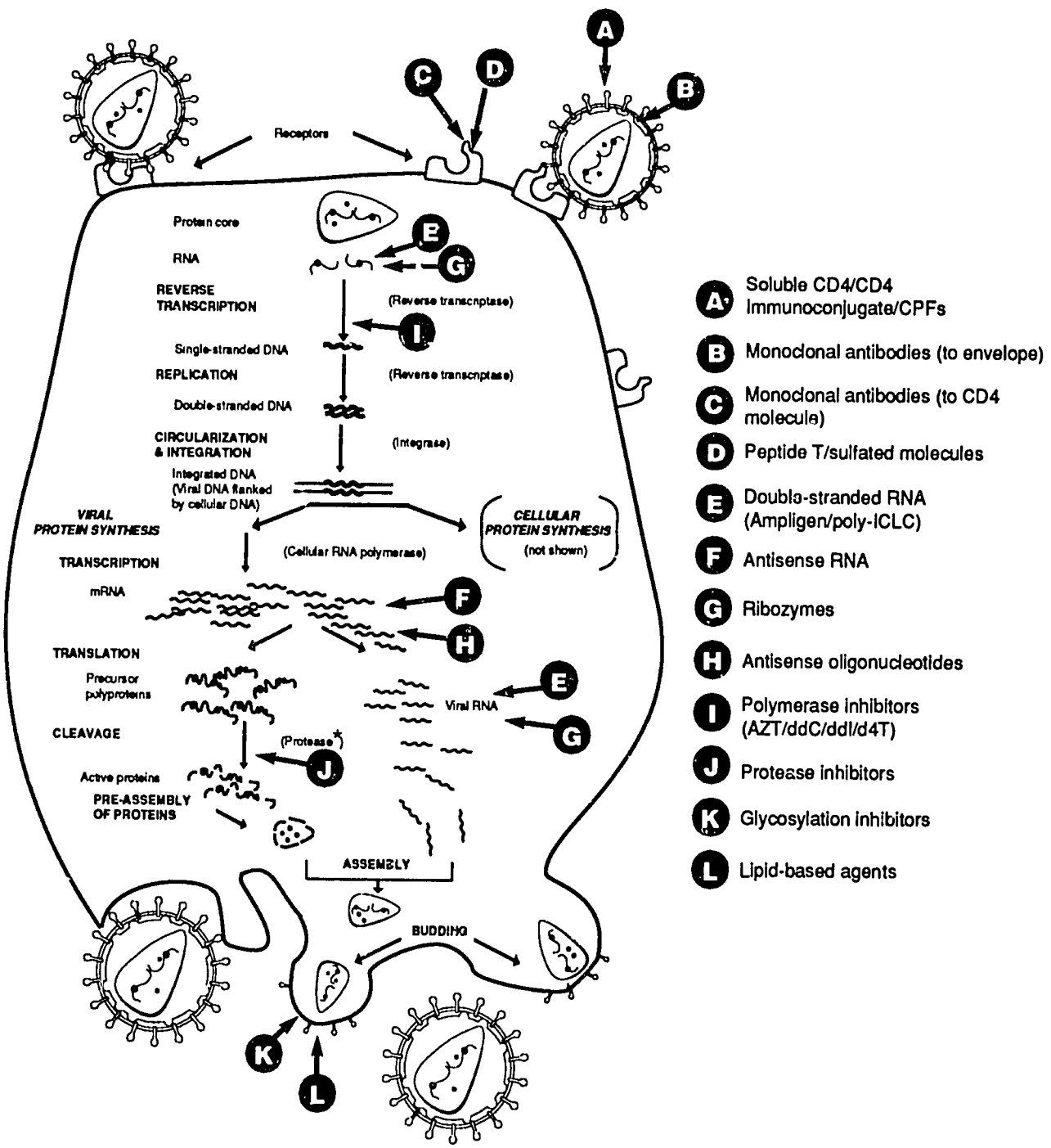
The physiologic properties of drugs are another important consideration in their design and development. Certain agents are thought to have at least theoretical advantages over others because of specific properties, such as lipid versus water solubility. Lipid solubility enhances the ability of a drug to cross cell membranes and, in the context of HIV infection, may allow an antiviral drug to enter the central nervous system in sufficient concentrations to combat HIV in the brain. Similarly, an agent's ability to cross the placenta may be of potential benefit (or concern) in HIV-infected pregnant women. Many drugs are being chemically modified in an effort to confer (or limit) these and other properties.

***In Vitro* Evaluation.** Before candidate anti-HIV drugs can be tested in animals or humans, their efficacy must be demonstrated in the laboratory. Researchers can measure the levels of viral enzymes like reverse transcriptase and viral antigens like p24 in cell cultures, which give at least a relative indication of whether or not a candidate drug is inhibiting viral replication. The pathologic effects of HIV on infected cells—cell death or abnormal giant cell (syncytia) formation—can also be monitored quantitatively. Reliable, quantitative DNA and RNA assays will likely be available within the next 3 years to measure specific HIV DNA or RNA sequences. (These tests, which are based on polymerase chain reaction, or PCR, technology are apparently still in the fine-tuning stage.)

New approaches for the *in vitro* testing of HIV drugs were presented at the Conference. Dr. David Volsky (St. Luke's/Roosevelt Hospital Center and Columbia University College of Physicians & Surgeons, New York) presented his team's data on an *in vitro* drug screening protocol that simultaneously evaluates the effects of potential antiviral drugs at different phases of the HIV life cycle by measuring various viral characteristics and cytopathic (cell-damaging) effects of the virus. Levels of HIV RNA and p24 antigen were measured, and the percentage of p24-positive cells and cell viability in cultures of both T cells and monocytes were determined. Immunomodulators and non-antiviral drugs—anti-infectives, for example—can also be tested in this way. Dr. Bruce Polsky (Memorial Sloan-Kettering Cancer Center, New York) and colleagues described a new anti-HIV drug screening system based on "reporter" gene activation. Reporter genes are genes whose activity (or "signal") can be readily detected. These genes can be inserted into a genome in proximity to the gene(s) of interest, in essence serving as markers. A reporter gene (lacZ) under the control of the HIV LTR (long terminal repeat, an HIV regulatory gene) was inserted into T cells, which were then exposed to HIV and, subsequently, to a specific drug. As expected, infection with concentrated HIV resulted in increased lacZ signal, and dilutions of HIV produced a stepped reduction in signal. The addition of different concentrations of the drug AZT (zidovudine) to the cell cultures resulted in dose-related reductions in the reporter gene's signal. The authors offered that their drug screening system allows for rapid and quantitative measurement of anti-HIV activity of potential therapeutic agents.

Figure 6.1. Target Sites of Anti-HIV Therapies

Several classes of drugs whose mechanisms of action directly interfere with gp120-CD4 binding or specific aspects of the HIV life cycle are being developed as potential AIDS therapies. Soluble CD4 molecules or CD4-immunoconjugates, CPFs, and monoclonal antibodies, for example, may prohibit HIV from attaching to (and subsequently infecting) susceptible CD4-bearing cells by binding to the CD4 binding region of HIV gp120. * Protease activity is shown here for illustrative purposes only. (See Table 6.1 for the mechanism of action of these and other candidate anti-HIV therapies currently in development.)



Because HIV appears to behave differently in different cell types, it seems probable that a given drug's mode of action, biochemical properties, and antiviral potency will also vary in different cell types. Many scientists have remarked that *in vitro* drug screening must include the use of different cell lines that can be infected with HIV—T lymphocytes, B lymphocytes, and monocytes/macrophages, for example.

Evaluation Using Animal Models. In an effort to bring the most effective anti-HIV agents to clinical trials quickly, animal model systems are being used to test the relative efficacies of various agents. Some investigators are using animal models in which HIV-related viruses cause an AIDS-like disease—for example, simian immunodeficiency virus in monkeys or feline immunodeficiency virus in cats—to predict the potential efficacy of specific agents in humans. A mouse model system that is susceptible to HIV infection is also being used to evaluate anti-HIV drugs. Dr. Joseph McCune (SyStemix, Inc., Palo Alto, California) discussed his team's recent success in demonstrating AZT and ddI (dideoxyinosine) efficacy against HIV infection in SCID-hu mice. (SCID-hu mice are a recently developed laboratory strain that do not possess an immune system but have received implanted human blood and lymphatic organs; see "Animal Models" in Chapter 1: VIROLOGY for detailed discussion.) The efficacy of both agents was demonstrated by quantitative analysis of proviral DNA using PCR—a technique that measures DNA levels in specimen samples (such as plasma) as an estimate of "virus load." Dr. McCune presented data showing that HIV-infected SCID-hu mice treated with AZT or ddI (at doses comparable to those presently used in humans) for 2 weeks had no detectable HIV DNA, in contrast to untreated animals. However, in SCID-hu mice treated for 2 weeks and then taken off treatment for 4 weeks, HIV was readily detected by PCR, thus demonstrating that AZT or ddI treatment could suppress HIV, but could not totally eliminate the virus.

Dr. Jui-Lien Li (Vical, Inc., San Diego, California) and colleagues compared the efficacy of standard AZT and liposome-encapsulated AZT in mice infected with a murine (mouse) retrovirus that causes immunodeficiency. Liposomes are membrane-like hollow spheres that are easily constructed in the laboratory, and offer several advantages as drug transporters. These spheres protect their contents—in this case, an AZT derivative—from degradation and loss of bioactivity. And because of their lipid composition, they readily adsorb onto cell membranes and release their drug directly into the cell's interior, acting as a highly efficient drug delivery system. Dr. Li and coworkers found that mice treated with liposome-encapsulated AZT had significantly higher plasma and tissue drug levels than those treated with non-encapsulated AZT. Furthermore, tissue levels of the liposome-encapsulated AZT derivative were retained for 24 to 48 hours instead of 4 to 6 hours.

***In Vivo* Evaluation.** Once an agent has shown biological activity *in vitro* and is demonstrated to be safe and effective in animals, and prior to comparative studies of drug efficacy in patients, its pharmacokinetics are studied in healthy human subjects. Pharmacokinetics is the study of drug action in the body: its absorption, bioavailability, distribution, localization, duration of effect (or half-life), and clearance (elimination from the body). The bioavailability of a drug—the degree to which a drug is available to the target tissue—is an important criterion in clinical trials. Bioavailability, and therefore drug effectiveness, may vary according to route of administration: for example, oral administration or injection by the intravenous, intramuscular, intraperitoneal, or subcutaneous routes. The minimum effective dose and maximum tolerated dose are determined in individuals belonging to the target population (for example, HIV-infected individuals) in Phase I safety trials.

In the United States, rigorous guidelines for the testing of all drugs are set by the Food & Drug Administration (FDA), which has recently made amendments to shortcut the ordinarily lengthy drug approval process for some agents used in HIV infection (see "Drug Approval Process"). Canada and other countries are also implementing policies that will speed the drug regulatory process.

The Drug Approval Process in the United States

The U.S. system of new drug approvals, regulated by the Food & Drug Administration, is perhaps the most rigorous in the world. The process by which a drug is tested and approved is outlined below.

Preclinical Testing
The promising agent is first subjected to extensive laboratory and animal testing to determine answers to two key questions: Is the compound biologically active? Is it safe? If the answers to both appear to be affirmative, the drug sponsor is ready to test in humans. This preclinical stage generally lasts from 1 to 2 years.

Investigational New Drug (IND) Status
Before human tests can start, the drug sponsor must file an IND application with the Food and Drug Administration (FDA) showing the results of all animal testing and how the drug is made. IND status becomes effective if the FDA does not disapprove the application in 30 days.

Human Testing (Clinical Trials)
Clinical drug trials consist of three phases, each involving larger numbers of people than the one before.

Phase I: Safety Studies and Pharmacological Profiling.
This phase determines the drug's pharmacological actions, its safe dosage range, how it is absorbed, distributed, metabolized, and excreted, in addition to its duration of action. These tests involve a small number of healthy subjects (not patients). Phase I clinical testing can usually be completed in less than 1 year.

Phase II: Pilot Efficacy Studies.
This phase consists of controlled studies involving approximately 200 to 300 volunteer patients to assess the drug's effectiveness. Simultaneous animal and human studies continue to determine the drug's safety. Phase II trials may require about 2 years to complete.

Phase III: Extensive Clinical Trials.
The testing now moves to larger numbers of volunteer patients, usually 1,000 to 3,000, in clinics and hospitals. These trials must confirm earlier efficacy studies and identify even low-incidence adverse reactions. Phase III clinical trials last about 3 years.

New Drug Application (NDA)
Following Phase III completion, the drug sponsor must file with the FDA an NDA, which contains all the information the sponsor has gathered. NDAs typically consist of thousands of pages. The submission must include the drug's chemical structure, the scientific rationale and purpose, animal and laboratory studies, results of all tests in humans, formulation and production details, and proposed labeling. On average, the FDA review and approval process of an NDA takes 2 to 3 years.

Approval
Once an NDA is approved, the company is required to periodically submit reports to the FDA, including adverse reaction data and production, quality control, and distribution records. For some drugs, the FDA requires affirmative post-marketing monitoring, or additional studies to evaluate the long-term effects.

Note: Early in 1989, the FDA implemented an Expedited Drug Approval plan to speed the approval of drugs for life-threatening and severely debilitating diseases. Under the plan, the FDA will work more closely with pharmaceutical companies during development and in evaluating clinical trials. The goal is to reduce the 7–10-year period it now takes to move a drug from the laboratory to the pharmacy. If a drug shows sufficient promise after Phase I, then Phases II and III could be combined to save 2 to 3 years from the 5–6-year clinical trial process. President Bush's cancer panel has undertaken a review of the FDA approval process for cancer and AIDS drugs. A final report was submitted to President Bush in August 1990.

Adapted from materials provided by the Pharmaceutical Manufacturers Association

Table 6.1. Catalog of Agents Presented at the Conference

AGENT	MECHANISM OF ACTION
Blockers of HIV-Cell Interaction	
CD4 Immunoadhesin	Binds to HIV; inhibits HIV/CD4 binding
CD4 Immunotoxin (Immunoconjugate)	Binds to HIV; inhibits HIV/CD4 binding
CPFs	Disrupts HIV envelope; inhibits binding
Monoclonal antibodies	Bind to CD4 receptor or to HIV envelope
Peptide T	Binds to CD4 receptor
Soluble CD4	Binds to HIV; inhibits HIV/CD4 binding
Soluble melanins	Block gp120/CD4 binding
Sulfated molecules	Interfere with HIV gp120/CD4 binding
RNA-Directed Agents	
Ampligen (mismatched dsRNA)	Activates RNA degradation pathway
Antisense RNA	Binds to HIV RNA; inhibits HIV replication
Antisense oligonucleotides	Binds to mRNA; inhibits HIV protein synthesis
GLQ223 (trichosanthin)	Inactivates ribosome function; inhibits HIV replication
Poly-ICLC	Activates RNA degradation pathway
Ribozymes	Cleave HIV RNA; disallow HIV replication
Polymerase Inhibitors	
Acriflavine derivatives	Inhibit reverse transcriptase
Acyclouridine (HEPT) derivatives	Inhibits HIV-1 reverse transcriptase
Acyclovir	Inhibits DNA synthesis
AZddU (azidouridine)	Inhibits reverse transcriptase
AZT (azidothymidine), or Zidovudine (ZDV)	Inhibits reverse transcriptase
BHC-89	Inhibits reverse transcriptase
Carbovir (carboxyclic didexoyguanosine)	Inhibits DNA synthesis
d4T(dideoxydidehydrothymidine)	Inhibits reverse transcriptase
ddA (dideoxyadenosine)	Inhibits reverse transcriptase
ddC (dideoxycytidine)	Inhibits reverse transcriptase
ddI (dideoxyinosine)	Inhibits reverse transcriptase
Foscarnet (phosphonoformic acid, or PFA)	Inhibits DNA synthesis
Puromycin aminonucleosides	Inhibits reverse transcriptase
PMEA (phosphonylmethoxy-ethyl-adenine)	Inhibits DNA synthesis
Ribavirin	Inhibits DNA synthesis
Vidarabine	Inhibits DNA synthesis
Protease Inhibitors	
Pepstatin	Inhibits protease activity
Synthetic peptide anti-HIV protease inhibitors	Inhibit HIV protease activity
Synthetic peptide derivatives	Inhibit HIV-1 and HIV-2 protease activity
Blockers of Viral Assembly	
DNM (deoxynojirimycin)	Inhibits glycosylation
Lipid analogues	Inhibits HIV envelope biogenesis

AGENT	MECHANISM OF ACTION
Natural Immunomodulators	
Colony stimulating factors (G-CSF; GM-CSF; M-CSF)	Stimulates maturation of progenitor (stem) blood cells
Erythropoeitin (EPO)	Stimulates maturation of red blood cells
Imreg-1	Stimulates IL-2 and gamma-IFN production
Interferons (alpha-IFN; beta-IFN; gamma-IFN)	Interferes with viral replication (via 2', 5'-A synthetase pathway)
Interleukin-2 (IL-2)	Activates T cells (formerly known as T-cell growth factor)
Lentinan	Enhances monocyte/macrophage functions; mechanism unknown
Thymopentin	Induces differentiation of precursor thymocytes (pre-T cells)
Tumor necrosis factor (TNF)	Possesses antiviral activity; mechanism unknown
Synthetic Immunomodulators	
AS-101 (ammonium trichlorotellurate)	Stimulates IL-2; IL-3; TNF; IFN; GM-CSF
DTC (diethyldithiocarbamate, or Imuthiol)	Possibly inhibits HIV tat gene product; possibly interferes with prostaglandin release
Miscellaneous	
1-aurothioglucose (AuT)	Inhibits HIV reverse transcriptase activity
Amphotericin B	Inhibits HIV production in macrophages; mechanism unknown
Anginlyc	Possesses immune enhancing properties
DHEA (dehydroepiandrosterone, EL-10)	Enhances immune cell function; mechanism unknown
Fluoroquinolones (oflaxacin and related fluoroquinolones)	Suppress HIV replication in infected cells; suppresses HIV cytotoxicity; mechanism unknown
Glycyrrhizin	Inhibits HIV replication; also stimulates IFN
Hypericin	Inhibits HIV production; mechanism unknown
Menaquinone-7	Inhibits HIV-induced syncytia formation
Naphthalenedisulfonic acid derivatives	Inhibit reverse transcriptase
Nonoxynol-9 (N-9)	Inactivates HIV *in vitro;* mechanism unknown
Oxophenarsine	Inhibits HIV infection of susceptible cells and suppresses HIV production from chronically infected cells *in vitro*
Ozone	Anti-infective with immunomodulatory effects; mechanism unknown
Papaverine hydrochloride	Inhibits HIV replication
Phthalocyanines	Inhibit HIV-1 reverse transcriptase; suppress HIV cytotoxicity
Porphyrins	Suppress HIV cytotoxicity
Quinolizine	Suppresses HIV reverse transcriptase activity
Somatostatin	Inhibits HIV replication in monocytes/macrophages *in vitro*
TIBO derivatives	Inhibit HIV-1 reverse transcriptase
Vitamin C	Inhibits HIV replication; mechanism unknown

At present, no definitive test or assay is available that tells us whether or not a particular anti-HIV drug is working in human beings. The technology for detecting HIV RNA and DNA has been improved to the extent that clinical investigators should soon be able to measure viral load—that is, concentration of virus—from patient specimen samples at different times during therapy and use these measurements as direct evidence for the efficacy of anti-HIV drugs. Until these tests are perfected, clinical researchers rely on indirect indicators of viral activity ("surrogate" markers)—usually CD4 cell counts and percentage of CD4 cells relative to total lymphocyte count. (CD4 cells, formerly called T4 cells, are the helper/inducer T lymphocytes infected and destroyed by HIV.) Levels of the HIV antigen p24 are often used as an indicator of actively replicating virus. Although no single surrogate marker has been validated as correlating with a patient's clinical disease stage, most clinical trials rely on one or more of these markers. Increases in CD4 cell counts and disappearance or suppression of p24 levels during therapy are evidence of potential clinical benefits. Transient versus sustained changes over time are also distinguished and used as an evaluation criterion.

Additional Considerations. Once the relative safety, efficacy, and dosage have been established for a given agent and the agent enters clinical use, longer term considerations must be addressed. In the treatment of HIV infection, untoward (side) effects of continuous long-term therapy are now being reported. And as Dr. Brendan Larder (Wellcome Research Laboratories, U.K.) and others pointed out, the emergence of drug resistance—such as the AZT-resistant HIV strains isolated from a subset of patients receiving long-term AZT therapy—has recently become a real concern for all drug developers. (See "AZT," below for more details on resistant HIV strains.)

A unique consideration for the planning of drug development has surfaced with respect to HIV infection. The natural course of HIV infection is such that the choice of a therapeutic regimen may vary according to the stage of infection—for asymptomatic patients, for patients with subtle but verifiable immune abnormalities but lacking clinical signs, for patients with early symptoms, and for patients diagnosed with ARC or AIDS. Until 1989, most clinical trials have been conducted in patients with full-blown AIDS. Now the focus of treatment is enlarging to encompass patients with early ARC and even asymptomatic infected persons with slight immune abnormalities.

The pharmacokinetics of various candidate antiviral and immunotherapeutic agents must also be studied in children and pregnant women, since it cannot be assumed that these measurements are the same as in nonpregnant adults. The toxicity of antiviral agents may also differ in pregnant women and children. (See Chapter 7: CLINICAL MANAGEMENT for details on treatment for HIV infection in children.) At present, evaluation of antiviral therapy (primarily with AZT) in pregnant women has been mostly limited to studies in which the drug was administered beginning in the third trimester of pregnancy.

COMBINATION THERAPY

The growing number of clinical trials employing more than one therapeutic agent reflects perhaps the frustrating lack of success that single-agent therapy has had in controlling HIV infection. Multiagent therapy offers several potential advantages:

- increases ability to gain access to HIV in different cell types
- confers ability to target different steps in the viral life cycle—combining drugs proven not to act antagonistically *in vitro* offers a greater potential for inhibiting HIV growth for a longer period of time
- generally allows lower single doses of each drug to be used, decreasing the chances of toxic effects
- reduces the risk of the evolution of drug-resistant HIV strains

Dr. Erik De Clercq (Rega Institute for Medical Research, Leuven, Belgium) pointed out that it may be particularly advantageous to combine a compound that interferes with an "early" event in the HIV life cycle (virus attachment to a host cell or reverse transcription, for example) with a compound that interferes with a "late" event (such as viral protein processing or budding and release). Such combinations should inhibit both the acute and chronic phases of infection. Infection of new cells by HIV can be blocked and, at the same time, cells already infected can be prevented from releasing viral proteins and progeny virions.

Coupling anti-HIV compounds with immunomodulators is also an attractive approach. Immunorestoration in HIV-infected patients is essential since HIV so thoroughly and systematically cripples the body's immune system. Table 6.2 catalogs the various combinations of anti-HIV drugs, and Table 6.3 outlines the combinations of antivirals and immunomodulators that were discussed at the Conference.

ANTIVIRAL AGENTS

Numerous antiviral agents are currently in various phases of pre-clinical or clinical testing. In the United States, the Pharmaceutical Manufacturers Association reports that 21 such compounds are being evaluated clinically. Worldwide, the number of agents being screened in the laboratory and tested in humans for anti-HIV activity is manyfold higher. Furthermore, with the stage set for the development of HIV designer drugs, more candidate drugs will likely enter the drug approval process in the next few years. (AZT—azidothymidine, or zidovudine—is the only AIDS drug approved by the U.S. Food & Drug Administration; it is manufactured and distributed as Retrovir by Burroughs Wellcome Company.)

[*A note about organization:* This section focuses on the mode of action and current knowledge of the biological activity of candidate antivirals for HIV infection. Clinical trial data is discussed in Chapter 7: CLINICAL MANAGEMENT. Many agents discussed in this chapter have multiple modes of action. For purposes of centralization, they have been arranged according to their primary application.]

AGENTS THAT BLOCK VIRUS-CELL INTERACTION

Electron microscopy, crystallography, molecular biology, and immunological studies have revealed several elements critical to HIV binding to susceptible host cells. Equipped with knowledge of the shape and amino acid makeup of both the viral envelope proteins (specifically, gp120 and gp41) and the cell surface receptor (the CD4 molecule) on susceptible cells, researchers have been exploring ways to block HIV infection of these cells by interfering with the envelope-receptor interaction. (Binding to a cell surface receptor is a prerequisite for HIV entry into a host cell; see Chapter 1: VIROLOGY and Chapter 2: PATHOGENESIS & IMMUNOLOGY.)

Among the several drug development strategies being pursued to block HIV binding to CD4-bearing cells is the design of agents that will directly interfere with "HIV receptors"—binding sites on the susceptible cell's surface (see "Soluble CD4," below). One novel strategy described at the Conference used a monoclonal antibody directed against the CD4 receptor, conjugated to pokeweed antiviral protein. (Monoclonal antibodies are derived from a specially sensitized antibody-producing B lymphocyte to recognize a specific antigen—in this case, the CD4 receptor. Pokeweed antiviral protein, extracted from pokeweed plants, inhibits the replication of certain plant and animal viruses.) Dr. Joyce Zarling (Oncogen Corporation, Seattle) and colleagues found that pokeweed antiviral protein, when conjugated to a monoclonal antibody that binds to the CD4 receptor, could inhibit HIV replication in CD4-positive cells *in vitro* at much lower concentrations than unconjugated pokeweed antiviral protein.

Another approach seeks to block HIV envelope glycoproteins (the "knobby" extensions on the virion) so that they will be unable to dock with the cell-surface receptor. Monoclonal antibodies can be designed to attach themselves to these envelope glycoproteins. Such monoclonal antibodies may be developed as potential therapeutic agents or as vaccines. Dr. John-Erik Hansen (Hvidovre Hospital, Denmark) and colleagues reported finding four envelope glycoprotein-specific monoclonal antibodies that could block infection of two strains of HIV in cultures of infected T cells. (Chapter 5. VACCINE DEVELOPMENT discusses monoclonal antibody-based vaccine strategies.)

Soluble CD4. A soluble, or fragmented, form of the CD4 receptor that serves as an entry portal for HIV can be produced by recombinant DNA technology. Theoretically molecules that mimic the CD4 receptor should be able to bind cell-free HIV (that is, circulating HIV) and thereby reduce the pool of virions available to infect susceptible cells. If enough soluble CD4 (sCD4) were available to bind a sufficient quantity of cell-free HIV, then the immune system might be able to defend itself against HIV and its pathological effects. Presumably a lower concentration of HIV virions means fewer infected CD4 cells (key regulators of the immune response), which should in turn equate with a more responsive immune system. Furthermore, it is likely that binding of HIV with sCD4 will permit the cellular CD4 receptor on T lymphocytes to carry out its normal immune function.

Early *in vitro* studies have shown that sCD4 not only inhibits infection of CD4 cells, but also inhibits cell fusion—the formation of syncytia, or giant multinucleated cell masses—that is caused by HIV infection. Syncytia formation, a covert means by which uninfected CD4-bearing cells become infected by HIV through cell-cell contact, is thought to be a major mechanism of cell infection and cell death. The finding of reduced syncytia formation suggests that sCD4, therefore, may also prevent the *spread* of infection.

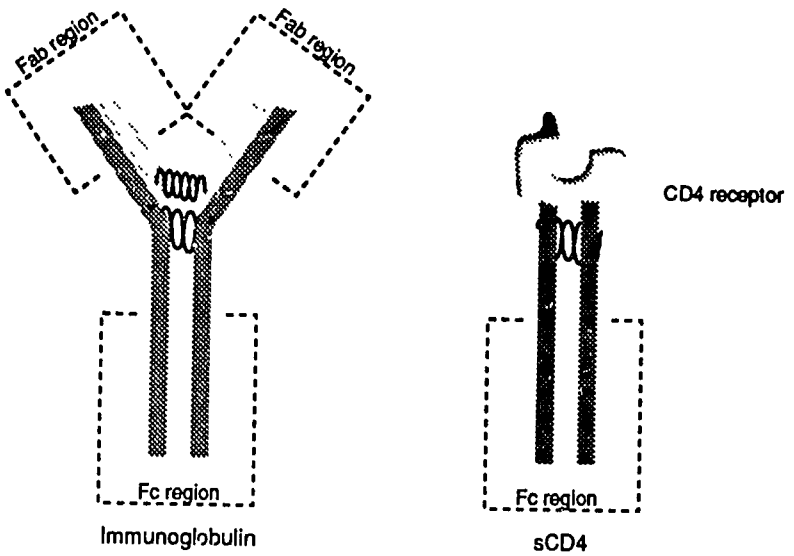
Clinical studies with sCD4 (administered by intravenous injection) have revealed that although the artificial receptor can bind HIV (complexes of sCD4 and HIV gp120 can be recovered from patients receiving therapy), the half-life of sCD4 in the blood is extremely short. In 1989, several clinical investigators reported that the biological activity of sCD4 at non-toxic doses was virtually nil. As reported by Dr. Kei-Lai Fong and coworkers (SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania), the drug appears to have a longer half-life when administered by subcutaneous injection, but its bioavailability is still very low. These findings have led researchers to design modified versions of sCD4.

A caveat should be noted with respect to sCD4 therapy: at least some HIV-infected patients appear to develop antibodies directed against the cellular CD4 receptor. (These "auto antibodies are produced by the body against its own, or "self," proteins or tissues that are mistakenly perceived as "non-self" or foreign.) It is not known whether sera from all HIV-positive individuals contain CD4 autoantibodies or whether the antibodies are transient. The clinical significance of CD4 autoantibodies remains unclear, and it is not known whether these autoantibodies can interfere with the activity of sCD4 or normal CD4 receptor function.

Some observers continue to question the theoretical basis of sCD4 therapy on other grounds. Larry Callahan (Food & Drug Administration, Bethesda, Maryland) and coworkers, for example, pointed out that many HIV-infected individuals have high concentrations of antibody to gp120, which when examined *in vitro* are found to inhibit binding of HIV to CD4 (essentially mimicking the effect of sCD4 therapy). These antibodies are unable to prevent onset of HIV-related disease in individuals possessing such antibodies, which suggests that administration of sCD4 will also be ineffectual as a therapeutic strategy.

Second Generation sCD4. Several independent groups are examining shorter, lower molecular weight sCD4 molecules, which may be more stable (that is, have a longer half-life) than the first generation sCD4. Dr. Paolo Marino Cereda (University of Pavia, Italy) and coworkers suggested that sCD4 molecules might require a critical level of structural complexity to

Figure 6.2. Schematic Representation of a CD4 Immunoconjugate



The antigen-binding portion (the Fab region) of an immunoglobulin molecule is replaced with the HIV gp120-binding portion of the soluble CD4 (sCD4) molecule. The resulting CD4 immunoconjugate (called CD4 immunoadhesin by researchers at Genentech, Inc., San Francisco), whose plasma half-life is increased over that of sCD4, is being clinically evaluated as a second-generation sCD4 molecule. Other types of immunoconjugates include bacterial toxins coupled with antibodies to HIV envelope proteins.

inhibit viral replication and syncytia formation: a 21-amino acid peptide was found capable of inhibiting syncytia formation, whereas a smaller (19-amino acid) CD4 peptide lacked this capability. (Peptides, or protein fragments, are composed of strings of amino acids.) The authors are seeking to further increase the therapeutic efficacy of these small CD4 peptides by chemical modification. (See also "Synthetic Peptides," below, for a description of low molecular weight molecules that contain phenylalanine, which has been implicated as the single-most important amino acid in the CD4 receptor molecule for HIV gp120 binding.)

Liposome conjugation offers another variation on the CD4 approach. Previous studies of liposomes, which readily attach to cell membranes, have shown that those of a certain composition are capable of cell killing. Dr. Kazuo Kumagai (Sumitomo Chemical Co. Ltd., Hyogo, Japan) and colleagues reported their *in vitro* results with an sCD4 molecule conjugated to a liposome, which was shown to possess inhibitory activity against HIV-infected T cells. Both nonconjugated liposomes and liposomes conjugated with sCD4 killed persistently infected cells, but the sCD4/liposome exhibited significantly greater cell killing activity.

Much attention has been focused on second generation sCD4 agents that combine sCD4 with bacterial toxins or fragments of immunoglobulin (antibody) molecules. Such hybrid molecules are touted for their increased half-life, which theoretically should increase the drug's therapeutic potential.

CD4 Immunoadhesin. Investigators at Genentech, Inc. (San Francisco) described the molecular fusion of a portion of the CD4 receptor with a portion of the human IgG immunoglobulin molecule, the Fc, or constant, region. (See Figure 6.2.) Dr. Daniel Capon (Genentech, Inc.) reported that, as expected, fusing the Fc region of the IgG molecule with sCD4 results in the transferral of specific properties of the Fc region to the hybrid molecule, which the researchers have called CD4 immunoadhesin. In addition to binding HIV gp120, CD4 immunoadhesins can bind to a complement protein (C1q) and to Fc receptors, which are found on certain immune system cells. Both of these binding capabilities are "borrowed" by the CD4 immunoadhesin from the Fc region of the IgG molecule. (Complement proteins, of which C1q is only one, are so-called because they complement antibody-medi-

ated immune responses; they function in numerous host defense mechanisms—notably, virus neutralization and cell lysis, or killing.) The most important borrowed property of the Fc region of the IgG molecule, however, is the lengthy serum half-life. CD4 immunoadhesin has a much longer half-life than that of sCD4: 30 hours compared to about 1 hour. From studies in rhesus monkeys, CD4 immunoadhesin was shown to be capable of crossing the placenta in a manner similar to that seen with normal IgG.

sCD4-toxin Hybrids. Most HIV-infected cells display gp120 (the viral envelope glycoprotein) on their surface, which has inspired yet another approach. Several research groups are working on a hybrid molecule that is made up of sCD4 (the portion that binds to gp120) and a bacterial toxin that can kill an infected cell. Previous studies have shown that the exotoxin secreted by the bacterium *Pseudomonas aeruginosa*, when conjugated to sCD4, can selectively kill HIV-infected T cells in culture and inhibit the spread of HIV from chronically infected CD4 cells to uninfected cells. Dr. Hiroshi Tsubota (New England Regional Primate Research Center, Southborough, Massachusetts), however, reported that such inhibition appears to be incomplete since viral replication can be detected following removal of CD4-exotoxin from these cultures.

Dr. Edward Berger and colleagues (National Institute of Allergy and Infectious Diseases, Bethesda, Maryland) reported preliminary observations of synergistic activity between this CD4-exotoxin hybrid and AZT or ddI. Uninfected T cells were added to cultures of chronically HIV-infected T cells. By adding CD4-exotoxin and AZT to the cultures, spread of HIV to the uninfected cells was blocked and p24 production suppressed. Similar results were observed with CD4-exotoxin and ddI.

Synthetic Peptides. Synthetic protein fragments, or peptides, that may be able to interfere with the CD4-HIV binding interaction are also being explored.

Peptide T. An octapeptide, Peptide T is made up of eight amino acids that correspond to a stretch of amino acids found in HIV gp120. Dr. Candace Pert and researchers at the National Institute of Mental Health (Bethesda, Maryland), who developed Peptide T in 1987, suggested that the peptide may bind to CD4 much as HIV gp120 does, occupying receptor sites that might otherwise serve as binding sites for gp120. Because Peptide T is itself a fragment of a natural peptide found in the brain (vasoactive intestinal peptide), laboratory and clinical studies are investigating the anti-HIV properties of Peptide T in the context of the central nervous system. *In vitro* studies are examining whether Peptide T can block HIV-induced neuronal damage (specifically, gp120 neuronal cell killing), and Phase I clinical trials are investigating possible neurologic benefits of Peptide T therapy (either intravenous or intranasal administration) for patients with diagnosed neurologic deficits.

CPFs. A recently published study of short (di- or tri-) peptides containing phenylalanine showed them capable of blocking gp120 binding to the CD4 receptor. (These authors previously found phenylalanine to be the amino acid critical to gp120 binding.) The di- and tri-peptides, called CPFs (N-carbomethoxycarbonyl-prolyl-phenylalanyl benzyl ester), could inhibit HIV infection of tumor cells and human T cells. Dr. Robert Finberg (Dana-Farber Cancer Institute, Boston) and colleagues reported that consistent inhibition of CD4-gp120 binding occurred when gp120 was preincubated with CPFs. Soluble (free-floating) gp120—fragmented from the virus—has been reported to be cytotoxic, and the authors suggested that, in addition to potential antiviral effects, CPFs may reverse both immunosuppression and toxicity caused by soluble gp120. Another practical benefit is the method of administration: molecules of small molecular weight, like CPFs, can usually be administered as an oral drug.

Sulfated Molecules. Molecules that contain sulfur—including polysaccharides (sugars) and polymers (synthetic high molecular weight compounds)—are also being studied for their ability to interfere with CD4-HIV gp 120 binding.

Polysaccharides. Drug screening studies carried out in 1987 and 1988 identified the sulfated polysaccharide dextran sulfate as a potent *in vitro* inhibitor of HIV replication. However, clinical studies revealed that the drug was poorly absorbed when administered orally. Subsequent studies showed that the anti-HIV properties of sulfated polysaccharides, which include inhibition of giant cell formation and of HIV antigen production in T cells, were directly related to the drugs' ability to interfere with HIV adsorption onto the cell surface. Dr. Loretta Low (Westmead Hospital, Sydney, Australia) and others have found that sulfated polysaccharides do not bind to HIV gp120 as was once suspected. Instead, Dr. Low's team found that two sulfated sugars, dextran sulfate and pentosan sulfate, apparently have different mechanisms of action: dextran sulfate disrupts the CD4-gp120 interaction by binding to the CD4 receptor, whereas pentosan sulfate apparently inhibits HIV infection via a CD4-independent mechanism.

Polymers. Studies of sulfated polymers, such as sulfated polyvinyl alcohol and acrylic acid with sulfated vinyl alcohol, were described by Dr. Erik De Clercq (Rega Institute for Medical Research, Leuven, Belgium) and coworkers. *In vitro* experiments showed that these polymers could block HIV adsorption to susceptible T cells at very low, non-toxic concentrations. They also observed inhibition of HIV-1 and HIV-2 replication, HIV antigen expression, and giant cell formation.

AGENTS THAT TARGET REPLICATION

Because the replicative cycle of HIV is a multistep process (beginning with reverse transcription and ending with the generation of new viral proteins and new viral RNA), several distinct sites can be targeted by diverse therapeutic agents:

- Reverse transcriptase inhibitors—Agents capable of inhibiting the enzyme that transcribes viral RNA to DNA in the infected cell include nucleoside analogues, such as AZT, and two new classes of agents, TIBO and HEPT derivatives. A subset of fluoroquinolone compounds, which may inhibit retroviral reverse transcriptases, reportedly possess anti-HIV activity.
- Ribosome inactivators—GLQ223 is a ribosome-inactivating protein that shuts down the cell's "translation" machinery, effectively preventing generation of new viral proteins.
- Integrase blockers—Agents that block the activity of the viral enzyme integrase, which catalyzes insertion of viral DNA into the host cell's DNA, will probably be developed once this viral enzyme is further characterized.
- DNA polymerase inhibitors—Broad-spectrum antiviral agents that inhibit DNA polymerase—such as foscarnet—continue to be studied for their efficacy in HIV infection. Foscarnet is known to be effective against DNA viruses such as the herpesviruses, and also against retroviruses like HIV.

Note: Agents that specifically target HIV's RNA are discussed separately in the following section, "Agents that Target Viral RNA."

The group in which the most progress has been made are the reverse transcriptase inhibitors, especially the nucleoside analogues (which resemble indispensable, naturally occurring building blocks of DNA). These agents include AZT, dideoxycytidine (ddC), dideoxyinosine (ddI), and azidouridine (AzdU). Because of AZT's relative success in treating AIDS and ARC patients and the preliminary data showing it to be effective in asymptomatic HIV-positive individuals, these agents are being researched at a stepped-up pace. With the recent recognition of AZT-resistant HIV strains (see "AZT," below), the other nucleoside analogues are being screened more closely.

Several research groups are investigating whether chemical modification of the dideoxy nucleosides can confer desirable biochemical properties on these compounds—such as greater membrane permeability, or fat solubility, which would enhance penetration into

the central nervous system. Dr. Takuma Shirasaka (National Cancer Institute, Bethesda, Maryland) and colleagues, in collaboration with Sanyo-Kokusaku Pulp Company (Yamaguchi, Japan), based on their findings of *in vitro* anti-HIV activity and increased lipophilicity (or fat solubility indexes), reported that chemically modified (halogenated) versions of AZT, ddI, and ddC are particularly attractive candidates for further development as anti-HIV drugs.

Other nucleoside analogues that differ in their chemical structure from the dideoxy compounds, such as the acyclic and carbocyclic nucleosides, are also being scrutinized in preclinical testing. These include PMEA (phosphonylmethoxy-ethyl-adenine), an acyclic nucleoside, and Carbovir, a carbocyclic nucleoside manufactured by Glaxo, Inc., (North Carolina).

AZT. The mechanism of action of AZT (and of several nucleoside analogues) is the termination of growing viral DNA chains. AZT exerts an antiviral effect in infected cells by interfering with the function of the viral enzyme reverse transcriptase: synthesis of viral DNA from viral RNA is cut short when reverse transcriptase mistakenly adds AZT, rather than a normal nucleoside, to a growing viral DNA chain. If viral DNA is not made, then no new virus particles can be produced. (See Chapter 1: VIROLOGY for full details on the reverse transcription phase of the HIV life cycle.)

When screened *in vitro* as an anti-HIV agent, AZT was found, as a triphosphate, to selectively inhibit viral reverse transcriptase. The cellular enzyme DNA polymerase, which, like reverse transcriptase, catalyzes the synthesis of DNA, was much less sensitive to AZT. Reverse transcriptase may be 100-fold more sensitive to AZT than is cellular DNA polymerase, possibly because, unlike cellular DNA polymerase, it cannot "repair" certain mistakes; reverse transcriptase is thus said to be error-prone. Given this discrepancy in sensitivity, it was presumed that viral DNA synthesis—and not cellular DNA synthesis—would be disrupted; healthy cells could therefore be expected to escape damage.

Toxic Effects. Clinical studies have shown that AZT can have significant toxic effects on blood cells, effects that require discontinuation of therapy or dose reduction. Adverse reactions to AZT include, but are not limited to, hematologic complications (mainly bone marrow suppression, sometimes leading to life-threatening anemia) and neurological effects (mainly motor dysfunction such as that seen in myopathy and peripheral neuropathy). About 40% to 80% of patients started on standard (high-dose) AZT therapy cannot tolerate the drug because of severe abdominal pains, diarrhea, nausea, anemia, neutropenia, and allergic reactions.

Drug-Resistant HIV Strains. A serious but not unexpected complication associated with AZT therapy is the development of drug-resistant strains of the virus—that is, HIV strains with reduced sensitivity to AZT. Recognized for the first time in 1986, AZT resistance is now known to result from multiple mutations in the gene that encodes reverse transcriptase. Dr. Brendan Larder and Sharon Kemp (Wellcome Research Laboratories, Kent, U.K.) reported last year that fully resistant HIV strains appear to require the accumulation of four mutations at specific sites in the reverse transcriptase gene and, in ARC/AIDS patients, the emergence of resistance is not detectable in drug sensitivity assays until after 6 months of AZT therapy.

Dr. Charles Boucher (Academic Medical Center, Amsterdam) reported—based on collaborative work with Dr. Larder examining the differential sensitivities of various HIV strains to AZT—that high-level drug resistance develops noticeably faster in ARC/AIDS patients than in asymptomatic individuals. This observation was borne out by Dr. Boucher's data on HIV strains isolated from asymptomatic individuals at various times over a 2-year treatment period. In 89% of cases, the isolated HIV strains had only a single mutation in a particular reverse transcriptase gene—one that confers AZT resistance at a slower rate than other mutations. Dr. Julio Montaner (St. Paul's Hospital, University of British Columbia, Vancouver) reported that the presence of AZT-resistant HIV may have little clinical significance in asymp-

tomatic patients. His team found that the development of AZT resistance, as measured through *in vitro* assays, was not associated with specific clinical events in asymptomatic persons. Dr. Montaner noted, however, that patients with low CD4 cell counts at entry to the study were more likely than others to develop AZT-resistant HIV strains after 6 months.

Dr. Hiroshi Mohri (Cedars-Sinai Medical Center, Los Angeles) described a highly disturbing observation: AZT resistance in HIV strains isolated from two untreated patients. This finding implies that drug-resistant HIV is being transmitted.

ddC. Dideoxycytidine (ddC), chemically related to AZT, is the most potent of the dideoxy nucleoside analogues. It is able to enter the central nervous system, which may explain, in part, the observations of neurotoxicity (such as peripheral neuropathy) in patients receiving high doses of ddC. Unlike AZT, ddC has been associated with almost no significant hematologic toxicity. Dr. Thomas Merigan (Stanford University Medical Center, California) described ongoing Phase I and II clinical trials, dose-response studies looking at safety and efficacy of ddC as single-agent chemotherapy (see Chapter 7: CLINICAL MANAGEMENT). Combination trials with AZT (both alternating and concomitant therapy) are now in progress. The manufacturer of ddC is Hoffmann-LaRoche (Nutley, New Jersey).

ddI. Dideoxyinosine, or ddI, reported to be a strong inhibitor of HIV replication in CD4 cells and monocytes *in vitro*, is currently in several Phase I, II, and III clinical trials using oral or intravenous administration. Dr. Raphael Dolin (University of Rochester Medical Center, New York) reported that although *in vitro* studies show ddI to be less potent than either AZT or ddC, this nucleoside analogue is also considerably less cytotoxic than either of the other agents. Several, but not all, investigators in the clinical trials reported decreases in levels of p24 and HIV RNA in ddI-treated patients. Pancreatitis and peripheral neuropathy appear to be the major dose-limiting toxicities, whereas toxicity in bone marrow cells and bone marrow progenitor cells (seen with AZT) appears to be rare. Dr. Robert Yarchoan (National Cancer Institute, Bethesda, Maryland) reported that toxicities are highly dose-dependent—that is, they may be avoided by reducing the dose. Bristol-Myers Squibb Company (New York) manufactures ddI as Videx.

d4T. Previous studies showed that another nucleoside analogue, dideoxydidehydrothymidine, or d4T, exhibits strong anti-HIV activity in HIV-infected CD4 cell lines. Dr. Douglas Boudinot (University of Georgia, Athens) and colleagues reported that in animal studies, d4T was found to penetrate into the central nervous system. Phase I clinical trials (using oral administration) are in progress to determine safety and toxicity; so far the minimum effective dose has not been defined. Bristol-Myers Squibb Company (New York) manufactures d4T.

TIBO and HEPT Derivatives. Dr. Rudi Pauwels (Rega Institute for Medical Research, Leuven, Belgium) described the discovery of a novel class of antiviral compounds, the TIBO derivatives (tetrahydro-imidazo-benzodiazepin-ones and -thiones). This series of synthesized derivatives is based on the prototypical benzodiazepine structure. (Benzodiazepines, a pharmacologic class of drugs, act as tranquilizers and include valium.) TIBO derivatives were found to be potent and selective inhibitors of HIV-1 reverse transcriptase. The absence of an inhibitory effect against HIV-2 or other animal retroviruses suggests that these agents have a mechanism of action different from that of other reverse transcriptase inhibitors. Dr. Michael Kukla (Janssen Research Foundation, Spring House, Pennsylvania) and collaborators at Rega Institute for Medical Research reported that *in vitro*, at clinically relevant doses, TIBO derivatives are as potent as AZT.

Acyclic uridine derivatives, the HEPT (1-[(2-Hydroxyethoxy)methyl]-6-phenylthiothymine) analogues, were also found to selectively inhibit HIV-1 replication. Dr. Masanori Baba (Fukushima Medical College, Japan) and colleagues reported that these new compounds could inhibit pathological changes induced by HIV infection in cultures of T cells and of human blood lymphocytes.

GLQ223. A purified preparation of trichosanthin (which is derived from a cucumber plant), GLQ223 seems to function as a ribosome inactivator. (A ribosome is a cell structure that is essential for the translation of messenger RNA into protein.) In 1989 several groups of researchers—at Genelabs, Inc. (Redwood City, California); University of California, San Francisco; San Francisco General Hospital; and Chinese University of Hong Kong—reported that GLQ223 was a potent inhibitor of HIV replication in both lymphocytic and mononuclear/macrophage cell cultures. This finding was particularly encouraging since few, if any, drugs in clinical trials have demonstrated anti-HIV properties in mononuclear cells (thought to be a reservoir of HIV in the body).

Dr. Michael McGrath (San Francisco General Hospital) reported at the Conference that when GLQ223 was added to cultures of HIV-infected macrophages, viral protein synthesis was "shut down." Cultured blood cells from AIDS patients also showed no new viral protein synthesis after incubation with GLQ223. HIV RNA levels dropped in both macrophage and blood cell culture systems. Presentations by scientists from Genelabs, Inc., showed that GLQ223 selectively targeted HIV proteins (and generally spared cellular proteins) in acutely infected T cells *in vitro*. At doses that significantly reduced the synthesis and accumulation of HIV proteins, total cellular protein synthesis was reduced only slightly. Selective degradation of viral RNA was also observed in treated HIV-infected T cells. Phase I clinical trials of GLQ223 (administered intravenously) are currently in place. Although the optimal dosage has not been determined, preliminary results from serum analysis reveal little effect on HIV.

AGENTS THAT TARGET VIRAL RNA

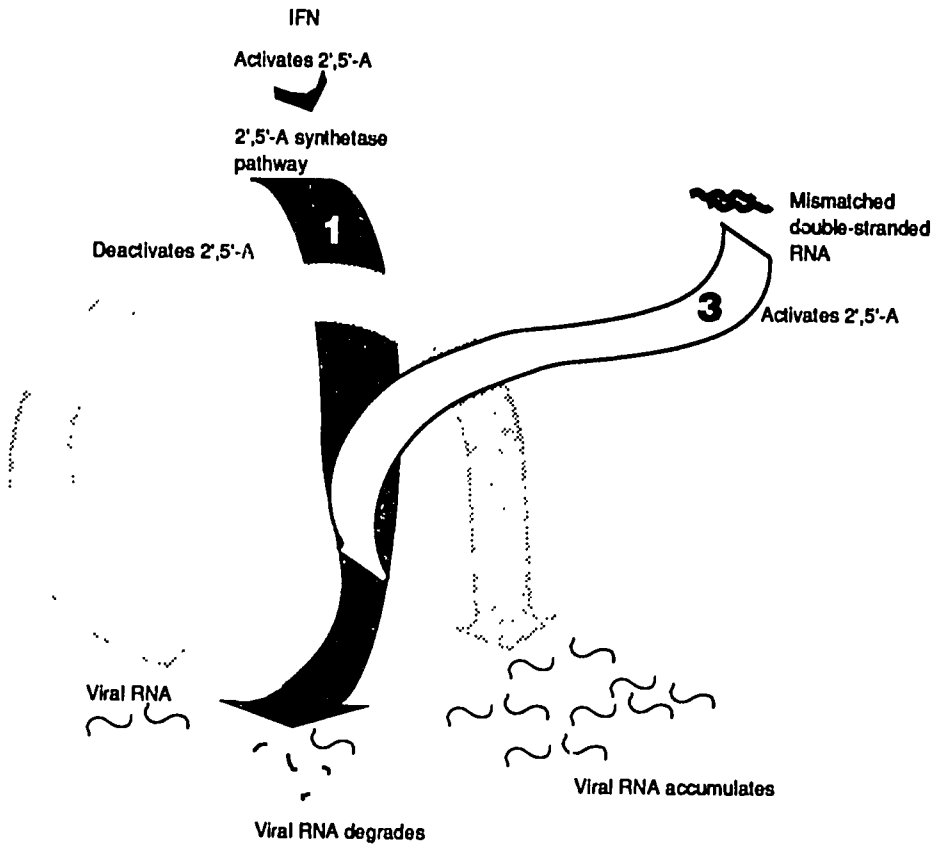
Polynucleotides. Mismatched double-stranded RNA (patented by HEM Research, Inc., Philadelphia, as Ampligen) and poly-ICLC (a synthetic RNA molecule that also contains a carbohydrate) have been shown to possess anti-HIV activity. A recent report of a new compound, double-stranded RNA complexed with dextran sulfate (called Retrogen D, developed by Washington Biolab, Inc., Creston, Iowa), shows that *in vitro*, the compound inhibits HIV-induced T cell death in a dose-related fashion. At the higher end of the dose range, which was still sub-toxic, 100% of T lymphocytes were protected (that is, the cells remained uninfected and metabolically active).

Ampligen. Previous *in vitro* experiments have demonstrated that T cells pretreated with mismatched double-stranded RNA (Ampligen) and subsequently infected with HIV had significantly lower levels of intracellular HIV RNA than untreated cells. Biochemical analyses have shown that, like interferon, double-stranded RNA binds to and thereby stimulates 2',5'-A synthetase, a cellular enzyme that induces degradation of RNA. It is hypothesized that in HIV-infected cells, the 2',5'-A synthetase pathway (ordinarily activated by interferon as a protective response to viral infection) is shut down by HIV RNA. Detection of significantly lower levels of HIV RNA in cultures of treated versus control cells, therefore, is indirect evidence that mismatched double-stranded RNA activates the 2',5'-A synthetase pathway, leading to degradation of viral RNA. (See Figure 6.3.)

Originally, pilot Ampligen studies, using Ampligen formulated in plastic bags, showed no difference in disease progression rates between Ampligen-treated patients and patients receiving placebo. However, differences in inhibition of HIV between glass-bottle formulations and bag formulations of Ampligen have been demonstrated. Furthermore, significant differences in activation of 2',5' A synthetase were also found.

In clinical trials, Ampligen (glass-bottle formulation) is being studied in 20 ARC or pre-ARC patients with depressed CD4 cell counts. Dr. David Strayer, (Hahnemann University, Philadelphia) and colleagues reported that patients receiving intravenous Ampligen therapy for a range of 4.5 to 36 months show decreases in p24 levels and increases in CD4 cell

Figure 6.3. dsRNA Activation of 2', 5'-A Synthetase Pathway



Interferon activates (1) the cellular enzyme 2',5'-A synthetase, which in turn activates the RNA-degrading enzyme, RNase L. However, it is hypothesized that viral RNA shuts down this pathway (2), thereby allowing accumulation of new viral RNA. Mismatched double-stranded RNA seems to competitively reactivate this pathway (3) and the subsequent degradation of viral RNA.

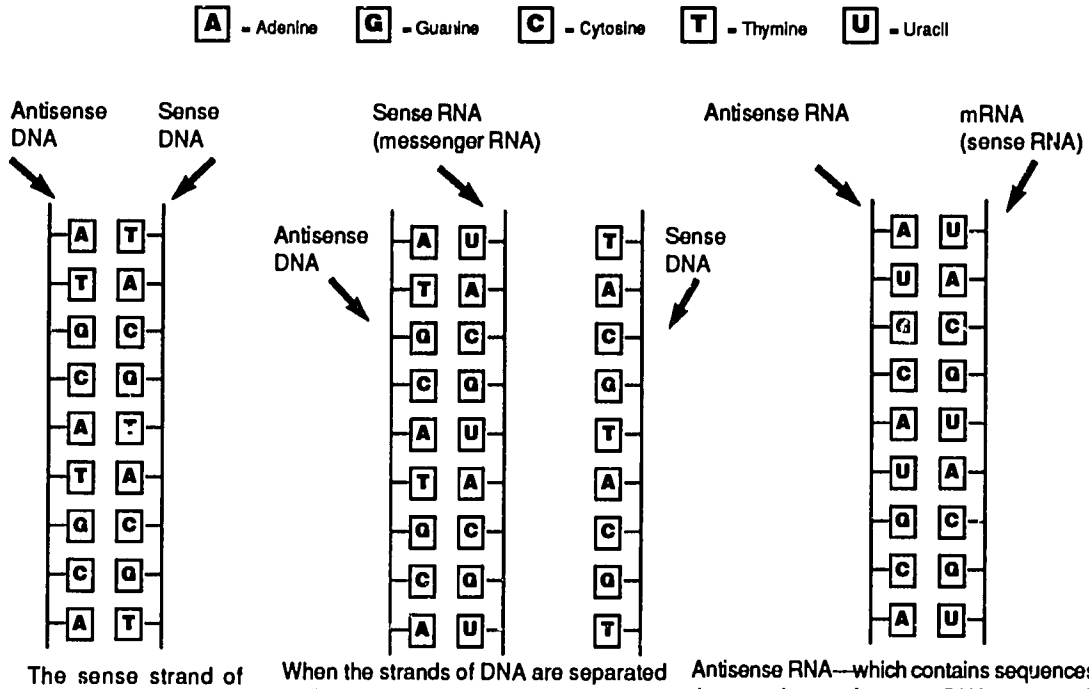
counts, compared with a matched placebo group with similar baseline CD4 cell counts, p24 level, and ARC symptoms. The data presented suggest that Ampligen can delay the development of opportunistic infections in this patient population: no opportunistic infections, lymphomas, or deaths occurred among patients in the treatment group. In contrast, 15 of 158 patients in the placebo group developed opportunistic infections.

In August, the U.S. FDA announced that on the basis of *in vitro* studies showing synergistic activity between Ampligen and AZT, a blinded multicenter clinical trial of the two agents will be initiated in late 1990.

[An interesting aside to the 2',5'-A synthetase-mediated antiviral response story is a Conference report presented by Drs. Werner Muller and Heinz Schroder (Institut fur Physiologische Chemie, Universitat Mainz, FRG). HIV replication was shown to be selectively inhibited in a series of *in vitro* gene therapy experiments using the human 2', 5'-A synthetase gene. DNA encoding the active form of the enzyme, under control of the HIV LTR (long terminal repeat, an HIV regulatory gene), was introduced into CD4-bearing T cells. When these cells were infected with HIV, the HIV *tat* gene product transactivated the LTR, yielding high levels of 2', 5'-A synthetase mRNA transcripts and activity as well as high cellular 2', 5'-A content. HIV reverse transcriptase activity was markedly decreased in cultures that received the 2', 5'-A synthetase DNA compared to control cultures. Furthermore, only 2% of cells were positive for p24 and p17 in the experimental cultures—compared with

Figure 6.4. Antisense RNA and DNA

Viral protein synthesis can be halted with antisense RNA or DNA molecules, which can be designed to complement (and therefore are capable of binding to) mRNA. (*Note:* In RNA molecules, thymine (T) is replaced by uracil (U).



The sense strand of double-stranded DNA encodes the genetic information for specific proteins.

When the strands of DNA are separated during transcription, mRNA is formed using antisense DNA as a template. After mRNA is synthesized, both antisense and sense DNA are degraded by cellular enzymes.

Antisense RNA—which contains sequences that complement the sense DNA strand and, therefore, complement the sense mRNA strand—will bind to mRNa. Such binding effectively prohibits normal translation of mRNA into proteins.

60% for untreated controls. The authors suggest, on the basis of their *in vitro* evidence, that it is conceivable that a gene therapy approach using 2', 5'-A synthetase-encoding DNA will confer on cells the capacity to efficiently suppress HIV replication.]

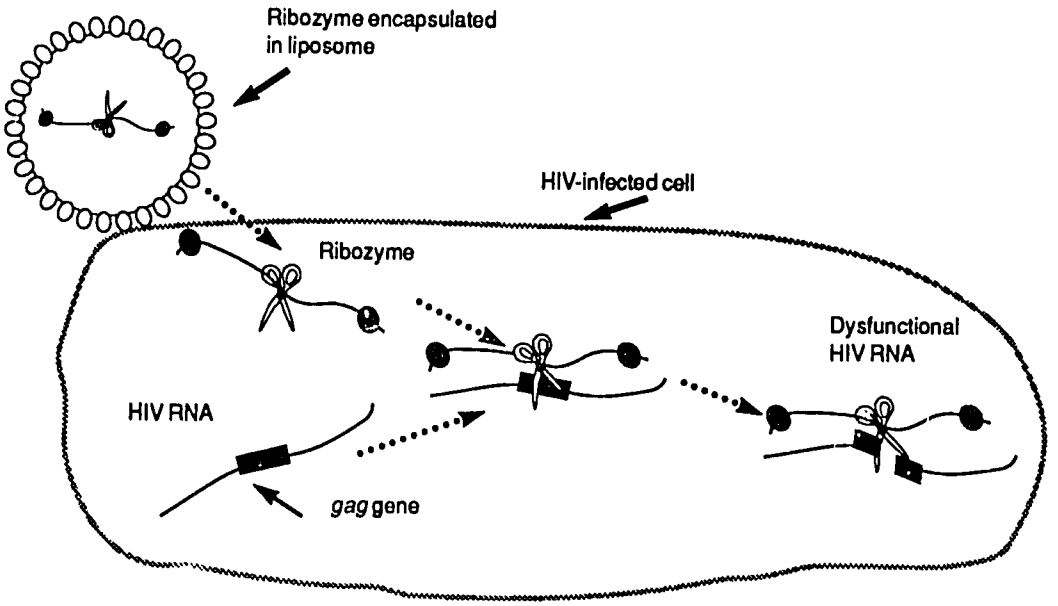
Poly-ICLC. This synthetic molecule (polyriboinosinic-polyribocytidlyic acid complexed with poly-lysine-carboxymethylcellulose) presumably exerts its anti-HIV effects in a manner similar to that of Ampligen—by activation of the 2',5'-A synthetase pathway. Phase I clinical trials of low-dose poly-ICLC administered intramuscularly, reported at the V International (Montreal) Conference, are still underway. Randomized controlled trials to explore efficacy (Phase II studies) are slated to begin in Puerto Rico by the end of 1990.

Antisense Molecules. Each double-stranded nucleic acid molecule (both DNA and RNA) consists of a "sense," or coding, strand and an "antisense," or noncoding, strand. The antisense strand serves as a template for the formation of a specialized form of RNA, called messenger RNA, or mRNA, during the transcription phase of protein synthesis. This mRNA—which contains a specialized nucleic acid sequence that complements that of the antisense strand, and which is in essence a sense strand—is necessary to the subsequent synthesis of proteins. In 1981 it was discovered that protein synthesis could be interrupted by taking advantage of the binding capabilities of sense and antisense strands through introduction of antisense molecules constructed with the sequence that complements and therefore binds to the mRNA strand. (See Figure 6.4.)

Because of the highly specific nature of the binding between two strands of a nucleic acid "duplex" (two-stranded) molecule, the individual strands are said to be complementary to

Figure 6.5. Anti-HIV Ribozyme

A liposome-encapsulated anti-HIV ribozyme is being delivered to an HIV-infected cell. (Liposomes, hollow spheres made of fat molecules, are particularly attractive drug-delivery systems as they are readily absorbed by the cell's membrane.) An RNA sequence, such as the HIV *gag* gene, is targeted, cut, and thereby disabled by the ribozyme construct.



each other. The four nitrogen-containing bases found in DNA and RNA molecules—cytosine (C), guanine (G) adenine (A) and thymine (T)/uracil (U)—pair with each other in a specific manner: A only pairs with T (or U, in the case of RNA), and C only pairs with G. Therefore, if the sequence of one strand is known, that of its complementary strand can easily be determined. Today many research groups are designing antisense molecules to correspond to known genetic sequences to serve as potential therapeutic agents for a number of human diseases.

Antisense Oligonucleotides. Short DNA segments, or oligonucleotides, which are complementary to mRNA (sense RNA) and therefore called antisense, can prevent mRNA-directed protein synthesis. When antisense oligonucleotides bind to mRNA, they render the mRNA incapable of generating proteins. (Note that DNA/RNA hybrid molecules—one DNA strand bound to an RNA strand—are normal "species" during the transcription phase of protein synthesis.) Antisense oligonucleotides can be chemically altered to change certain of their properties; for example, phosphate-containing antisense oligonucleotides are less susceptible to degradation by cellular enzymes and are able to cross membranes more easily. Dr. Lia Smit (Academic Medical Center, Amsterdam) and coworkers reported that HIV replication was inhibited when tissue culture cells were exposed to both sense and antisense phosphate methylated oligonucleotides. Not all HIV strains were sensitive to treatment: replication of two strains of HIV-2 (RUT and LAV-2) could not be blocked.

Antisense RNA. RNA molecules constructed to contain sequences complementary to those of sense DNA strands (and their corresponding mRNA) are called antisense RNAs (see Figure 6.4). Binding of antisense RNA to mRNA prevents translation of the mRNA into proteins in much the same way antisense oligonucleotides do.

Several teams have been manufacturing antisense RNAs directed toward specific HIV sequences that encode essential HIV genes. Dr. Georg Sczakiel and colleague (Deutsches

Krebsforschungszentrum, Heidelberg, FRG) and Drs. Andrew Rhodes and William James (Oxford University, U.K.) independently reported that HIV replication could indeed be reduced in cells treated with antisense RNA. Dr. Sczakiel's antisense RNA was constructed to target the HIV *gag* gene; the antisense RNA was then injected into the nucleus of cells together with normal proviral HIV DNA. A maximum inhibition of 70-75% (7 to 10 days post-infection) was observed, with inhibition being completely lost by 3 weeks. Dr. Rhodes and colleague used a retroviral vector (a virus that carries recombinant—in this case, HIV antisense—genes in its genome) to introduce antisense RNA into HIV susceptible cells. Several different antisense RNAs were tested for their ability to inhibit HIV replication and it was found that the more successful ones contained RNA sequences complimentary to sequences of HIV *tat* and *env*, reducing viral replication by more than 70% during the first week after infection.

Ribozymes. In the early 1980s, certain ribonucleic acid (RNA) molecules were found capable of behaving like enzymes by catalyzing the cleavage of their own molecules. Thomas Cech (University of Colorado, Boulder) and associates coined the term "ribozyme" to describe such RNAs. Later it was discovered that ribozymes can also snip apart (cleave) other molecules—not just themselves. (Dr. Cech and Sidney Altman, from Yale University, received the 1989 Nobel Prize in chemistry for their independent discovery of these catalytic RNAs.)

Earlier this year, Dr. Nava Sarver (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland), in collaboration with investigators at City of Hope Medical Center, Duarte, California, published work using designed ribozymes that recognize, hold in position, and snip specific HIV RNA sequences. Several groups are now looking at delivery systems for ribozymes. (See Figure 6.5.) At this year's Conference, Dr. William James and Paul Crisell (Oxford University, U.K.) reported on a ribozyme that was shown capable of cleaving HIV RNA sequences corresponding to the *tat* gene in T cell cultures. The authors are now attempting to use viral vectors to introduce ribozyme genes into cells.

PROTEASE INHIBITORS

The HIV protease enzyme—one of the virus's enzymes and the one responsible for the maturation of new infectious viral particles—provides another target for therapeutic intervention. At the molecular level, protease (or proteinase) is essential to HIV protein processing: protease cleaves large, inactive precursor polyproteins into functional viral proteins. Therefore, agents that interfere with normal HIV protease function would necessarily disallow the generation of biologically active viral proteins. Indeed, studies have shown that viral particles without protease or with genetically altered protease are rendered noninfectious. (See Chapter 1: VIROLOGY for a detailed discussion of HIV protease function.)

Since the recent discovery of the three-dimensional structure of the HIV protease molecule (by researchers at Merck, Sharp, & Dohme Research Laboratories, West Point, Pennsylvania, reported in 1989), at least six biopharmaceutical companies and several government institutions have been designing HIV-specific protease inhibitors. Work has been published this year by scientists at SmithKline Beecham (King of Prussia, Pennsylvania), The Upjohn Company (Kalamazoo, Michigan), and Abbott Laboratories (Chicago); however, only scientists from Roche Products Ltd. (U.K.) reported their progress in the custom design of HIV protease inhibitors at the Conference. Dr. Noel Roberts, of Roche, discussed his team's findings showing that a series of peptide-based HIV protease inhibitors are highly selective for HIV protease and do not inhibit the closely related human (aspartic) proteases. From *in vitro* experiments (using CD4-bearing T cells), these HIV protease inhibitors were found to possess strong antiviral activity as determined by p24 antigen assays, but they were not cytotoxic.

BLOCKERS OF VIRAL ASSEMBLY AND BUDDING

Agents that block viral assembly and budding can prevent the release of new virions from the infected cell. These compounds include agents that interfere with the processing of the HIV envelope proteins gp120 and gp41, as well as agents that can damage the membrane of the infected cell, interfering with viral assembly and budding at the cell surface.

Glycosylation Inhibitors. Compounds that inhibit the necessary processing of sugar molecules on the HIV envelope may disrupt viral envelope organization, possibly rendering the virus less infectious. For example, it appears that HIV usurps the cellular enzyme glucosidase, which is responsible for cleaving oligo- (or poly-) saccharides, to cleave its own envelope glycoproteins at the time of budding. Agents that inhibit glucosidase function may therefore prove therapeutically effective.

One such drug that inhibits glycosylation, deoxynojirimycin (DNM), has been shown to exert anti-HIV activity in *in vitro* studies, but only to a slight degree. Certain glycoprotein carbohydrate-processing inhibitors, in fact, have been reported to enhance HIV infection in CD4 cell cultures. Ongoing studies are seeking to further characterize glycosylation inhibitors in different HIV-susceptible cell types.

Lipid-based Agents. Also being tested are lipid-based agents capable of damaging the cell membrane, which could disrupt HIV envelope acquisition. A lipid analogue that contains phosphocholine and exhibits anti-HIV activity at concentrations that do not kill host cells was described by Canio Marasco (University of North Carolina School of Pharmacy, Chapel Hill) and colleagues. *In vitro* studies showed that the agent had additive anti-HIV effects when combined with AZT.

IMMUNOTHERAPEUTIC AGENTS

Because AIDS is a progressive debilitating disease of the immune system, researchers have been seeking ways to rebuild the natural immune defenses of HIV-infected persons. Some approaches that are being investigated include:

- Passive immunotherapy (also-called immunoglobulin therapy). Plasma is taken from HIV-infected donors with high titers of antibodies to HIV and transfused into recipients with low antibody levels.
- Cell therapy. The patient's own immune system cells are stimulated to proliferate, with the aim of enhancing their immune response against HIV and opportunistic pathogens.
- Active immunotherapy. Immunomodulators are administered to HIV-infected individuals to reconstitute a compromised immune system; this strategy is the focus of the bulk of current research on immunotherapy.
- Immunotherapy with immunogens. Substances that stimulate an immune response are administered to individuals who have been exposed to HIV. This approach, which is being tested in HIV-infected persons in different stages of disease, is discussed in Chapter 5: VACCINE DEVELOPMENT.

PASSIVE IMMUNOTHERAPY

Although safety and pharmacokinetic studies have shown passive immunotherapy to be safe—one research group noted that passive immunotherapy is "remarkably free of side effects"—ongoing clinical studies have shown that the long-term clinical benefits of immunoglobulin therapy are variable. Several Phase I clinical trials are in progress in the United States, Canada, the United Kingdom, and Germany. In these studies, plasma collected from asymptomatic HIV-infected individuals (a stage at which antibody titers are high but productive virus is presumably lacking) is transfused into ARC or AIDS patients;

control groups (seronegative individuals injected with infected plasma) are not used in these studies to avoid the possibility of transmitting the virus. Donor plasma is selected based on the presence of high HIV antibody titers and the absence of HIV or its antigens as determined by antigen screening and/or culture.

Dr. Abraham Karpas (Cambridge University, U.K.) and coworkers presented data from their study of 4 ARC and 6 AIDS patients who were p24-positive at study entry and who began receiving passive immunotherapy in March 1988. One death occurred after 5 months of therapy, but most patients' constitutional symptoms (such as weight loss and lethargy) improved in the first 6 months of continuous therapy. In the second half of the year, however, several patients developed opportunistic infections or showed general clinical deterioration. Of the 9 remaining patients, 5 died between the 11th and 17th months of the study. The authors pointed out that, in general, the ARC patients had sustained improvement compared to the AIDS patients, which suggests that passive immunotherapy may be most effective when started soon after the onset of HIV-associated symptoms.

This finding is supported by a study of the effect of the therapy on HIV RNA or DNA in recipients' sera. Dr. Indira Hewlett (FDA, Bethesda, Maryland) and colleagues reported that no viral nucleic acid sequences were detected in any of 7 patients with AIDS and 3 with ARC after the initial transfusion of HIV immune globulin. HIV DNA and RNA had been detected in all of the study patients prior to the initiation of therapy. At 10-11 months of immunotherapy, HIV nucleic acid sequences were detected in 3 of the 7 AIDS patients, but in none of those with ARC.

CELL THERAPY

As immunologists are learning more about the immune system and the immune response to specific disease-causing microorganisms, it has become increasingly clear how important cell-mediated immunity (as opposed to humoral, or antibody, immunity) is. The cellular branch of the immune system includes a population of killer cells that can recognize specific antigens on cell surfaces. These killer cells can identify and dispose of infected cells that bear foreign or "non-self" antigens on their surface. (See Chapter 2: PATHOGENESIS & IMMUNOLOGY for a detailed discussion of cytotoxic T lymphocytes.)

Pioneering work led by Dr. Steven Rosenberg and other scientists at the National Cancer Institute (Bethesda, Maryland) has culminated in clinical trials in which patients are given back an expanded population of their own (autologous) cytotoxic (cell-killing) T lymphocytes, also known as CTLs. At the Conference, John Armstrong and colleagues (University of Pittsburgh) reported preliminary observations in an ARC/AIDS patient whose cytotoxic T cells were removed, activated by interleukin-2 (IL-2), and then returned to the patient via intravenous infusion. (IL-2 is a naturally occurring hormone-like substance known to induce the proliferation of certain immune system cells.) The University of Pittsburgh researchers found that this patient, who has completed 6 months of therapy, has tolerated the therapy very well with no significant side effects. A second patient has started therapy, and 4 more ARC/AIDS patients are to begin therapy before the end of the year.

ACTIVE IMMUNOTHERAPY

The administration of substances with the potential to rebuild the immune system is called active immunotherapy. These substances, known as immunomodulators, or biologic response modifiers, were the focus of a great deal of cancer research in the 1970s. Some immunomodulators are also being tested in the laboratory for anti-HIV effects. Some have entered clinical trials as either single-agent or adjunctive therapy for AIDS or HIV-related conditions. Since most naturally occurring immunomodulators are nonspecific stimulators of immunity, their precise mechanisms of action in HIV infection continue to be studied *in vitro* while clinical trials

Table 6.2. Combination Therapies: Antivirals

Combination	Schedule	Conference Reports
AZT & acyclovir	Concurrent administration	*In vitro* findings of synergistic anti-HIV activity of these 2 agents (reported in 1986) led to initiation of clinical trials in 1988. This year, Dr. Robert Coombs (University of Washington, Seattle) compared AZT alone with AZT plus acyclovir as given to patients with constitutional disease (CDC class IVa). He reported that no discernible difference in anti-HIV effects was found, as determined by p24 levels or plasma viremia. Dr. Coombs added, however, that because of the small sample size (22 patients), the trial does not have the power to fully exclude possible beneficial effects of acyclovir. Dr. Jan Mulder (Municipal Health Services, Amsterdam) and coworkers reported their long-term findings on asymptomatic, p24-positive patients who received combination therapy for 118 weeks. After 60-117 weeks, disease progression was noted in 2 of 6 patients on AZT/acyclovir and in 4 of 18 patients receiving AZT alone. The authors concluded that disease progression still occurs among patients receiving AZT and that acyclovir does not seem to provide any additional benefits.
AZT & ddC	Alternating regimen; concurrent regimen (both under evaluation)	Dr. Gail Skowron (Brown University, Providence, Rhode Island) gave an interim analysis of a large, ongoing Phase II study (AIDS Clinical Trials Group) evaluating different regimens of AZT with either high- or low-dose ddC. In reporting results of this study, Dr. Skowron pointed out that the adverse effects of single agents appeared to be reduced when administered in a weekly or monthly alternating regimen. Higher dose ddC may show greater ability to sustain rises in CD4 cell counts and suppression of p24. In separate arms of this trial, an intermittent schedule (with neither AZT nor ddC administered for 1 week) did not appear to be as effective as the alternating regimen. Dr. Tze-Chiang Meng (UCSD) and colleagues reported that concurrent low-dose AZT/ddC is well tolerated; continued follow-up will reveal whether initial increases in CD4 cell counts are sustained.
AZT & ddI	Not applicable (*in vitro* studies)	Victoria Johnson (Massachusetts General Hospital/Harvard University, Boston) reported her team's findings of additive anti-HIV properties between AZT & ddI in T cell cultures exposed simultaneously to HIV and the 2-drug combination. Assays for p24 on post-infection day 10 showed significant reduction in p24 levels; no additive toxicities were noted.
AZT & antisense oligonucleotide	Not applicable (*in vitro* studies)	Dr. Ting-Chao Chou (Memorial Sloan-Kettering Cancer Center, New York) and coworkers found AZT and an antisense oligonucleotide (S-dC-28) to act antagonistically with respect to anti-HIV effects when tested in T cell cultures. (S-dC-28 and dextran sulfate showed slight synergism.) The authors plan to study AZT and S-dC-28 at lower doses for possible synergistic activity.
AZT & dsRNA	Not applicable (*in vitro* studies)	Kengo Gohchi (Hôpital Bichat-Claude Bernard, Paris) and coworkers reported that AZT with double-stranded RNA (poly-ICLC) showed synergistic anti-HIV activity in monocyte/macrophage cultures, but not in T cell (MT4) cultures.
CD4-toxin hybrid & AZT or ddI	Not applicable (*in vitro* studies)	Dr. Edward Berger (NIAID, Bethesda) and colleague found that CD4-*Pseudomonas* exotoxin acts synergistically with either AZT or ddI to protect uninfected lymphocytes in HIV-infected cell cultures.
sCD4 & ddI	Not applicable (*in vitro* studies)	Based on observation of *in vitro* anti-HIV synergism between these 2 drugs (as determined by p24 assays), Dr. Victoria Johnson (above) recommended that this combination be considered for clinical testing.

evaluate their efficacy and toxicity. Although it is necessary to identify the specific immunopotentiating effects and mechanisms of action of the individual immunomodulators, most researchers believe that the real benefits of immunotherapy will be accrued in combination with antiviral compounds. Such combinations are outlined in Table 6.3.

The naturally occurring immunomodulators are, for the most part, cytokines or growth factors (See "Colony Simulating Factors," below, for a discussion of immunomodulating growth factors.) Cytokines, soluble proteins released by various cells, act as chemical messengers, giving specific signals to target cells. Some cytokines allow the maturation of specific target cells, while others augment or suppress a variety of immune responses. Many of these proteins have multiple functions, depending on the cell type that receives their signal. It is becoming clear that many of the cytokines act synergistically with others to mediate their effects. Thus, a proper balance of these soluble messengers is critical for a coordinated and effective immune response. In many HIV-infected individuals this balance is thought to be disrupted, so that the use of cytokines as therapeutic agents seems appropriate.

The relative success of immunomodulators may depend on the stage of disease at which therapy is initiated. As more and more studies have shown greater benefits for mildly symptomatic or early ARC patients, immunomodulators are being viewed as an important early intervention. It is presumed that patients in earlier stages of HIV-related disease have suffered less damage to the immune system than patients with advanced disease, and thus may be more capable of responding to immunological stimulation.

Interferons. Cytokines produced naturally in response to viral infection may provide protection against HIV infection. Researchers aptly named one of these substances "interferon" in 1957, when they confirmed that these natural substances do indeed interfere with viral growth. The human interferons—of which there are three classes: alpha, beta, and gamma—confer a nonspecific antiviral state on neighboring uninfected, or unexposed, cells. Interferons also possess antiproliferative (antitumor) and immunopotentiating effects.

Gamma Interferon. Production of gamma interferon, a potent macrophage activation factor, is depressed in HIV-infected patients. (Macrophages are cells that have two major immunological functions: killing invading microorganisms and tumor cells, and aiding T lymphocytes in the recognition of foreign antigens as non-self.) Because gamma interferon shows little anti-HIV activity *in vitro*, studies of its use as an immunomodulator have largely been limited to combination studies (with tumor necrosis factor and granulocyte-mononuclear colony stimulating factor.

Beta Interferon. Besides its modest inhibitory effects on HIV replication, beta interferon has also been found to augment natural killer (NK) cell activity and cytotoxic T cell activity, specifically antibody-dependent cellular cytotoxicity (ADCC); see Chapter 2: PATHOGENESIS & IMMUNOLOGY. Dr. Richard Pollard (University of Texas Medical Branch, Galveston) and colleagues reported preliminary results of a Phase II safety and efficacy trial of subcutaneously administered beta interferon in 52 HIV-positive patients with CD4 cell counts below 400/mm^3. Three dose regimens were evaluated, and patients in each dosage group had to reduce or discontinue therapy because of toxicity. A statistically significant decline in serum HIV antigen was observed in the patients who completed more than 28 weeks of treatment. Recombinant human beta interferon (marketed as Betaseron by Triton Biosciences, California) is being tested in combination with AZT in ARC/AIDS patients, as well as in patients with AIDS-related Kaposi's sarcoma.

Alpha Interferon. Because alpha interferon was already being used in the treatment of some cancers, this immunomodulator was tested in AIDS patients with Kaposi's sarcoma as early as 1984. Alpha interferon, manufactured as a genetically engineered product, is marketed as Roferon-A (by Hoffmann-LaRoche & Company), Intron A (by Schering Plough), and

Wellferon (by Burroughs Wellcome). Although patient response to alpha interferon therapy varies considerably, several independent investigators report that alpha interferon–treated patients with AIDS-related Kaposi's sarcoma exhibit partial or complete tumor regression, longer survival, and a lower incidence of subsequent opportunistic infections. The dose-related antitumor effects of alpha interferon are very dramatic in some cases. Alpha interferon and AZT therapy continues to be studied in patients with Kaposi's sarcoma. Little enthusiasm has been found for alpha interferon as a therapy for AIDS in general, since early studies in patients with advanced AIDS concluded that the agent held no clinical value in these patients. However, Dr. Clifford Lane (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) pointed out that recent studies in HIV-infected patients without AIDS show that alpha interferon may indeed be beneficial—presumably because these patients have a less severely damaged immune system than those with AIDS. (See Table 6.3 and Chapter 7: CLINICAL MANAGEMENT.)

Several groups of investigators have speculated that alpha interferon's anti-HIV effects take place during the late stages in the viral life cycle—assembly, budding, and release. Laboratory studies have shown that alpha interferon inhibits HIV replication in cultures of CD4 cells at concentrations achievable in patients. Data presented by Dr. Howard Gendelman (Henry M. Jackson Foundation, Rockville, Maryland) and coworkers, however, indicate that alpha interferon behaves differently in monocytes and macrophages than in T cells. Monocytes treated with alpha interferon at the time of virus challenge show no sign of HIV infection, which suggests that alpha interferon interrupts one or more events that occur early in the viral life cycle, before the formation of proviral DNA. And when alpha interferon is added to monocyte cultures chronically infected with HIV, a gradual decrease in p24 levels is noted, along with declines in reverse transcriptase activity and viral mRNA levels. The authors believe that alpha interferon induces viral restriction, or latency, since large quantities of proviral DNA are present with no evidence of active transcription (that is, no new viral mRNA).

Colony Stimulating Factors. Natural, hormone-like substances that promote maturation of certain blood cell types from precursor "stem cells" are being tested in AIDS patients and other HIV-infected persons with low blood cell counts. Both T and B lymphocytes come from a common blood cell ancestor—a stem cell—that originates in the bone marrow. Growth factors called colony stimulating factors (CSFs) can accelerate the maturation of such stem cells into specialized blood cell types. (CSFs are a class of cytokine, chemical messengers produced and released by immune cells.) The major CSFs, named for the type of cell whose maturation they promote, include:

- mononuclear-CSF (M-CSF),
- granulocyte-CSF (G-CSF),
- granulocyte-mononuclear (GM-CSF)
- erythropoeitin (EPO)

Although secreted from kidney cells rather than immune system cells, the hormone erythropoeitin is classified as a growth factor (specifically, a CSF) because it stimulates the growth of erythrocytes (red blood cells). EPO and the other CSFs normally function to replenish blood cells lost through natural processes. Therapeutic use of CSFs seeks to exploit this replenishing ability to compensate for the destruction of blood cells by HIV and by certain therapeutic agents, such as AZT.

Currently, investigators do not agree about whether EPO might be of therapeutic benefit in AIDS. Professor Wolfgang Scherer (Rudolf Virchow University Hospital, Wedding, FRG) and associates reported that EPO levels in HIV-positive patients are not statistically different from seronegative individuals. Based on their findings, these researchers concluded that the anemia (marked reduction in red blood cells) observed in some patients with HIV infection, including patients on AZT therapy, is not caused by an absolute or relative lack of EPO.

Table 6.3. Combination Therapies: Antivirals & Immunomodulators

Combination	Schedule	Conference Reports
AZT & AS-101	Concurrent administration (1200 mg AZT per day; 3 mg/m^2 AS-101 3 times/week)	Dr. Henry Masur (NIAID, Bethesda) and colleagues reported preliminary findings from a combination Phase I trial in patients with CD4 cell counts less than 200/mm^3 or less than 20% of circulating lymphocytes. Although no hematologic toxicity has been noted, neither have any immunpotentiating effects in patients who have received therapy for 12 weeks. Adverse reactions to AS-101 included skin rashes, garlic body odor, and fever.
AZT & alpha-IFN	Concurrent administration (600 mg AZT daily; 1 x 10^6 units IFN daily)	In an ongoing study in patients with early HIV infection (CD4 cell counts greater than 500/mm^3) comparing this combination with component single agents, Dr. Clifford Lane (NIAID, Bethesda) reported that, so far, all 3 treatment groups have shown decreases in p24 levels. Whether this drop will be sustained must be determined. Victoria Davey (NIAID, Bethesda) reported that of the 120 patients enrolled in this randomized placebo-controlled trial, none have reached study end points: CD4 decline, % CD4 declines, or development of an opportunistic infection. The mean tolerated doses determined from patients completing 28 weeks of therapy were 600 mg daily (AZT) and 2 x 10^6 units daily (IFN). The relative efficacy of this combination should be analyzable after another 6 months.
AZT & acemannan	Concurrent administration (1000 mg AZT daily; 1000 mg acemannan daily)	Danielle Weerts (St. Pierre University Hospital, Brussels) and coworkers reported preliminary findings from a 24-week double-blind randomized trial in ARC patients comparing the combination with acemannan alone. Acemannan appeared to be well tolerated at a daily dose of 1000 mg either alone or in combination with AZT; hematologic toxicity of AZT, however, was not modified by adjunctive therapy with acemannan.
AZT & erythropoeitin (EPO)	Concurrent administration (EPO given 3 times/week)	In their placebo-controlled trial, John Carey (Case Western Reserve University, Cleveland) and colleagues found that subcutaneous administration of EPO to anemic patients on AZT therapy does not appear to have any effect on p24 levels.
AZT & G-CSF	Concurrent administration (G-CSF given in daily dose of 2 mcg/kg)	Using G-CSF as adjunctive therapy in 12 ARC/AIDS patients, Poll Van der Wouw and colleagues (Academic Medical Center, Amsterdam) found that G-CSF (by subcutaneous injection at a dose of 2 mcg/kg) effected an increase in white blood cell, monocyte, and neutrophil counts (neutrophils rose to levels greater than 3000/mm^3). Counts returned to pretreatment levels after discontinuation of G-CSF (at end of study). Tolerable side effects were noted in some patients.
AZT & GM-CSF	Concurrent administration (GM-CSF in daily dose of 0.3 mcg/kg)	Ross Hewitt (SUNY, Buffalo) and coworkers reported that in 9 of 11 patients receiving continuous GM-CSF therapy (for AZT-induced neutropenia) their absolute neutrophil counts increased to at least twice their pretreatment levels. A 33% decline in p24 levels was noted in 4 patients after 28 days of concurrent therapy. Although therapy was generally well tolerated, 4 patients withdrew from the trial due to development of malaise, fever, or nausea. Gene Morse (SUNY, Buffalo) and coworkers noted increased AZT bioavailability in these patients. Trial participants were receiving 300–1200 mg AZT daily.

Combination	Schedule	Conference Reports
AZT & IL-2	AZT therapy alone for 8 weeks, AZT/IL-2 for 4 weeks, another 8 weeks of AZT therapy alone	Although previously restricted to patients with Kaposi's sarcoma, this combination trial now includes asymptomatic patients with CD4 cell counts greater than 200/mm^3. John Bartlett (Duke University Medical Center, Durham, North Carolina) and coworkers reported that both drugs are well tolerated when administered as described. Specific immunologic parameters, such as NK cell activity, LAK activity, and non-MHC restricted cellular cytotoxicity, rose dramatically in 70% of the group during the IL-2 part of therapy, but absolute CD4 cell counts only rose slightly and the increase was not sustained. Similar findings for cell-mediated immune parameters were noted in a separate clinical trial with asymptomatic patients with CD4 cell counts greater than 400/mm^3 conducted at Stanford University Medical Center (California) by David H. Schwartz and colleagues. Both studies concluded that further study is warranted.
AZT & GM-CSF & alpha-IFN	Concurrent administration (daily GM-CSF initiated when neutrophils fall below 1000/mm^3)	Richard Davey and coworkers (NIAID, Bethesda) reported that low-dose GM-CSF (1 µg/kg/day) effectively raises mean neutrophil counts in patients on AZT/IFN therapy. Significant toxicities, however, are noted at higher doses. At 2 weeks into GM-CSF therapy (by subcutaneous administration), mean neutrophil counts rose from pretreatment levels of 872/mm^3 to 6686/mm^3 (in patients on low dose AZT—600 mg daily) or from 695/mm^3 to 4505/mm^3 (for patients on high dose AZT—1200 mg daily). The authors commented that the antiviral effects of AZT/IFN were not compromised by addition of GM-CSF. Similar findings were reported in patients with Kaposi's sarcoma by Susan Krown and colleagues (Memorial Sloan-Kettering Cancer Center, New York).
AZT & IV IgG	Concurrent administration	Ulrike Brunkhorst and associates (Hannover Medical School, Germany) are evaluating IVIgG adjunctive therapy in 25 ARC/AIDS patients on AZT. Pretreatment serum Ig levels were elevated in all study participants and analysis of cell surface marker revealed that late B cells/plasma cells were increased while early, proliferable B cells were decreased. At 3 and 6 months into the therapy, the authors hope to have safety and efficacy data.
Antisense oligo-nucleotide & alpha-IFN	Not applicable (*in vitro* studies)	Dr. Ting-Chao Chou (Memorial Sloan-Kettering Cancer Center, New York) and coworkers found slight to moderate anti-HIV synergism in T cell cultures exposed to HIV and to which an antisense oligonucleotide (s-dC-28) and IFN were subsequently added. Evaluation of the anti-HIV properties of the combination at varying concentrations are planned.
ddI & alpha-IFN	ddI for 6 weeks, ddi/IFN concurrently for 28 weeks	A Phase I safety trial, just started at NIAID, will evaluate 2 doses of ddI (167-250 mg twice daily and 250-375 mg twice daily) and 4 doses of alpha-IFN (1,5,10, and 15 x 10^6 units). *In vitro* findings of synergistic anti-HIV effects (without additive cytotoxicity) for this combination were reported by Victoria Johnson (Massachusetts General Hospital/ Harvard University, Boston).

The discovery of CSFs has attracted the attention of oncologists and researchers studying the toxic effects of cancer chemotherapy on blood cells. Because the large majority of HIV-infected individuals have decreased white blood cell counts, particularly CD4 cell counts, CSFs are being evaluated for their ability to boost production of these blood cell types. Furthermore, evidence that normal proliferation of bone marrow stem cells (the precursors of some white blood cells) is depressed in HIV-infected patients also supports the potential therapeutic value of CSF administration in these patients. CSFs are thought to be especially promising in HIV-infected patients with lymphoma or Kaposi's sarcoma, since in these patients cancer chemotherapy causes severe neutropenia (a decrease in a subset of white blood cells) and/or anemia; clinical trials are ongoing.

Experimental Immunomodulators. Table 6.1 includes all agents being developed and tested for their potential immunomodulating effects. Some immunomodulators have also been found to possess antiviral effects. Discussed below are some of the substances presented at the Conference.

Acemannan. Two presentations at the Conference this year reported preliminary results from clinical safety and efficacy trials of the polysaccharide acemannan, a purified extract from the aloe vera plant. Both the natural and the purified plant products have been postulated to stimulate immune function—particularly that of monocytes and macrophages involved in the inflammatory process. Antiviral effects of acemannan were reported last year by Dr. Maurice Kemp (Texas A & M University, College Station) and colleagues: HIV-infected T cell cultures treated with acemannan showed reduced infectivity. These investigators are now trying to determine whether acemannan's antiviral activity is the result of inhibition glycosylation of the viral envelope proteins. Clinical trials reported by Danielle Weerts (St. Pierre University Hospital, Brussels) and colleagues and by Reg McDaniel (Dallas-Fort Worth Medical Center, Grand Prairie) found acemannan to be well tolerated in asymptomatic and ARC/AIDS patients; no significant toxicities were noted.

AS-101. The synthetic organometallic compound ammonium trichloro tellurate (AS-101) is reported to have potent immunomodulating properties. Stimulation of production of interleukin-2 (IL-2), IL-3, tumor necrosis factor, interferons, and GM-CSF were originally noted both *in vitro* and *in vivo* by researchers at Bar Ilan University, Israel. In the United States, AS-101 is being studied as part of combination therapy with AZT (see Table 6.3).

Imreg-1. An extract from healthy white blood cells, Imreg-1 (the trade name registered by Imreg, Inc., New Orleans) has been shown to stimulate IL-2 and gamma interferon production *in vitro*. (IL-2 is a cellular growth factor that stimulates the proliferation of certain T lymphocytes.) The effects of Imreg-1 *in vivo* include increased IL-2 production, partial or complete restoration of skin responsiveness to immunologic challenge, and a slower rate of CD4 cell depletion. Robert Zackin (Imreg, Inc., Cambridge, Massachusetts) and colleagues presented data from a multicenter clinical trial of Imreg-1 in ARC patients who received therapy for more than 6 months. Compared to patients receiving placebo, patients on Imreg therapy were noted to have fewer AIDS-related events.

Imuthiol. The sulfur-containing compound diethyldithiocarbamate, or DTC (marketed as Imuthiol by the Merieux Institute, France), binds to heavy metal ions and free oxygen radicals, which are generally toxic to cells. Imuthiol is also thought to inhibit the synthesis of prostaglandin, a hormone with immunosuppressive effects. It has been hypothesized that, because of its ability to bind to heavy metal ions, Imuthiol may inhibit the zinc- or magnesium-dependent HIV *tat* gene product, which is known to increase the rate of HIV replication.

Dr. Evan Hersh (University of Arizona, Tucson) and colleagues reported preliminary results of a placebo-controlled trial of oral Imuthiol in 191 AIDS and ARC patients. Imuthiol-treated patients had fewer AIDS-related events, including development of opportunistic infections and drops in the CD4 cell count. However, the death rate was the same for both treatment

and placebo groups. Earlier this year, researchers from Bernard Nocht Institute for Tropical Medicine (Hamburg, FRG) and members of the German DTC Study Group reported that, in a placebo-controlled clinical trial in 60 pre-ARC or pre-AIDS patients, intravenous Imuthiol administration was associated with a significant delay in the progression to AIDS.

Thymopentin. This synthetic peptide, which contains amino acid sequences corresponding to those found in a natural thymic hormone, is being tested for its immune enhancing properties—specifically, its ability to induce differentiation of thymocytes, the precursor cells that differentiate into immunologically active T lymphocytes. Results from clinical trials reported at the Conference showed that, in contrast to placebo groups, HIV-infected asymptomatic or mildly symptomatic patients treated with thymopentin had a response in CD4 cell counts, percentage of CD4 cells, and CD4/CD8 cell ratios: levels were either maintained at or increased above baseline (their value at study entry).

COMBINATION THERAPY

ANTIVIRAL AGENTS

Combination therapies using antiviral drugs with differing mechanisms of action are thought to hold the most promise for controlling and combating HIV infection. Combination drug therapies, in comparison to single agent therapies, should increase the possibility of successfully fighting HIV for several reasons:

- lower single doses can be used, which, in turn,
- decreases the prospect of side effects and
- decreases the risk of emergence of drug-resistant virus strains.

A synergistic interaction (enhancement of either agent's biologic activity) has been shown to result *in vitro* from various combinations of antiviral agents—for example, between AZT and antiviral agents known to disrupt DNA polymerase function, such as acyclovir. Table 6.2 outlines the combinations currently under investigation, both *in vitro* and in clinical trials.

ANTIVIRAL AND IMMUNOTHERAPEUTIC AGENTS

Because HIV is capable of destroying the immune system so thoroughly, combining antiviral agents with immunomodulators is considered to be particularly relevant for the treatment of HIV-positive individuals. As more information about the AIDS virus and its pathogenesis accumulates, it has become apparent that immunorestoration in the patient is an integral component of therapy. Matching antiviral agents with natural or synthetic immunomodulators may

- preclude the development of drug-resistant viral strains,
- allow for lower single doses of the individual agents, which may
- diminish short- and long-term side effects, and
- counteract the bone marrow or blood related toxicities frequently associated with many of the anti-HIV agents now in development.

Combination clinical studies are not initiated until each of the individual agents in the combination have been fully evaluated, since it is imperative to know each agent's full range of effects before beginning combination studies. *In vitro* experiments must demonstrate synergistic activity without additive toxicity. Some studies now in progress show that combination therapy using intermittent or alternating, rather than concurrent, administration of the individual agents imparts clinical and immunologic benefits to the patients. Table 6.3 summarizes the combination therapies currently being explored.

CHAPTER 7

Page 169 **BACKGROUND**
169 Disease Course
170 Diagnosis
171 **DISEASE PROGRESSION**
171 Factors Affecting Disease Progression
173 Laboratory Markers of Disease Progression
179 **PATIENT CARE STRATEGIES**
181 **ANTIVIRAL THERAPIES**
181 AZT
186 Dideoxycytidine (ddC)
186 Dideoxyinosine (ddI)
187 Soluble CD4
187 Peptide T
188 GLQ223 (Compound Q)
188 **OPPORTUNISTIC INFECTIONS**
189 Pneumocystis Carinii Pneumonia
191 Cytomegalovirus Disease
193 Toxoplasmosis
194 Mycobacterial Infections
197 Mycoplasma
197 Bacterial Diseases
204 Systemic Fungal Diseases
205 **ORAL INFECTIONS AND LESIONS**
206 **CANCERS AND NEOPLASMS**
206 Kaposi's Sarcoma
208 Lymphomas
209 Other Cancers
209 **NUTRITION**
210 HIV and the Gastrointestinal Tract
211 Cachexia
211 Nutritional Status and Immunological Function
211 Assessment of Nutritional Status
212 Nutritional Intervention
213 **CARE DELIVERY**
213 Hospital-Based Care & Associated Costs
215 Outpatient Services & Associated Costs
216 Early HIV Intervention
218 Changing Patterns of Care
221 Costing Studies
223 **PEDIATRIC HIV INFECTION**
224 Clinical Assessment
224 Clinical Manifestations
225 Disease Progression and Prognosis
226 Treatment and Clinical Trials
228 Prevention

CLINICAL MANAGEMENT

BACKGROUND

Advances are being made in the clinical management of HIV-related disease, albeit not at a pace sufficient to assist all those with progressive disease: HIV is still outdistancing its pursuers. However prophylactic options and early therapeutic intervention are growing in availability and potential benefits. Further complicating the already massive challenge—to both the patient and the clinician—is the interaction of the physiological, psychological, social, and economic factors related to the HIV epidemic. Effective clinical management of the HIV-infected patient requires a thorough understanding of the natural history of HIV disease and treatment options available. The devastating consequences of medical complications, such as opportunistic infections and cancers, in an already immunocompromised patient mandate heightened anticipation and, when possible, treatment. Persons who suspect they have been exposed to HIV or who engage in (or, in the last 10 years, have engaged in) AIDS risk behaviors are urged to be tested for HIV as early as possible since new clinical trial information has demonstrated that persons treated early in the disease course reap greater benefits than those in later stages of disease (see, in particular, "AZT," below).

This chapter reviews current concepts regarding the natural history of HIV disease, summarizing information on disease progression, clinical trials of antiviral agents, and recommendations for prophylaxis and treatment of opportunistic infections as reported at the Conference. Also covered are nutritional issues, patient care strategies, and costs associated with care for HIV-infected individuals. (Note that while clinical trial data relating to antiviral agents are discussed in this chapter, details on the mechanisms of action of these and other agents being tested for efficacy against HIV are presented in Chapter 6: ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS.)

DISEASE COURSE

Current data and cumulative clinical experience demonstrate that HIV causes a chronic, progressive immune deficiency disease with variable manifestations. In broad strokes, HIV disease can be described as a continuum, or spectrum, of "silent" immunological changes and clinical events that tend to occur in sequential stages. The rate of movement along this

continuum varies extensively for each individual and may be influenced by a number of factors—notably, mode of transmission, age and health at the time of infection, co-existing infectious diseases, and, perhaps, the pathogenicity of the infecting HIV strain or variant. (As discussed in detail in Chapter 2: PATHOGENESIS & IMMUNOLOGY, the pathogenicity—that is, the ability to cause disease—of HIV strains may vary. Indeed, long-term survivors have been shown to harbor HIV isolates that are much less pathogenic than isolates found in individuals who have died of HIV-related disease.) Nevertheless, the so-far predictable outcome of HIV infection is the development of increasingly severe immune deficiency.

At the time of initial exposure to HIV, persons may develop a transient flu-like illness that lasts about 3 to 14 days or longer. Physicians first recognized an acute-illness phase in retrospective cases studies—cases in which the time of exposure was known (in transfusion-associated HIV infection, for example). Some individuals experienced the illness after a lag period of about 3 to 6 weeks after exposure. Most other cases cannot be traced to the time of initial exposure, but acute illness has been documented in approximately 20-30% of cases.

Figure 7.1 depicts the progressive disease stages observed in HIV infection. (See Appendix C: CDC & WALTER REED STAGING CLASSIFICATION SYSTEMS OF HIV INFECTION for details on stage-specific symptoms and characteristics.)

HIV disease, in all its phases, is a chronic illness of a duration that depends on many influencing factors, including therapeutic intervention. The median time from initial infection to development of disease is currently estimated to be about 8 years. In some HIV-infected populations (specifically, a subset of homosexual and bisexual men participating in the San Francisco's Men Health Study), 10 or more years may elapse after infection before 50% of the population develops advanced disease; some individuals may remain in apparent good health for more than 12 years.

Once advanced disease occurs, survival depends on the specific complication(s) that arise and on the effectiveness of and access to therapy, but it may often exceed 2 to 3 years. The occurrence of opportunistic infections or neoplasms in HIV-infected persons, in general, significantly hastens the disease process. Treatment may both prolong the presymptomatic phase and improve survival once symptoms occur. HIV disease, then, can be characterized as a chronic long-term illness with an estimated survival period of 5–15 years.

DIAGNOSIS

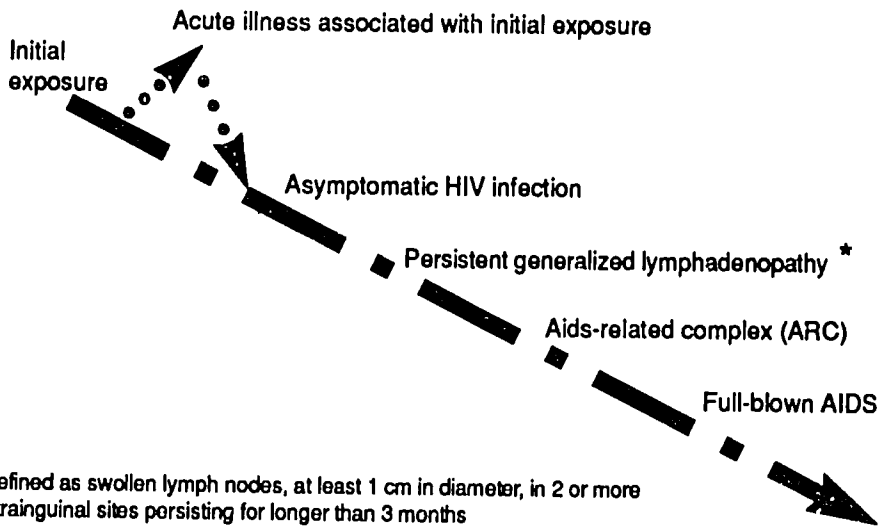
Diagnosis of HIV infection is generally based on the results of HIV-antibody tests (detection of antibodies specific to HIV indicate the presence of the virus). Other types of tests are in development, but their use may be limited to research purposes or to special circumstances—such as the diagnosis of pediatric (neonatal) HIV infection. (See Chapter 4: DIAGNOSTIC TESTS FOR HIV for a detailed discussion on the various types of commercially available tests as well as new tests in development.)

A diagnosis of AIDS is made when there is laboratory evidence of HIV infection (a positive, confirmed antibody test) and clinical evidence of specific diagnosable conditions that have been designated as indicative of AIDS (see Appendix A: CENTERS FOR DISEASE CONTROL DEFINITION OF AIDS). Note that in some instances, an AIDS diagnosis is based on laboratory evidence of HIV infection and a *presumptive* diagnosis of indicator disease(s).

An AIDS diagnosis can also be made in special cases in which laboratory confirmation of HIV infection is lacking; the diagnosis is then based on declining levels of a key immune parameter—CD4 cell count (500/mm^3 or less, provided other causes of immunodeficiency are ruled out)—and a definitive diagnosis of an indicator disease. (In the context of AIDS research, the term CD4 cell—previously T4 cell—refers to the helper/inducer subset of T cells; CD4 is a cell surface molecule found on these T cells.)

Figure 7.1. Stages of HIV-Related Disease

Approximately 3-6 weeks after exposure to HIV, some individuals develop an acute flu-like illness. This phase resolves itself and is followed by an asymptomatic period during which immunological changes occur—primarily affecting the number and function of subsets of immune cells. Considerable variation is seen from patient to patient and many factors are thought to influence disease progressions to PGL, ARC, and AIDS; however, a "typical" progression is outlined as follows.



*defined as swollen lymph nodes, at least 1 cm in diameter, in 2 or more extrainguinal sites persisting for longer than 3 months

Note that some controversy surrounds the stringency of the criteria for an AIDS diagnosis using the CDC definition: Although the 1985 definition was updated in 1987 to be more inclusive, many clinical investigators feel that the current definition should be further expanded to include infections or symptoms that although not necessarily commonly encountered, are nevertheless clearly HIV related. Women with HIV infection, for example, may develop pelvic inflammatory disease, a manifestation not currently included in the CDC definition. Also omitted from the list of indicator diseases in the CDC definition is *pulmonary* tuberculosis—a condition which is increasingly being diagnosed in HIV-infected individuals. In the United States, where a national health plan does not exist, but Medicaid provides a safety net for the disabled, not being diagnosed with AIDS can mean denial of disability and therefore federal health benefits. Other reasons for concern with the criteria for an AIDS diagnosis include gauging the magnitude of the consequences of HIV infection.

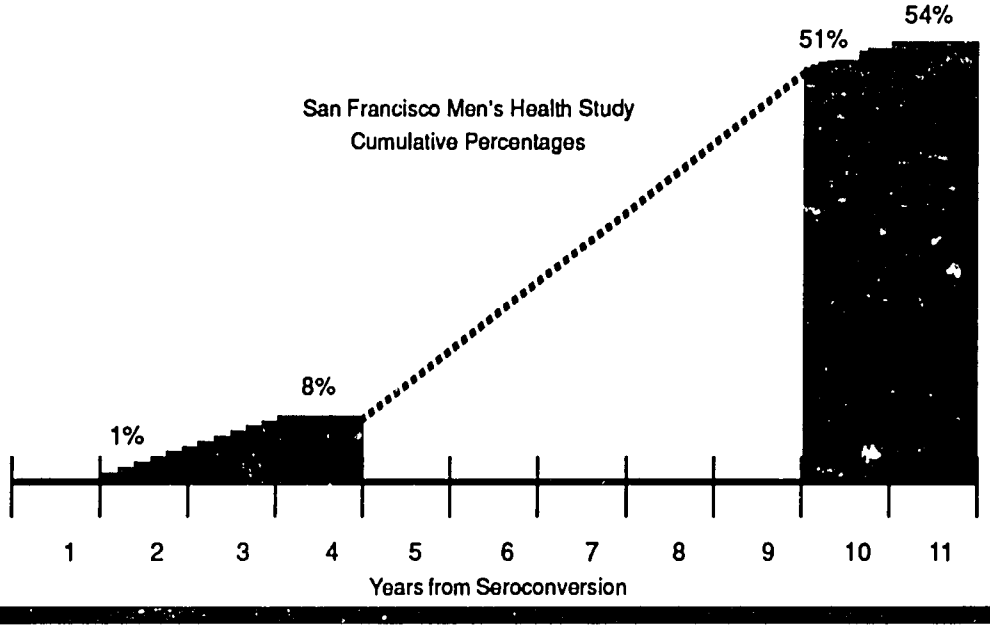
DISEASE PROGRESSION

FACTORS AFFECTING DISEASE PROGRESSION

In the course of any chronic disease, the passage of time is both the holder of hope and the patient's greatest adversary. HIV disease has proved to be no exception. Previous studies addressing time as an independent factor have noted both the regularity of HIV disease progression and the tremendous variability in the rate of progression. At the Conference, Dr. Alan Lifson (Department of Public Health, San Francisco) observed that, based on an ongoing cohort study of homosexual and bisexual males with known dates of seroconversion, the risk of immunodeficiency increases with duration of HIV infection; see Figure 7.2. (Cohort studies are those which follow, over time, a well-characterized group, such as persons with a common risk factor.)

Figure 7.2. Duration of Infection and Disease Progression

Based on the San Francisco Men's Health Study of homosexual/bisexual men with known seroconversion dates, Dr. Alan Lifson (Department of Public Health, San Francisco) reported that of 489 men, 1% progressed to AIDS within 2 years, 8% within 4 years, 51% within 10 years, and 54% within 11 years (cumulative percentages). Some men in the cohort were still immunocompetent (defined as having CD4 cell counts greater than 500/mm^3) 9 years after seroconversion. Dr. Lifson observed that the risk of immunodeficiency increases with duration of HIV infection. Although these observations were made on a defined population, the findings are, with few exceptions, considered to be representative of the time course of HIV disease progression.



Other factors influencing HIV disease progression, in addition to duration of infection, reported at the Conference included:

- *Age.* HIV-infected hemophiliacs older than age 35 were noted to progress to AIDS at a faster rate than younger hemophiliacs (Dr. Nicola Schinaia, Instituto Superiore di Sanita, Rome); Dr. Andrew Phillips and colleagues (Royal Hospital & School of Medicine, London) used 25 years as the age criterion but still observed this age-related phenomenon—that older hemophiliacs develop AIDS within a shorter time frame than younger hemophiliacs. Among intravenous drug users (IVDUs), Dr. Stanley Weiss (University of Medicine & Dentistry of New Jersey, Newark) and coworkers and, independently, Dr. Roy Robertson (Edinburgh Drug Addiction Study) and associates noted that older HIV-infected IVDUs also have a significantly higher risk of developing AIDS.
- *Immunogenetic Markers.* Genes involved in defining an individual's immune system, the HLA (histocompatibility) genes, were shown to be significantly different between asymptomatic HIV-infected men and men persistently symptomatic or men with AIDS. The HLA DR-beta gene appears to be more common among asymptomatic men; persistently symptomatic men or men with AIDS have some different form of this gene (Leslie Louie, University of California, Berkeley).
- *IV Drug Use.* Cessation of IV drug use was demonstrated to be positively correlated with decreased progression rate (Dr. Rainer Weber, University of Zurich).
- *Cigarette Smoking.* Based on cohorts of homosexual and bisexual men, smoking was found to adversely affect HIV disease progression (Rachel Royce and Warren Winkelstein, University of California, Berkeley and, independently, Lawrence Park and colleagues, MACS, Bethesda, Maryland).

Formal and informal discussion about these and other factors influencing disease progression continues. Some researchers argue that the nature of the virus itself largely dictates the timetable of the disease course, and that the factors implicated in influencing disease progression identified thus far play only a minor role. HIV strains with varying degrees of pathogenicity, for example, may account for most of the variability observed in disease progression. Drawing on data from a review of the literature as well as from his own experience, Dr. Godfried J.P. van Griensven (Amsterdam Municipal Health Service) reported that infection acquired through unprotected sexual intercourse with a person with AIDS appears to be a strong determinant of a faster rate of disease progression. Dr. van Griensven (and others he cited) posit that HIV recovered from persons with AIDS is likely to be more virulent than isolates from asymptomatic HIV-infected persons.

Alternatively, nonbiologic factors, such as socioeconomic factors, may be involved in determining disease progression, as was clearly demonstrated by several presentations: In the United States, for example, minorities and economically disenfranchised HIV-infected people tend to have less access to appropriate clinical care; and medical care, when they do receive it, is more likely to be given later in the course of the disease process. (See "Care Delivery," below.)

Many clinicians believe that psychological and spiritual health contribute positively to maintaining a patient's physical health, whereas a negative attitude and poor self-care are detrimental. Producing scientific evidence of such influences has been difficult, perhaps because of the subjective nature of the effects being measured. Nevertheless, findings reported by Dr. Margaret Kemeny (University of California School of Medicine, Los Angeles) and coworkers, demonstrate that chronic depression may precede and even contribute to immunodeficiency (measured by declines in specific immunological parameters). Dr. Kemeny's team showed that 25 of 35 (71%) depressed HIV-infected homosexual men followed for 5 years had a 20% decline in their CD4-cell percentages compared to 36 of 70 (51%) of nondepressed controls.

LABORATORY MARKERS OF DISEASE PROGRESSION

Certain measurable biological and clinical laboratory parameters have been found to correlate with HIV disease stages and rate of disease progression. Some of these parameters have considerable utility in characterizing disease status and progression and may be helpful in planning patient care. A few of them have become important indicators in clinical decision making; for example, CD4 cell counts help determine when to initiate AZT (zidovudine) therapy or prophylaxis for *Pneumocystis carinii* pneumonia (PCP). Others still under study include virus-specific parameters, such as p24 antigen levels, which are thought to reflect HIV activity in the body. With further characterization and correlation with disease stage, these parameters might prove useful in monitoring the efficacy of specific therapeutic agents or vaccines; unfortunately, the precise utility of many of these markers remains uncertain and controversial. The numerous correlations noted thus far have value primarily as indicators of the patterns of HIV-related disease; their application in daily clinical practice, however, may be quite limited. Table 7.1 catalogs the various markers currently under evaluation.

Immunological Markers. A large number of laboratory parameters continue to be investigated for their reliability in serving as markers of immune activation. Most are not specific for HIV, but appear to show a positive correlation with advancing disease stages. (Note that although the metabolite neopterin is considered a nonspecific marker of immune activation—levels are significantly elevated in a great majority of persons with infectious diseases and cancer—for categorization purposes, it is discussed below; see "Metabolic Markers.") HIV-specific immunological markers currently being evaluated include antibody responses to specific HIV antigens—for example, antibodies to p24 antigen and antibodies to nonstructural HIV antigens (such as *nef*, *rev*, *tat*, *vpu*, and *vpr*). Discussed here are markers that have been particularly well characterized.

Table 7.1. Laboratory Markers of HIV Infection

Immunological Markers		Virological Markers	Metabolic Markers
CD4 cell count	CD4 cell %	p24 antigenemia	Neopterin levels
CD8 cell count	CD8 cell %	HIV isolation (viremia)	Steroid hormones levels
CD4: CD8 ratio	Soluble CD8 levels	HIV DNA/RNA detection	
Beta-2-microglobulin levels	Interleukin-2 receptor levels	Circular (unintegrated) HIV DNA detection	
Cutaneous antigen reactivity	Monocyte-tissue factor expression	Reverse transcriptase levels	
Antibody levels (antibody to p24; antibody to nonstructural proteins)	Alpha interferon class (acid labile vs. stable)		

CD4 Cell Counts and Percentages. The most studied and probably the most useful clinical gauge of immune status in HIV-infected individuals, CD4 cell counts help guide decision making regarding administering antiviral agents and initiating prophylaxis for opportunistic infections. Because HIV preferentially infects and kills CD4 cells, it is not surprising that CD4 cell counts drop precipitously with progressive HIV disease. (The normal range of CD4 cell counts, in healthy individuals, is 800–1200/mm^3.) The Centers for Disease Control (CDC) Classification System and the Walter Reed Staging Classification System include CD4 cell counts as a differentiating criterion for disease stages (see Appendix C).

At the Conference, Dr. Steven Lagakos (Harvard University, Boston) and coworkers presented data showing that lower baseline CD4 cell counts predicted both a more rapid decline in CD4 levels and clinical progression to advanced HIV disease. Professor Court Pedersen (University of Copenhagen) reported an association between the severity of the initial decline in immune function immediately following HIV infection and the rapidity of subsequent disease progression. His study found that patients whose first year of HIV disease was characterized by lower CD4 cell counts were more likely to experience rapid disease progression. Using a retrospective approach, Suzanne Crowe (MacFarlane Burnet Centre for Medical Research, Melbourne) and colleagues reported a relationship between disease progression and declining CD4 counts that affirms the current practice of prescribing antiviral therapy when the CD4 cell count falls below 500/mm^3 and prophylactic therapy for PCP when below 200/mm^3.

Nevertheless, depending exclusively on CD4 counts to guide decision making clearly has its limitations and hazards: Nicole Denomme and colleagues (Ottawa General Hospital/University of Ottawa AIDS Study Group) and Mark Packard and coworkers (Duke University Medical Center, Durham, North Carolina) both presented data showing significant "intraperson" variability in serial CD4 cell counts collected over a short-term interval (1 to 2 months). Dr. Packard's group found the greatest variability in individuals with asymptomatic disease; patients with AIDS showed the least variability. (Dr. Denomme's team only studied asymptomatic HIV seropositives.)

When serial *percentages* of CD4 cells were evaluated (that is, the percentage of CD4 cells relative to the total of circulating lymphocytes) for the same time period, however, intraperson variation was significantly less. Both research groups recommended that clinicians use CD4 percentage values rather than absolute counts. These studies substantiate the frequent observation that the percentage of CD4 cells is much less volatile than the absolute CD4 count.

Lymphocyte Counts. An interesting study of HIV-infected asymptomatic and non-AIDS patients by Michael Para and colleagues (Ohio State University AIDS Clinical Trial Unit,

Columbus) suggested that ordinary absolute lymphocyte counts offer a reliable alternative to the more expensive CD4 assays in certain circumstances. Dr. Para's team demonstrated that lymphocyte counts exceeding 1500/mm^3 correlated with a CD4 level of at least 200/mm^3. Although the authors did not find a lymphocyte count that reliably correlated with a CD4 level greater than 500/mm^3, they did find that a lymphocyte count of less than 1450 reliably indicated a CD4 level of less than 500/mm^3. (WBC, platelet count, and hemoglobin added no further discrimination.) This is an especially important finding for clinicians caring for patients with limited access to care or limited financial resources because of the lower laboratory costs.

Beta-2-Microglobulin. A protein detectable in serum, beta-2-microglobulin is tightly linked with a class of human major histocompatibility proteins found on the surface of certain white blood cells. (The major histocompatibility proteins play a role in the recognition of "self" antigens found on every cell in the body.) Conference studies this year supported previous findings that increased beta-2-microglobulin levels correlate with advanced disease stage and that monitoring these levels may serve as evidence of HIV disease progression. Dr. John Fahey and colleagues (Multicenter AIDS Cohort Study, Los Angeles), for example, noted that beta-2-microglobulin levels were 30% higher in men at the time of diagnosis of AIDS, than in matched cohort members who did not develop AIDS. Based on a 6-year study of 131 HIV-positive homosexual men, Dr. Alexander Kramer (University Tubingen, FRG) and colleagues reported that AIDS incidence rates were significantly higher among homosexual men with high beta-2-microglobulin levels (greater than 3 mg/l) than in those with medium levels (2-3 mg/l), and those with normal levels (less than 1 mg/l); measurements were taken 1 year after (known or estimated) seroconversion.

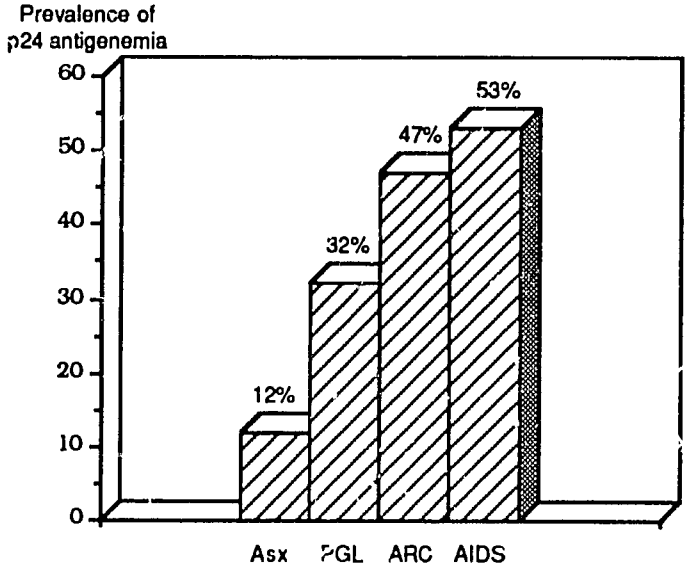
Dr. Robert Clarkson (HIV Immunology Unit, Edinburgh) and associates reported significantly elevated levels of beta-2-microglobulin among both HIV-negative (2.89 mg/l) and HIV-positive IVDUs (4.24 mg/l); HIV-positive IVDUs in later stages of HIV disease showed the highest levels (4.87 mg/l). Katherine Davenny and coworkers (Montefiore Medical Center, Bronx, New York) also noted strikingly elevated levels of beta-2-microglobulin in seronegative and seropositive IVDUs. Although asymptomatic HIV-positive IVDUs had higher mean beta-2-microglobulin values than their HIV-negative counterparts, the finding of abnormally high values in asymptomatic HIV-negative IVDUs in and of itself suggests that non-specific markers may be signaling other causes of immunodeficiency and may therefore confound clinical assessment. (Dr. Davenny's team also documented abnormal values in CD4 cell counts and CD4: CD8 ratios for the asymptomatic seronegative IVDU group.)

Virological Markers. Detection of virus or viral components (antigens, DNA, or RNA) in serum or plasma samples from individuals in various disease stages—or from a single individual at different times—has thus far not yielded a reliable measure of viral load or viral activity in the body. Isolation of virus from blood cells and detection of DNA or RNA requires what seems an inordinate amount of time and technical expertise (see "Viremia," below). Continued studies on HIV antigen levels in HIV antibody-positive persons have revealed certain patterns that may aid in predicting disease progression. Clinical researchers are especially interested in finding a reliable virological marker that accurately reflects disease progression (or stasis) in the body, thereby providing a means by which to monitor the efficacy of a particular therapy.

Antigenemia. Viral antigens—specifically HIV's core antigen, p24—can be detected in the serum of infected individuals but apparently only at particular points during the disease course. HIV's p24 antigen is first detectable in the blood within 1 to 2 weeks of initial HIV infection, well in advance of the presence of detectable serum antibodies. However, for reasons not entirely clear, p24 then disappears and remains undetectable through the asymptomatic phase of disease in most patients. HIV p24 antigen then reappears, usually years later, and this reappearance is associated with increasing short-term risk of serious clinical illness. Diagnostic tests that detect p24 have come into wider use during the past year,

Figure 7.3. Association Between p24 Antigenemia and HIV Disease Stage

Free p24 antigen, but not immune-complexed p24, was found to increase in prevalence with advancing disease progression. Free p24 antigen was found in 3 (12%) of 25 asymptomatic (Asx) patients, in 15 of 47 (32%) patients with progressive generalized lymphadenopathy (PGL), in 7 of 15 (47%) patients with ARC, and in 10 of 19 (53%) patients with AIDS. (Based on a report by Dr. Luc Kestens, Institute of Tropical Medicine, Antwerp, and colleagues.)



and more clinicians now use them to assess the likelihood of disease progression among asymptomatic as well as symptomatic HIV-infected individuals. (See Chapter 4: DIAGNOSTIC TESTS FOR HIV for a detailed discussion on p24 antigen tests.)

The appearance and decay of serum p24 levels during the course of HIV disease dictates cautious interpretation of test results; a positive or negative result must be interpreted relative to the patient's disease course. In monitoring patients with established disease, HIV antigen testing is more useful than during asymptomatic stages, but, again, offers no absolutes: the appearance of antigen (and, especially, of a rising titer) suggests that disease progression is occurring at a substantial pace, but the absence of HIV antigen does not rule out that same possibility.

Two intriguing presentations looked at serum p24 antigen as "bound" antigen (p24 antigen complexed with antibody) and as "free" antigen. (A laboratory protocol whereby bound p24 can be released from the antigen-antibody complex precipitated from HIV-positive sera allows for the differential detection of p24 in its free and bound forms.) Dr. Luc Kestens (Institute of Tropical Medicine, Antwerp) and colleagues noted that p24 antigen detection in serum increases significantly after p24 antigen-antibody complexes (or immune complexes) are dissociated. Furthermore, analysis of sera from 106 HIV antibody-positive individuals revealed that bound p24 (antigen-antibody complexes) are associated with low CD4 cell counts:

CD4 cell count	<200 cells/mm^3	200-400 cells/mm^3	>400 cells/mm^3
p24 antigen ("free")	14/29 (48%)	8/23 (33%)	13/54 (24%)
p24 antigen-antibody complex	18/29 (62%)	8/23 (33%)	16/54 (30%)
free or complexed p24	21/29 (72%)	14/23 (58%)	18/54 (33%)

When the authors looked at free and bound p24 in relation to clinical stage, both forms of p24 antigen were detectable at every stage. An increasing prevalence of free p24 antigen correlated with disease progression (see Figure 7.3). Although bound p24 antigen was not significantly associated with clinical stage, it may be of interest to determine the prognostic value of immune-complexed p24 in asymptomatic individuals.

Dr. Roger Detels and coworkers (Multicenter AIDS Cohort Study, Los Angeles) also noted that free p24 antigen (either alone or as part of p24 immune complexes) is associated with disease progression and that the highest titers of immune-complexed p24 were always found in asymptomatic individuals. However, the authors reported that, based on their findings in 400 seropositive men, the predictive value for AIDS using either free or complexed p24 was less than that for other markers studied (beta-2-microglobulin, CD4 cell counts, IgA levels, and neopterin).

Dr. Betsy Ohlsson-Wilhelm and collaborators (Pennsylvania State University College of Medicine, Hershey) reported that quantitation of cell-associated p24 by flow cytometry may also provide a means by which to monitor the degree of HIV infection *in vivo*. HIV-infected lymphocytes (producing HIV p24 protein, and therefore harboring actively replicating HIV) can be quantified using flow cytometry; the number of infected cells can then be compared to total CD4 cell counts. Dr. Ohlsson-Wilhelm's team looked at 10 individuals in late-stage disease before and after AZT therapy: ratios of circulating p24-positive lymphocytes to total CD4 lymphocytes varied with time and decreased in some patients after initiation of AZT therapy. The authors suggested that their data reflect the episodic nature of HIV replication during disease progression and should, therefore, also serve as a caveat: Alternating periods of high replicative activity and relative quiescence mean that multiple baseline samples should be taken, before and after initiation of therapy, to avoid misjudging HIV activity in patients.

Viremia. Because virus cannot be consistently isolated from serum or plasma of known HIV-infected (antibody-positive) individuals, viremia has not been considered a reliable marker of disease progression. Dr. Michael Saag (University of Alabama, Birmingham) and coworkers, however, reported that the *ability* to isolate virus from the plasma correlates well with disease stage: Among HIV-infected adults, virus could not be detected among any of the 17 asymptomatic subjects studied, but was detected among 4 of 33 ARC patients and 15 of 16 AIDS patients. And when examined according to CD4 cell counts, virus could not be isolated from any of the 16 samples with counts greater than 600/mm^3; 1 of 21 samples with counts equal to 400–600/mm^3 had detectable virus titers; 4 of 13 samples with counts equal to 200–400/mm^3 and 14 of 16 samples with counts less than 200/mm^3 had detectable virus titers. Furthermore, among viremic patients subsequently treated with AZT, 8 of 8 demonstrated a decrease in virus titers.

Dr. Mathez Dominique (Hôpital Raymond Poincare, Garches, France) and colleagues reported on a novel *ex vivo* system, in which blood cells from HIV-infected individuals are killed by irradiation *in vitro* and then cultured with stimulated normal lymphocytes. HIV expression can be quantified as "radiation-resistant scores," which were used to estimate viral load. More significantly, HIV expression was detected in all retrospective samples from 56 patients with AIDS-related illnesses or lymphopenia. (Serum p24 antigen was only detectable in 70% of those patients.) Furthermore, samples from asymptomatic individuals with persistently normal CD4 cell counts collected over 2 to 5 years had low HIV expression/radiation resistant scores. Ongoing studies with this technique may demonstrate its utility as a surrogate marker for clinical end points in treatment efficacy trials.

Much effort is currently being expended to fine-tune the relatively new gene amplification technique—polymerase chain reaction (PCR)—which it is hoped, will allow HIV DNA or RNA levels to be quantified. Researchers speculate that clinical milestones directly correspond to viral activity in the body (such as periods of high viral replication, which can be demonstrated by increased amounts of DNA or RNA). Indeed, Dr. Richard Wang (Armed Forces Institute of Pathology, Washington, D.C.) and colleagues reported a significant difference in the amount of HIV DNA (using the HIV *env* gene as probe to quantify DNA copies) between seropositive subjects in early stage HIV disease (CDC Stage II/III) and seropositives in late-stage disease (CDC Stage IV). Mildly symptomatic subjects had a mean number of copies of 67, compared to a mean number of 804 for late-stage disease subjects.

A singular presentation on the ability to detect and quantify HIV RNA from infectious viral particles in serum of HIV-infected patients was reported by Dr. Mark Holodniy (Stanford University School of Medicine, California) and colleagues. Among HIV-infected individuals not receiving therapy, RNA was detected (using HIV *gag* gene) in 0 of 5 asymptomatic subjects, 4 of 5 ARC patients, and 4 of 5 AIDS patients.

As evidenced by the many discussions at the Conference, PCR technology still requires refinement. (Chapter 4: DIAGNOSTIC TESTS FOR HIV discusses PCR technology in detail.) Although it is highly touted by basic science researchers for its extremely sensitive theoretical capabilities, at this time PCR does not consistently yield accurate results. In the study conducted by Dr. Wang and associates (cited above), for example, only 24 of 25 seropositives (antibody-positives) were also PCR positive. Nevertheless several research teams have been able to show a statistical correlation between disease stage and HIV DNA or RNA *detection*. Dr. Peter Neumann (Federal Centre for AIDS, Ottawa) and colleagues found that although PCR failed to detect HIV in all HIV antibody-positive subjects, seropositive individuals with negative PCR results tended to have less severe disease (in terms of CD4 cell counts and clinical stage).

Metabolic Markers. Some normal body metabolites are known to vary in concentration with changes in immune status. Information gained largely from studies with tissue or organ transplant (immunosuppressed) patients is being evaluated for its relevance to HIV-related disease.

Neopterin. The metabolite neopterin—a relatively obscure biomolecule that belongs to a class of nitrogen-containing compounds called pteridines—continues to be closely studied for its value as a predictor of HIV disease progression. The biochemistry of neopterin reveals this compound's subtle relationship to infections and disease states: neopterin is produced and secreted by certain immune cells on stimulation by gamma interferon, which is released from activated T cells. Numerous research teams have demonstrated that neopterin levels are elevated in all persons with HIV infection; however, evidence of a direct effect on neopterin levels by HIV has been lacking. Data presented by Dr. Christel Uittenbogaart (University of California School of Medicine, Los Angeles) and colleagues suggest that increased serum neopterin levels may, in fact, be the direct result of stimulation of immune cells by HIV. Dr. Uittenbogaart's team reported that activation of monocytes and B cells with cytokines (such as gamma interferon or interleukin-2) results in increased neopterin production by as much as 25-fold. Their *in vitro* studies also showed that B cells stimulated with inactivated HIV leads to increased neopterin production. Increased serum neopterin levels in HIV-positive individuals, then, may reflect direct activation of the immune system by HIV—rather than reflecting nonspecific immune activation.

While clearly elevated in all persons with HIV infection, significant differences in these elevated levels of neopterin concentration at different disease stages remains to be established. Several Conference presentations demonstrated that correlation with advanced disease stage can be only made when weighed in conjunction with other markers of immune activation. Dr. Haynes Sheppard (California Department of Health Services, Berkeley) and coworkers, for example, reported that elevated neopterin levels were predictive for development of AIDS for patients who also had low p24 antibody titers. (See also "Multiple Parameters," below.)

Steroid Hormones. Dr. Nevena Christeff (INSERM, Paris) and colleagues reported findings based on evaluation of levels of certain steroid hormones in HIV-positive men at different disease stages during a 2-month period. In comparison to uninfected men, all HIV-positive men had significantly increased serum cortisol, progesterone, and estrogen levels. Progesterone concentrations were decreased in patients with more advanced disease, and elevated concentrations of androgens were only observed in patients in the early stages of HIV disease (CDC Stages II and III). The authors suggested that these steroid changes may

be involved in the pathophysiology of immunodeficiency processes and/or clinical symptoms such as weight loss. (Indeed, the combination of high cortisol and progesterone concentrations and low serum progesterone levels in HIV-positive patients may explain the slight decrease in mineralocorticoids in these subjects.)

Multiple Parameters. Several presentations looked at multiple laboratory values in an effort to identify the most reliable (individual) markers as well as combinations of markers. Dr. Robert Andersen (ViRx, Inc., San Francisco) and colleagues monitored levels of CD4 cells, neopterin, beta-2-microglobulin, p24 antibody, and p24 antigen in a cohort of 421 homosexual/bisexual men, 118 of whom developed AIDS during a 4-year follow-up period. The authors found that as HIV disease progresses, CD4 cell levels decline while beta-2-microglobulin and neopterin levels increase. In contrast, antibody to HIV p24 remained relatively stable, although persons with AIDS had the lowest titers at diagnosis and at 2 years prior to their diagnosis.

Viral p24 antigen was measured as percent of cohort group with detectable antigen:

- Among 32 men who seroconverted during the study period, 5.9% were p24-positive at 6 months after seroconversion.
- Among 271 HIV-positive men, 10.4% were p24-positive at initial evaluation; that rose to 15.6% after 4 years.
- Among 118 men who developed AIDS, 39% were p24-positive at 2 years prior to diagnosis, whereas at time of diagnosis, 56% had evidence of p24 antigen.

Similar trends were reported by numerous other research teams for various markers during disease progression. Based on findings from a cohort of male sexual partners of men with HIV disease, Dr. Randall Coates (University of Toronto) and colleagues found CD4:CD8 ratios, increasing levels of IgA antibodies, and development of minor opportunistic infections (such as thrush, zoster, and oral hairy leukoplakia) to be significant independent predictors of progressive disease.

Dr. Dennis Osmond (San Francisco General Hospital) and colleagues found that in addition to elevations in beta-2-microglobulin and neopterin levels, an increase in the soluble CD8 level also proved to be a strong prognostic marker. In their study of 185 HIV-seropositive homosexual/bisexual men (asymptomatic, with CD4 cell counts of 200–500/mm^3) followed since 1983–84, these three markers plus soluble interleukin-2 receptor were found to be highly correlated with one another. When beta-2-microglobulin, neopterin, and soluble CD8 were evaluated in combination with p24 antigen, the three immune-activation markers were observed to predict AIDS risk (progression to AIDS) independent of p24 and CD4 cell counts.

PATIENT CARE STRATEGIES

Providing care for persons with HIV infection and persons with AIDS involves recognition and documentation of HIV infection as well as recognition of myriad symptoms associated with AIDS indicator diseases. Indeed, primary care clinicians of all specialities (including family physicians, emergency physicians, and gynecologists) as well as physician's assistants and nurse practitioners should also be prepared to recognize and initiate management of complicating opportunistic infections.

World Health Organization, Geneva, in collaboration with several international groups has drafted clinical management guidelines to provide a framework for a consistent and logical approach for the diagnosis and treatment of HIV infection and AIDS. These guidelines or algorithms, described as "appropriate care guidelines," (available from WHO) may be especially useful for developing countries adopting national AIDS programs, of which clinical management has necessarily become an expanding component.

Patient Care Continuum

In direct response to the new care challenges and economic problems brought on by the HIV epidemic, Caremark Inc. (Lincolnshire, Illinois) has created a new locus of care where HIV-infected individuals can receive a variety of services that are appropriate to their disease status and—of equal import—are cost-effective. Fueling the need for these multifaceted clinical centers is the breadth of effects of HIV disease on the individual and on the health care system.

Caremark Connection centers are in essence a practical alternative to hospitalization, offering a continuum of care for persons with all stages of HIV disease. Available services include:

- **Prescribed medical treatments.** Therapies for various HIV-related complications can be administered by the health care staff at the facility or at the patient's home.
- **Investigational drug clinical trials.** Investigational therapies can be administered under controlled circumstances at the site, where the staff draws serial blood samples, as required, and supervises patient compliance.
- **Skilled health care staff.** Nurses, dieticians, pharmacists, physical therapists, social workers, and counsellors work closely with the patient and primary physicians, preserving the case management approach to care.
- **Personalized care plans.** The center's health care staff works closely with the patient's physicians to integrate all facets of care with ongoing patient assessment and counselling. The needs and preferences of both patients and physicians dictate who, when, and where therapy is administered.
- **Nutritional education, evaluation, and support.** Patient assessment includes anthropometric measurements, biochemical indices, dietary history, weight changes, and lifestyle needs.
- **Financial advocacy.** Reimbursement specialists act as the link between physician, patient, and third-party payors to help secure payments, solve reimbursement problems, and investigate alternative sources of funds.
- **Networking social support services.** The staff can link patients with various community services, including help with daily living arrangements and financial assistance.
- **Home delivery.** When the patient is too ill to travel to the facility, prescriptions, adjunctive therapy, and/or nutritional supplements can be brought to the patient's home for administration by the nurse or by the trained patient or the patient's trained care givers.

As of publication, 12 Caremark Connection sites were open in these U.S. cities: San Francisco, Seattle, Phoenix, Denver, St. Louis, Boston, Philadelphia, Chicago, Houston, and Atlanta.

As our HIV/AIDS knowledge base grows, diagnosis and treatment of HIV disease, and of the associated individual opportunistic infections, improves. These advances, along with accumulating clinical experience, allow clinicians to offer treatments earlier in the disease course. New antiviral and immunotherapeutic agents are being developed and brought to clinical testing phases more rapidly than for any other human disease condition. Various strategies for optimizing the potential benefits of these drugs are being explored: lower dosages, use as prophylactic treatment, combination therapies, for example, have shown unexpected benefits.

As discussed in "Care Delivery," below, new strategies for caring for HIV-infected persons continue to evolve as patients, with their physicians and other care providers, learn what is most efficient, comfortable, and cost-effective. Out-of-hospital care options —notably, outpatient clinics or home care—have been shown to be viable alternatives to lengthy, uncomfortable, inconvenient, and expensive hospital stays. One innovative alternative-care approach is being offered by the Caremark Connection (Caremark Inc., Lincolnshire, Illinois); see "Patient Care Continuum," above.

ANTIVIRAL THERAPIES

Antiviral chemotherapy is now established as a central element in a comprehensive plan for managing HIV-related disease. Although the arsenal of antiviral agents is growing, AZT was the first and most widely prescribed anti-HIV agent for individuals with HIV infection or AIDS. Numerous other agents, some similar to AZT in mechanism of action and chemical structure, are in various phases of preclinical or clinical testing. Still other agents are in the early (laboratory) stages of development; several of these show specific anti-HIV properties *in vitro* at doses achievable in humans. Basic science studies of antiviral agents are discussed in detail in Chapter 6: ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS. (Immunotherapeutic agents, also called immune boosters or biologic response modifiers—including synthetic and naturally occurring compounds—are those intended to aid rebuilding of the immune system; to date not much success has been achieved with these compounds. However, Dr. Clifford Lane at National Institute of Allergy & Infectious Diseases, Bethesda, Maryland, reported that new clinical trial data from a placebo-controlled study suggests that alpha interferon given in early-stage HIV infection postpones development of opportunistic infections. Early studies concluding that alpha interferon was of no value in AIDS were based on AIDS patients whose pretreatment CD4 cell counts were below 100/mm^3.)

AZT

The preponderance of clinical experience and the majority of clinical trials have established the utility and safety of AZT (azidothymidine or zidovudine, ZDV) for AIDS patients as well as for HIV-infected individuals with few or no symptoms. AZT therapy is no longer reserved for those with an AIDS diagnosis; it is now recommended for HIV-infected individuals who are either asymptomatic or mildly symptomatic and have CD4 counts below 500/mm^3.

The original AZT pilot study (in 1986) and numerous other clinical trials have clearly shown that patients with AIDS who receive AZT therapy fare significantly better than their untreated counterparts; reported benefits include:

- improved immunological function
- prolonged survival time
- elevation of depressed platelet counts (amelioration of thrombocytopenia)
- a lessening of HIV-related neuropsychological manifestations, in some instances

In 1989, data from a 2-year clinical trial demonstrated that AZT also benefits HIV-infected people with mild or no symptoms (CD4 cell counts less than or equal to 500/mm^3). The primary effect of AZT in these patients was postponement of advanced immunodeficiency: as discussed below, significantly fewer AZT-treated individuals with CD4 cell counts at or below 500/mm^3 progressed to ARC or AIDS than did matched control subjects. Although most clinicians now agree that AZT is appropriate for HIV-infected individuals with CD4 counts equal to or less than 500/mm^3, many questions remain—perhaps the most important is whether all such patients should receive AZT, or whether certain markers of disease progression should be used instead. Active clinical research regarding AZT therapy continues to address several questions:

- Which patients with early stage HIV disease should receive AZT therapy? All patients with fewer than 500 CD4 cells/mm^3, or defined subsets of those patients? At what point, and using which criteria, should therapy be initiated?
- What is the proper dose of AZT? Does this differ for patients at different phases of HIV disease?
- How long should AZT therapy be continued?
- What are the important short and long-term toxicities? How should they be managed?
- What is the clinical significance of AZT resistance?
- What is the role of other antivirals in AZT therapy? Do combinations improve treatment results? Do they potentiate or limit toxicity?

AZT in Early HIV Disease. Several Conference reports validated the findings of the AIDS Clinical Trials Group studies, in which mildly symptomatic or asymptomatic HIV-infected individuals with CD4 cell counts less than 500/mm^3 were demonstrated to benefit from AZT. These studies, known as the ACTG trials 016 and 019, were summarized in 1989 and reported by Dr. Margaret Fischl (University of Miami School of Medicine) and Dr. Paul Volberding (University of California, San Francisco) at this year's Conference. The ACTG trial 016 sought to determine the efficacy of AZT in *early* HIV disease: of 711 subjects, 351 received placebo while 360 were given 200 mg AZT orally every 4 hours over the 2-year study period. When AZT therapy was initiated early in the course of HIV infection, progression to advanced disease seemed to be delayed: a significantly higher number of placebo recipients developed advanced ARC or AIDS, or died during the 2-year study (34 placebo recipients versus 12 AZT recipients). The AZT-treated group also showed significant increases in CD4 cell counts and decreases in p24 antigen levels. (The ACTG trial 019 evaluated low- versus high-dose AZT therapy, see "Doses & Dosing Schedules," below.)

Although few clinicians argue with the evidence for AZT's efficacy in treating advanced HIV disease, some controversy exists regarding the use of AZT in earlier stages. Clinical trials have not uniformly and unequivocally confirmed the observations of the above-cited ACTG studies: neither the U.S. Veterans' Hospital multicenter study nor the Concorde trials in progress in Europe have thus far shown clear and convincing benefit to early AZT treatment for all patients. Some evaluators believe that the ACTG data obscure the possible presence of stratifications in benefit; they suggest that some, but not all, patients with early stage disease might improve with AZT. Some investigators are exploring the utility of markers that might identify the patients most likely to benefit from early AZT therapy—those at higher risk of disease progression. Unfortunately, as yet, no consensus has been reached about such markers.

Furthermore, researchers cannot yet prove that early AZT therapy extends *total* survival time for people with HIV infection, although many clinicians presume that this is true and that AZT brings other positive effects not measured by survival time alone. Over the next 1 to 3 years, the results of extended clinical research trials now in progress should provide greater clarity.

Doses and Dosing Schedules. The currently recommended dose of AZT is 500 or 600 mg/day in divided doses, usually 100 mg orally every 4 hours while awake. (Most clinicians no longer require patients to take a sixth dose in the middle of the night, an inconvenience necessary to achieve an equally divided total daily dose of 600 mg.) Previous studies, mostly treating patients with advanced disease, had used higher doses of AZT—usually 200 mg orally every 4 hours around the clock for a total daily dose of 1200 mg. (This previously standard dose of 1200–1500 mg/day was based primarily on pharmacokinetic projections of the dose most likely to be effective—that is how the drug would be distributed through the body and be cleared.) Patients receiving 1200 mg of AZT per day, however, had significant toxicity: unacceptable bone marrow toxicity, manifested chiefly by unrelenting anemia requiring (and often producing dependency on) transfusions and by sustained neutropenia (low neutrophil count).

Subsequent clinical trials have demonstrated that in treating early stage disease, lower doses of AZT are at least as effective and associated with significantly fewer adverse effects. Several studies presented at the Conference confirmed these findings for patients with asymptomatic or mildly symptomatic HIV infection. In summarizing the findings from the ACTG study 019, in which lower dose AZT was evaluated in mildly symptomatic or asymptomatic individuals who had CD4 cell counts below 500 cells/mm^3, Dr. Paul Volberding (University of California, San Francisco) reported that 500 mg/day was safer than a 1500 mg dose while producing a similar slowing in the 1-year progression rate to AIDS. Of 428 trial participants receiving placebo, 33 developed AIDS, as did 11 of 453 on the 500 mg/day

dose, and 14 of the 457 in the 1500 mg/day group. (Dose-related adverse effects are discussed below under "Toxicity.") Similar findings were presented by Dr. Dominique Salmon (Hôpital Bichat-Claude Bernard, Paris), who was reporting for the French AZT Cooperative Study Group: 600 mg per day (200 mg every 8 hours versus 200 mg every 4 hours) was noted to produce fewer adverse effects and was just as effective as the standard 1200 mg/day dose for patients with either ARC or AIDS.

Numerous (smaller) efficacy trials conducted with different patient populations reported at the Conference showed similar findings of equivalent drug efficacy with lower dose AZT. Dr. Michel Carles (University Hospital, Nice) and colleagues, for example, found that 900 mg per day was as efficacious as and better tolerated than standard 1200 mg/day dose in a prospective randomized study of 36 patients with HIV disease (CDC stage IV). In a study of patients with advanced HIV disease who had experienced at least one case of *Pneumocystis carinii* pneumonia, Dr. Margaret Fischl (University of Miami School of Medicine) reported her team's findings that a lower daily dose (200 mg every 4 hours for 4 weeks, followed by 4 weeks of 100 mg every 4 hours) was as effective in postponing other severe complications due to immunodeficiency—with substantially fewer cases of anemia or neutropenia—as the standard (high) daily dose of 1200 mg. On questioning at the Conference regarding the possible efficacy of an even lower daily dose of AZT, Dr. Fischl observed that the intracellular concentration of AZT is what is important and that, in her opinion, the optimal dose of AZT is probably between 200 mg and 500–600 mg/day. A study that sought to evaluate low-dose AZT in combination with the antiviral agent acyclovir found 300 mg/day of AZT (alone) to be as efficacious as 600 mg/day of AZT (alone); the addition of acyclovir did not appear to confer greater benefits. Presenting virologic data on the 22 patients (CDC Stage IVA) in the study, Dr. Robert Coombs (University of Washington, Seattle) showed that plasma viremia titers and p24 levels were significantly—and equally—suppressed by both the 300 mg and 600 mg daily doses.

Clinical studies investigating the question of intermittent versus continuous therapy have not so far provided any clear answers with regard to "which is better." Many patients receiving AZT experience an increase in CD4 cell counts, sometimes by more than 500/mm^3 during treatment. It is possible that temporary discontinuation of AZT at that point might be of both economic and clinical benefit; intermittent therapy could potentially delay or limit the evolution of drug resistance and might lessen long-term toxicity. It is equally possible, however, that the benefits of AZT would be reduced by periodic withdrawal of the drug, and that continuous therapy would enable patients to experience its full benefit. In practice, most clinicians do not discontinue AZT despite increases in CD4 counts. Similarly, no data has been presented to encourage a "pulsed" approach to giving AZT in an intermittent fashion.

Toxicity. The major toxicity associated with AZT administered at high doses is hypoplasia of the bone marrow with cytopenias (that is, destruction of bone marrow with decreased cell counts, especially of red blood cells, producing anemia). Patients receiving high doses of AZT require frequent assessment of bone marrow function throughout therapy. Among patients treated earlier and with lower doses, such bone marrow toxicity has been uncommon.

Similarly, the myopathy (disturbance of muscle function) associated with the originally defined standard AZT dose (1200 mg/day) has been less frequent and less severe at lower doses. With the exception of minor toxicities often characterized as inconvenient (such as slight nausea without vomiting and mild headache), AZT given at lower doses (300 to 500 or 600 mg/day) rarely produces short-term side effects.

Based on the ACTG trial 019 discussed above, a double-blind randomized control trial comparing two doses (1500 mg and 500 mg) of AZT and placebo, patients receiving AZT had no greater incidence of most side effects than did control patients receiving placebo. Reporting

toxicity findings from this study, Dr. Matthew Koch (Research Triangle Institute, North Carolina) and colleagues found that the *pattern* of toxicity of AZT at each dose (1500 mg and 500 mg) was similar in asymptomatic subjects and patients with ARC and AIDS, but the *frequency* of toxicity was lower. The risk of severe anemia was small in the lower dose subjects; in the higher dose group, the risk was greatest in the first 3 months of treatment.

Myopathy has been associated with HIV infection itself (see Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION) and AZT therapy. Several studies have described AZT-induced myopathy as proximal muscle weakness and elevations of the enzyme creatine kinase, usually developing after several months or more of AZT therapy. This myopathy usually resolves in most patients after the drug is discontinued, and when AZT therapy is resumed, the myopathic syndrome usually recurs. Dose reduction improves this myopathy in many patients (both clinically and biochemically), and some patients whose AZT therapy was stopped because of myopathy do not have a recurrence if AZT is subsequently given in much smaller doses.

Length of Treatment. Once a patient begins taking AZT, most clinicians feel uncomfortable discontinuing it absent clear evidence of treatment failure or serious toxicity. As yet, no trials have defined the optimum duration of therapy. Since AZT postpones, but does not prevent, progression of HIV-related disease altogether, there may be clinical grounds for stopping the drug at some point; that point, however, is not known.

Continuous therapy with AZT, however, does raise several concerns. Because of the relatively short experience with AZT in HIV infection, the long-term consequences of therapy have not been defined. Perhaps one indication, discussed by Dr. Robert Yarchoan (National Cancer Institute, Bethesda, Maryland), is the finding of increased incidence of B cell lymphomas (specifically, non-Hodgkin's lymphoma) among patients receiving continuous AZT therapy. It is possible, Dr. Yarchoan said, that this recently observed increased incidence is a direct effect of AZT-related lengthened survival time: patients living longer, because of AZT therapy, have a greater chance of developing lymphomas, which are a known complication of immunodeficiency states.

The emergence of drug resistant HIV strains is also a concern in regard to continuing AZT indefinitely. Many clinicians worry that instituting therapy earlier may "use up" the beneficial effects of AZT, produce resistance, and then leave the patient with fewer options once more severe disease develops. The limited information available thus far about AZT resistance does not resolve this concern, but does suggest that AZT treatment over 1 to 3 years is associated with some degree of drug resistance, (see discussion below).

Finally, there is an important issue of costs. Even at a lower dosage, and even given reduced prices (lowered in 1989 by Burroughs Wellcome), AZT is an expensive agent over the long term. Medical economics holds that any therapy not be continued—and especially not a costly one—when its benefit can no longer be demonstrated. But in the case of AZT, the limitations of benefit are unclear; accordingly, concern about cost alone cannot justify discontinuing treatment.

AZT-Resistant HIV Strains. Following the not unexpected discovery of AZT-resistant HIV strains in 1989, numerous investigators began looking at the drug sensitivity of HIV strains isolated from patients on AZT therapy. As pointed out by Drs. Brendan Larder (Wellcome Research Laboratories, Beckenham, U.K.), Charles Boucher (Academic Medical Center, Amsterdam), and Marty St. Clair (Wellcome Research Laboratories, North Carolina), although AZT resistance can be demonstrated *in vitro*, no clinical correlations have been noted—that is, AZT-treated patients from which AZT-resistant strains have been isolated do not appear to have a clinically worse profile. However, *in vitro* findings reveal greater AZT resistance in HIV strains isolated from ARC or AIDS patients who have been receiving AZT for 6 months than in HIV isolated from asymptomatic individuals who

have been on therapy for 2 years; these findings do suggest that HIV isolated from asymptomatic patients is more susceptible to AZT and has a limited capacity to develop resistance. Some scientists argue that viral replication in asymptomatic individuals is being kept in check—and consequently the virus has less chance to develop drug resistance. Whether this may have clinical benefit is not known.

Quite predictably, the finding of AZT-resistant HIV strains from patients has added a greater sense of urgency in bringing other antiviral therapies to clinical testing phases. Alternatively, combination therapy using AZT with other agents, such as dideoxycytosine (ddC) or dideoxyinosine (ddI), is thought to reduce the likelihood of AZT resistance. Dr. Hiroaki Mitsuya (National Cancer Institute, Bethesda, Maryland) and colleagues reported that HIV isolated from patients receiving AZT and ddC in an alternating regimen for 1 year did not show any change in sensitivity to either AZT or ddC when compared to pre-treatment HIV strains.

AZT Combination Therapy. Clinical trials of AZT in combination with other antiretrovirals (ddC or ddI, for example) or immunomodulators (alpha interferon, for example) are seeking to establish whether additive clinical benefits can be gained. Such trials have involved a relatively small number of patients and data accumulated thus far appear inconclusive with respect to efficacy. Dr. Robert Yarchoan (National Cancer Institute, Bethesda, Maryland) reported that some patients have been on an alternating regimen of AZT and ddC for as long as 3 years. Although a weekly alternating regimen was originally tried, Drs. Thomas Merigan and Gail Skowron (Stanford University School of Medicine, California) reported that, based on studies involving 131 ARC or AIDS patients who had detectable levels of p24 antigen, a high-dose *monthly* alternating schedule now appears to be optimal (as determined by sustained suppression of p24 antigen and increased CD4 cell counts). Dr. Merigan cautioned, however, that the data need to be evaluated against AZT therapy alone.

Bone marrow toxicity has been significantly less in all alternating regimens than that observed with continuous AZT therapy, but neuropathy is observed in all treatment regimens, particularly during weekly or monthly high-dose ddC treatment. Preliminary analysis of the Stanford trials indicated that an alternating regimen of AZT and ddC was less toxic and more effective (as measured by clinical benefit, CD4 cell count improvement, and p24 antigen suppression) than either drug alone. Such an approach may be absolutely necessary with ddC, since doses that produce the greatest therapeutic benefit are typically unacceptably toxic. Given that AZT's primary toxicity is myelosuppression, whereas the dominant toxicity of ddC is neuropathy, the combination of the two agents might at least be expected not to produce additive side effects. Dr. Samuel Bozzette (University of California, San Diego) and colleagues reported findings from their comparison study of alternating versus intermittent AZT/ddC therapy in ARC and AIDS patients who had recovered from AZT-associated hematologic toxicity: Although different dosing schedules, including an intermittent schedule that incorporates a drug-free period, appear to significantly alter the individual toxicity profiles of AZT and ddC, the best regimen has yet not been determined.

Dr. Tze-Chiang Meng (University of California, San Diego) and colleagues reported preliminary findings from their study of 24 patients who received ddC and AZT, administered concurrently in low doses (7 different regimens): Overall, the combination had beneficial effects on markers of disease progression (CD4 cell counts and p24 antigen levels) and was generally well tolerated. Toxicities included peripheral neuropathy (1 patient), pancytopenia (1 patient), and hemoglobin toxicity (2 patients); 2 trial participants have discontinued therapy because of toxicity.

Dr. Victoria Davey and colleagues (National Institute of Allergy & Infectious Diseases, Bethesda, Maryland) compared tolerance and toxicity of AZT/alpha interferon combination therapy with that of single agent therapies (AZT alone and alpha interferon alone) in early

HIV infection. In this study, 120 individuals with CD4 cell counts of at least 500/mm^3 were randomized to receive one of the three therapy regimens:

- AZT alone at 1200 mg/day
- alpha interferon alone at 5 x 10^6 units/day
- AZT at 600 mg/day and alpha interferon at 1 x 10^6 units/day

All three treatment groups showed a decrease in circulating p24 antigen, with patients on combination therapy showing the most sustained falls. Of 30 patients receiving therapy for as long as 1 year, 1 patient receiving alpha interferon has reached a study endpoint (decline in CD4 cell count to less than 200/mm^3 or CD4 count representing less than 20% of total lymphocytes or development of an opportunistic infection). Attrition from the study due to subjective intolerance was the same for all three groups, although the AZT/alpha interferon group had a greater frequency of subjective and laboratory toxicities. With dose adjustments, Dr. Davey reported that each of the therapies are expected to be equally tolerable. Data collected over the next 6 months from this ongoing study should give some indication of the relative effects of the three therapies. Other AZT combination therapies being evaluated in clinical trials include AZT/acyclovir; AZT/AS-101; AZT/interleukin-2; and AZT/acemannan. (See Tables 6.2 and 6.3 in Chapter 6: ANTIVIRAL & IMMUNOTHERAPEUTIC AGENTS.)

DIDEOXYCYTIDINE (DDC)

This "dideoxy" nucleoside analogue is the most potent of all such compounds, indeed, its minimal effective dose is 1/100th that of AZT. Although early clinical trials of dideoxycytidine (ddC) revealed significant neurotoxicity, subsequent descending dose-response studies have shown that low dosages (optimal, .01 mg/kg every 8 hours) are associated with specific antiviral effects, such as p24 suppression, with little toxicity. (Mild neuropathy may be seen in some patients, but it is much less frequent and is reversible. No hematologic toxicity such as that associated with AZT has been observed.) Dr. Thomas Merigan (Stanford University, California) reported that improved immunological parameters, such as increased CD4 cell counts, have also been demonstrated in ongoing safety and efficacy Phase I/Phase II studies. A clinical trial with (previously untreated) advanced ARC/early AIDS patients is now in progress, the aim of which is to compare the benefits of ddC directly with those of AZT. As a single agent, ddC is also being evaluated in patients who are intolerant to or failed to respond to AZT therapy and who are intolerant to ddI.

DIDEOXYINOSINE (DDI)

Dideoxyinosine (ddI), another antiviral compound shown to have activity against HIV *in vitro*, was made available in 1989 for limited clinical use by its manufacturer, Bristol-Myers Squibb Company (New York). Approximately 20,000 U.S. patients have been enrolled in this expanded-access program, according to Dr. Raphael Dolin (Rochester Medical Center, New York). In Canada, more than 400 ARC and AIDS patients have participated in an open-access ddI program. Among these Canadian trial participants, Wendy Ungar and colleagues (Bristol-Myers Squibb Pharmaceutical Group, Ottawa) reported that the most frequent reason for requesting ddI was hematologic intolerance with AZT. The current role of ddI in clinical practice is as a substitute, or replacement, for AZT in patients who have failed to respond to or showed signs of disease progression on AZT therapy, could not tolerate the drug, or had unacceptable side effects.

Many reports concerning ddI at the Conference dealt with its pharmacokinetics, dosing, safety, and efficacy. Dr. C. A. Knupp (Bristol-Myers Squibb, Syracuse, New York) and coworkers demonstrated that ddI administered to patients receiving ddI orally or parenterally (twice daily) across dose ranges of 0.8-33 mg/kg (oral) and 0.4-16.5 mg/kg (IV) yields favorable bioavailability and renal clearance.

Dr. Neil Hartman (National Cancer Institute, Bethesda, Maryland) and associates, however, cautioned that while several different oral formulations of ddI produce therapeutic levels in the blood, absorption varies greatly, depending on both the specific formulation and the time since the study subjects' last food intake: ddI in solution with oral antacids, for example, was noted to have higher bioavailability than buffered ddI tablets.

Based on pooled clinical trial data with patients receiving more than 30 weeks of ddI, the currently recommended dose is 7–10 mg/kg/day. Dr. Timothy Cooley (Boston University School of Medicine) and colleagues presented efficacy data from their Phase I trial of 36 ARC and AIDS patients: sustained increases in CD4 cell counts were observed as well as sustained suppression of p24 antigen. Dr. Shizuko Aoki and coworkers (National Cancer Institute, Bethesda) showed that ddI at therapeutic dose levels reduces HIV DNA in peripheral blood mononuclear cells, as demonstrated by PCR data. Based on a Phase I trial with 58 ARC and AIDS patients who have been taking ddI for longer than 72 weeks, Dr. Robert Yarchoan and colleagues (National Cancer Institute) found that not only is ddI well tolerated, but it also appears to improve cognitive functioning in patients with HIV-associated neurological disease. (Pharmacologic studies have demonstrated that ddI crosses the blood-brain barrier and enters the central nervous system.)

Dose-limiting toxicities, as outlined by Dr. Dolin, include peripheral neuropathy (involving the legs, similar to that associated with ddC) at doses exceeding 10 mg/kg/day and pancreatitis. Until data from direct comparison studies with AZT are available and analyzed, ddI therapy will probably remain as an alternate antiviral therapy for AZT.

SOLUBLE CD4

Therapy with soluble (sCD4 or recombinant CD4) is an interesting anti-HIV approach: Ideally, this strategy would disallow HIV from binding to the CD4 molecule on uninfected CD4 cells, effectively preventing infection of such cells. The principle involved is competitive binding: since HIV binds to the CD4 molecule on the surface of susceptible cells, introduction of sCD4 as a therapeutic agent means that it will compete with the natural CD4 receptor for HIV binding sites (specifically, the CD4 binding region of gp120). The initial enthusiasm for sCD4, however, has waned because of human pharmacokinetic studies demonstrating the need for frequent dosing (sCD4 has a half-life of only about 45 minutes) and considerable toxicity. Furthermore, no sustained clinical and immunological benefits were consistently observed.

Development of "second generation sCD4" agents (primarily sCD4 conjugated to a portion of an IgG antibody molecule, called CD4 immunoadhesin) have attempted to overcome these problems. Drs. Ann Collier (University of Washington, Seattle), Robert Yarchoan (National Cancer Institute, Bethesda), and Clifford Lane (National Institute of Allergy & Infectious Diseases, Bethesda) all showed that intravenous sCD4-IgG therapy was well tolerated and produced a much more favorable pharmacokinetic profile than sCD4 alone. Similarly, Dr. Teri Hodges (New England Deaconess Hospital, Boston) and colleagues found that intramuscular administration of sCD4-IgG produced therapeutic levels similar to effective drug concentrations *in vitro* when given only once a week, and that patients tolerated the treatment well.

PEPTIDE T

This peptide drug contains eight amino acids that correspond to a stretch of amino acids found in HIV gp120. Researchers at the National Institute of Mental Health (Bethesda, Maryland), who developed Peptide T in 1987, suggested that the peptide may bind to CD4 much as HIV gp120 does, occupying receptor sites that might otherwise serve as binding sites for gp120. Furthermore, because "free" HIV gp120 has been shown to be toxic to certain cells *in vitro*—notably, neuronal cells—use of Peptide T as a therapeutic tool continues to be explored.

Data from Phase I clinical trials presented by Kenneth Mayer (Fenway Community Health Center, Boston) and colleagues and Dr. Michael McGrail (University of Southern California, Los Angeles) and colleagues demonstrated that Peptide T produces improvement in neuropsychiatric symptoms and mood. Dr. Mayer's team found that Peptide T was well tolerated when administered via nasal inhaler three times daily. Although immunological and virological markers were not observed to improve consistently, Dr. Peter Haseltine (University of Southern California, Los Angeles) and coworkers showed in a 24-week Phase I study that Peptide T could be given without serious toxicity to ARC and AIDS patients.

GLQ223 (COMPOUND Q)

Enthusiasm for GLQ223—a purified extract from cucumber roots that has shown anti-HIV properties *in vitro*—has waned during the last year because of the absence of clinical data showing efficacy; concern regarding its toxicity also remains. (The *unpurified* version of GLQ223 is called Compound Q; the U.S. FDA put an import alert on the drug in mid-1989. Community-based clinical trials with Compound Q conducted in the United States, in which there were several deaths, have been heavily criticized.) Pharmacokinetic studies reported by Giorgio Gatti (University of California School of Pharmacy, San Francisco) and colleagues showed that, in ARC and AIDS patients receiving intravenous administration, GLQ223 undergoes rapid distribution followed by a slower elimination phase with a half-life of about 3 hours. However, Dr. James Kahn (San Francisco General Hospital) and colleagues demonstrated that the serum concentrations obtained *in vivo* do not approach those shown to be effective *in vitro*. In Dr. Kahn's study, patients receiving GLQ223 experienced clinically important and even life-threatening toxicity. Further clinical studies are needed to establish efficacy for this antiviral therapy.

OPPORTUNISTIC INFECTIONS

Opportunistic infections continue to cause most of the deaths, hospitalizations, and disabling episodes related to HIV disease. Systemic opportunistic infections, and those affecting the central nervous system, present a number of challenges in diagnosis and management. It is still true that many patients with HIV infection first seek medical care not because of a direct manifestation of HIV itself, but because of a complicating infection; in fact, the existence of underlying HIV infection may not even be suspected originally. Many HIV-infected patients have not been tested for antibody to HIV or had a clinical evaluation until immunological function is in substantial decline.

Diagnosing specific opportunistic infections is often done in the context of an already immunocompromised even seriously ill patient. Dr. Mary Young and colleague (Georgetown University Hospital, Washington D.C.), for example, showed that among urban women, 75% of these female patients seek care for the first time with CD4 cell counts at or below 500/mm^3 and 39% with CD4 cell counts under 200/mm^3. Given the nonspecific nature of many of the manifestation of opportunistic infections, and the added complexity of the underlying immunodeficiency, specific accurate diagnosis can be difficult.

Indeed, Dr. Jose Pando and coworkers (VA Medical Center, University of Miami, Florida) showed that opportunistic infections are commonly misdiagnosed or go undiagnosed altogether: In an autopsy review of 51 cases, opportunistic infections causing death had been accurately diagnosed by the clinician 43% of the time and suspected 36% of the time, but in 20% of the cases, opportunistic infections were completely unsuspected. The authors pointed out that therapy was available for about 60% of the missed diagnoses. Certainly these findings suggest the need for more comprehensive and aggressive approaches to diagnosing opportunistic infections. However, some opportunistic infections, even if accurately diagnosed, cannot be effectively treated. And therapy in other

opportunistic infections may improve the immediate prognosis without bringing significant benefit to the patient's quality of life or survival, while causing both further economic strain and potential treatment-related toxicity.

Several studies attempted to correlate the first diagnosed opportunistic infection with HIV risk category in hopes of anticipating specific opportunistic infections and planning prophylaxis. Dr. A. Laporte and colleagues (Ministry of Health, Paris) reported that although *Pneumocystis carinii* pneumonia (PCP) and cerebral toxoplasmosis were the most frequent initial opportunistic infections, no clear trends with respect to HIV risk category were evident. When comparing emergency department patients seen for an infectious complication by HIV risk category (homosexual/bisexual men versus IV drug users), Dr. Debbie Fleetwood and colleagues (Johns Hopkins University, Baltimore) noted that although no correlation with HIV risk category could be drawn, the specific system affected (neurologic versus pulmonary, for instance) differed significantly between previously symptomatic patients and those with their first HIV-related episode.

Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION discusses opportunistic infections restricted to the central nervous system, including toxoplasmosis, cryptococcosis, CMV encephalitis, and neurosyphilis.

PNEUMOCYSTIS CARINII PNEUMONIA

Pneumocystis carinii pneumonia (PCP) remains the most common single cause of serious morbidity and mortality among the opportunistic infections complicating HIV disease. The first life-threatening opportunistic infection in more than two-thirds of patients with HIV disease, each episode of PCP is still associated with a 20 percent chance of death. Recurrence rates after successful therapy are high, even in the presence of AZT treatment. Improved strategies for preventing first and repeat events of PCP and better therapies for established disease will have enormous impact on the quality of life of people with HIV disease as well as on survival.

Clinicians caring for patients with PCP have searched for prognostic markers that might assist identification of patients at risk for respiratory failure and death. Between 15% and 20% of patients with PCP progress to respiratory failure; that percentage might be reduced by earlier recognition of clinical or laboratory characteristics that predict poor outcome. In a study of collagenic markers (in bronchoalveolar washings and serum), Dr. Fausto Capella (Hôpital Saint-Antoine, Paris) and coworkers found that serum antigen levels of a product of Type III collagen (Type III procollagen N terminal peptide) may predict the early onset of pulmonary fibrosis in patients with PCP. In this study, increased levels of this peptide were found in the sera of patients with fatal versus nonfatal PCP. Dr. Walter Blumenfeld and colleagues (University of California, San Francisco) noted that low "cyst density" (which indicates a greater proportion of more virulent parasitic forms) of *Pneumocystis carinii* in tissue samples was associated with unfavorable outcomes.

Treatment of PCP. Although the antibiotic pentamidine effectively treats PCP when administered intravenously, it is a toxic and expensive therapy that usually requires inpatient management. Patients as well as the health care system would benefit from less expensive, less dangerous treatments, especially if administered in an outpatient setting. This year's Conference included some encouraging information about such possibilities. Dr. Julian Falutz and colleagues (Montreal General Hospital), for example, showed that almost half (47%) of the group of 49 patients with PCP treated between 1987 and the end of 1989 were initially managed outside the hospital; in 75% of these, inpatient therapy was never required.

Dr. Bruce Montgomery (University of California, San Francisco) and colleagues showed that trimethoprim/sulfamethoxazole effectively treated PCP when given as 15 mg/kg/day in four divided doses, initially intravenously and then, after 5 days, orally. Patients who

were stable when transferred to oral therapy could then be managed as outpatients. However, they also found that patients treated with aerosol pentamidine (600 mg delivered once a day by Respirgard II nebulizer) were less likely to have serious side effects or to discontinue therapy (because of adverse effects).

The frequency of side effects with trimethoprim-sulfamethoxazole also led to attempts to use other drug combinations; clindamycin with primaquine were investigated by several groups. Dr. Emil Toma and colleagues (Hôtel-Dieu de Montréal and Université de Montréal), in a very preliminary report, and Iris Rohde and coworkers (University Hospital, Berlin), in a retrospective review, found that clindamycin/primaquine was as effective as trimethoprim/sulfamethoxazole, at least in patients with less severe PCP (Po_2 greater than 60 mm Hg, temperature less than 40° C, heart rate less than 120/min.). Patients given clindamycin/primaquine gained some advantage in fewer side effects, although that combination did produce methemoglobinemia. David Jones and coworkers (Indiana University School of Medicine, Indianapolis) found that the clindamycin/primaquine combination does not alter the kinetics of AZT and does not require any change in AZT dosing and management.

Prevention of PCP. Aerosol pentamidine continues to generate much interest as a prophylaxis for PCP. Although Pierre Chopard (Geneva University Hospital) and coworkers found no benefit in using aerosol pentamidine (given as 300 mg every 28 days) in their placebo-controlled study of 177 patients with CD4 counts under 200/mm^3, the majority of studies validate the utility of aerosol pentamidine in primary and secondary prophylaxis of PCP. As a primary prophylaxis, Dr. Catherine Leport (Hôpital Bichat-Claude Bernard, Paris) reported that co-trimoxazole is recommended for patients whose CD4 cell counts fall below 200/mm^3 or below 15–20% of total lymphocytes.

Margaret Johnson and coworkers (Free Hospital and School of Medicine, London) studied the effect of inhaled pentamidine on specific cell populations found in bronchoalveolar washings. They noted a significant difference in the proportion of monocytic cells in samples from HIV-infected patients in whom aerosol pentamidine was effective as compared with patients not receiving aerosol pentamidine and uninfected individuals. Dr. Johnson's team concluded that the HIV-infected patient may be susceptible to PCP due to a depletion of lung monocytes and that aerosol pentamidine may work by increasing the population of such cells.

A new concern, which surfaced in the last year, was the potential for the development of extrapulmonary pneumocystis disease in patients receiving aerosol pentamidine as a prophylactic for PCP. As suggested by Dr. Gary Noskin and colleagues (University Medical College, Chicago), it is possible that patients with disseminated pneumocystis disease have more virulent strains of the organism or that *P. carinii* regularly causes a systemic illness, the extrapulmonary components of which become visible when aerosol pentamidine suppresses disease in the lung. Dr. Steven Scheibel and associates (Community Health Network, Rochester, New York) identified 3 episodes of "breakthrough" extrapulmonary pneumocystis disease among 25 patients given aerosol pentamidine; they found that very low CD4 cell counts (less than 50/mm^3) predisposed patients to this occurrence. Based on retrospective review of charts, radiological studies, and pathological specimens of AIDS patients with disseminated pneumocystis disease, in which 4 of 7 patients with cystic PCP had disseminated *P. carinii* infection, Dr. Irwin Feuerstein and colleagues (National Institutes of Health, Bethesda, Maryland) recommended that immunosuppressed patients with one of the following risk factors be screened for disseminated pneumocystis disease:

- PCP with cystic features
- PCP that develops in a patient while on aerosolized pentamidine
- pneumothorax (either spontaneous or complicating an infiltrate)
- PCP with mediastinal widening

Dr. Feuerstein's team concluded that because disseminated *Pneumocystis carinii* infection can be completely subclinical (without clinical manifestations) with normal laboratory studies or nonspecific radiology, it is probably more common than currently believed.

Nanda Gopinath and coworkers (University of Toronto) reported their findings of a characteristic ultrasound appearance of *P. carinii* tissue infection in 2 cases of disseminated *P. carinii*. Another 2 cases were identified following ultrasound screening among 18 asymptomatic HIV-infected individuals receiving prophylactic aerosolized pentamidine. Several observers commented on the possible utility of periodic systemic therapy (with parenteral pentamidine, dapsone, or trimethoprim-sulfamethoxazole) to reduce the likelihood of disseminated disease in patients receiving aerosolized pentamidine prophylaxis, but as yet no broad consensus has been reached as to the best agent, dose, or regimen.

Since parenteral pentamidine sometimes induces glucose intolerance (in as many as 22% of patients), Dr. Hetty Waskin (Duke Medical Center, Durham, North Carolina) and coworkers studied the effect of aerosolized pentamidine on glucose metabolism. They found that prophylactic aerosolized pentamidine is much less likely to cause significant abnormalities of blood sugar, and that no patients receiving only aerosolized pentamidine for pneumocystis prophylaxis developed treatment-associated diabetes mellitus.

Other than breakthrough disease, the major problem with aerosolized pentamidine is bronchospasm at the time of administration (also called pentamidine-induced bronchoconstriction). Dr. Jean Quieffin and colleagues (St. Paul's Hospital, Vancouver) found bronchospasm occurred in 30% of a group of 31 subjects receiving aerosolized pentamidine. Dr. Timothy Leigh (Westminster Hospital, London) and colleagues observed a drop in FEV_1 (the volume of air exhaled at 1 second after maximum inspiration) in all patients receiving aerosolized pentamidine, averaging 21% but being greater in smokers. In this study, pre-treatment with terbutaline (10 mg nebulized 2 minutes prior to administering aerosolized pentamidine) significantly reduced the effect of aerosolized pentamidine on FEV_1.

While the concern about disseminated disease may have reduced the overall enthusiasm for aerosolized pentamidine as the sole prophylactic regimen, little evidence was presented to suggest more viable alternatives. Oral dapsone was found to be as effective as aerosolized pentamidine for primary prophylaxis of PCP in a study of 33 patients conducted by Monica Slavin and coworkers (Fairfield Hospital, Melbourne). Similar findings were reported by Ramon Torres and colleagues (St. Vincent's Hospital and Medical Center, New York) in their comparative evaluation of 251 patients receiving either oral dapsone (100 mg twice weekly) or aerosolized pentamidine (100 mg every 2 weeks) as primary or secondary prophylaxis. Both drugs were well tolerated with few patients discontinuing because of adverse effects: in the dapsone treatment arm of this randomized trial, rash and hepatic dysfunction were more common; cough, bronchospasm, dysgeusia, shortness of breath, chest tightness, and sore throat were more common in the aerosolized pentamidine segment (bronchospasm was managed successfully with bronchodilators in some patients). The authors concluded that the advantages of dapsone (low cost, low toxicity, and ease of compliance with intermittent regimen) may outweigh those of aerosol pentamidine since efficacy appears to be similar.

CYTOMEGALOVIRUS DISEASE

In the immunocompromised person, infection with cytomegalovirus (CMV) has the potential to cause retinitis (inflammation of the retina) and blindness, and many of its other manifestations may undermine survival. At the Conference numerous studies focused on the question of the most appropriate therapy for CMV disease.

CMV Retinitis. Accurate ways to predict the occurrence of CMV retinitis are still being sought; in the meantime, experience has shown that the likelihood of development of CMV disease in any site increases as immunological function deteriorates. Studies by Dr. Karen

Stewart and coworkers (Fairfield Hospital, Melbourne) and, independently, John Zurlo and coworkers (National Institutes of Health and Food & Drug Administration, Bethesda, Maryland) confirm this clinical observation with data demonstrating that the incidence of positive CMV cultures increases as CD4 cell counts decrease. Documenting the presence of CMV in body fluids and secretions may be important in anticipating retinitis: in their prospective study, Dr. Stewart's team found that HIV-infected patients who developed CMV retinitis had CMV viremia prior to showing evidence of retinitis.

Initial Treatment. Within the last 5 years, two promising drugs have been brought to clinical testing phases for CMV disease: Ganciclovir has been approved by the FDA for use in the United States and foscarnet is expected to be licensed soon. Judith Feinberg (National Institute of Allergy & Infectious Diseases, Bethesda) and colleagues reported data accrued under the treatment investigational new drug (IND) mechanism (a regulatory drug protocol that allows access by any licensed U.S. physician to a promising investigational drug with evidence of safety). Based on 1,125 patients with sight-threatening retinitis, the data show that IV ganciclovir (5 mg/kg twice a day for 14 days) can delay retinitis progression in a significant proportion of patients; in this 6-month evaluation, 56% had not progressed by the study's end. Only 6% of patients treated withdrew because of adverse events, with neutropenia as the most common serious toxicity; however, most patients in the study had previously received AZT therapy.

Dr. Catherine Leport (Hôpital Bichat-Claude Bernard, Paris) reported that concomitant administration of granulocyte-mononuclear colony stimulating factor (GM-CSF) and ganciclovir is being tried in an effort to counteract hematologic toxicities; preliminary findings revealed that 16 patients were able to tolerate ganciclovir 5-10 mg/kg/day with concomitant GM-CSF. Dr. David Hardy (University of California, Los Angeles, School of Medicine) gave preliminary results on a randomized (ACTG) trial in which the combination was compared with ganciclovir as a single agent. Although small (25 patients), the trial showed the ganciclovir/GM-CSF combination to be associated with fewer occurrences of neutropenia: 6 patients on ganciclovir had absolute neutrophil counts below 750, compared with 3 patients on ganciclovir/GM-CSF.

Although potentially quite toxic, foscarnet is also somewhat effective in treating CMV retinitis. Dr. Elisabeth Dohin and colleagues (Hôpital Pitie-Salpètriére, Paris) found that intravenous administration of 200 mg/kg/day, in either two or three doses, produced complete resolution of retinitis in 60 of 61 study patients with acceptable toxicity. Mild, reversible nephrotoxicity occurred in 11% of the patients; hydration during treatment is important in reducing the frequency and severity of this complication. Other investigators have observed more frequent toxicity, especially renal insufficiency, and one study (at Hôpital Bichat-Claude Bernard, Paris) raised the possibility of genital ulceration as a complication of foscarnet therapy. Nevertheless, most agree that these negative effects can be prevented or controlled by more cautious monitoring and dose modifications. Furthermore, foscarnet may have a role in managing patients whose CMV retinitis is resistant to ganciclovir therapy. Dr. David Parenti (George Washington University Medical Center, Washington, D.C.) and coworkers, for example, reported on two patients who had progression of retinitis despite high-dose ganciclovir therapy; these patients responded favorably to foscarnet therapy (60 mg/kg/day for 14 days, followed by 90 mg/kg/day): both ceased shedding virus (had negative CMV cultures), and retinitis progression did not occur until week 12 or week 25 of foscarnet treatment.

Maintenance Therapy. Following initial treatment of CMV retinitis, maintenance therapy is necessary to prevent relapse. In general, patients who have had documented CMV retinitis will require indefinite therapy. The challenges of maintenance therapy differ from those of initial therapy and include:

- expense
- logistics of drug administration
- long-term toxicity
- patient acceptance and compliance

At this year's Conference, a number of presentations addressed these issues. Both ganciclovir and foscarnet have been shown to be useful agents in maintenance therapy. In the ganciclovir treatment IND summary study (cited above), patients received maintenance therapy consisting of 35 mg/kg/week indefinitely. Data reported by Dr. Ian Jennens and coworkers (Fairfield Hospital, Melbourne), however, suggest that lower doses may be as effective: a regimen of 10 mg/kg three times a week was found to prevent relapse as well as 5 mg/kg five times a week, while offering savings in costs and time. The potential for significant neutropenia with ganciclovir therapy is a concern in using it for maintenance therapy, especially since most patients will also be receiving AZT.

Intermittent foscarnet therapy, given with hydration, is effective in preventing relapse of retinitis and may also be adaptable for home care. Christine Katiama and colleagues (Hôpital Pitie-Salpètriére, Paris) showed that two daily doses of 90–100 mg/kg of foscarnet with 2 hours of hydration by infusion was more effective than lower doses (such as three daily doses of 60 mg/kg) in inhibiting relapse; nephrotoxicity, a potentially serious complication, was rarely observed in this study. Mark Jacobson (University of California, San Francisco and San Francisco General Hospital) reported his team's findings that a somewhat higher dose (120 mg/kg/day) was more effective than lower doses and did not increase the frequency of nephrotoxicity. Since foscarnet does not commonly cause myelosuppression, it may be a particularly useful agent for patients who also take AZT, especially if AZT-induced cytopenias occur.

Neither ganciclovir nor foscarnet can prevent all recurrences of CMV retinitis. Dr. Graeme Molye (Westminster Hospital, London), for example, reported his team's finding of a 25% relapse rate with either drug. Resistance to ganciclovir can be demonstrated *in vitro* and is sometimes associated with clinical progression. Dr. Lawrence Drew (Mount Zion Hospital and Medical Center, San Francisco) reported that 13.6% of patients receiving ganciclovir for more than 3 months excreted moderately or highly resistant strains of CMV and that excretion of resistant strains was associated with disease progression. However, clinical deterioration occurred in some patients in the absence of demonstrable ganciclovir resistance *in vitro*.

Other Manifestations of CMV Disease. Infection with CMV can also cause damage to the digestive tract or lungs. CMV commonly goes unrecognized as a pathogen in gastrointestinal illness among patients with HIV infection. Using upper and lower endoscopy, Dr. Walter Heise and colleagues (Auguste-Viktoria-Hospital, Berlin) found lesions caused by CMV in 34% of 250 AIDS patients who had gastrointestinal complaints; 57 of the 85 patients underwent acute and maintenance therapy with ganciclovir, which was effective in treating these lesions in most patients (52 of 57). Dr. Douglas Dieterich (St. Luke's Hospital, New York) reported that ganciclovir was effective in treating CMV colitis in his team's study of 62 AIDS patients with colitis. These and other previous studies suggest the need for early endoscopy and evaluation of tissue for CMV in patients who have gastrointestinal symptoms, such as dysphagia, diarrhea, or abdominal pain.

TOXOPLASMOSIS

A disease caused by a parasite (*Toxoplasma gondii*) commonly found in cat feces, toxoplasmosis rarely affects individuals with a properly functioning immune system. This opportunistic infection is diagnosed in approximately 20% to 50% of AIDS patients worldwide; its morbidity almost always involves the CNS. (See also Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION.) As shown by Dr. Sophie Matheron and colleagues (Hôpital Bichat-Claude Bernard, Paris) and others, the occurrence of toxoplasmosis among patients with HIV infection is directly related to the degree of immunodeficiency. Dr. Matheron's team reported that AIDS patients with toxoplasmosis had CD4 cell counts in the range of 0–103/mm^3 com-

paired with a range of 10–190/mm^3 for AIDS patients without toxoplasmosis. When studied by disease stage, they found 105 of 285 (37%) AIDS patients and 13 of 80 (16%) ARC patients to have toxoplasmosis. Similarly, Murielle Eliaswicz (Saint-Antoine Hospital, Paris) and coworkers showed that patients whose CD4 cell counts fall to 150/mm^3 are at high risk for toxoplasmosis: at the time of diagnosis, 104 of 133 (78%) AIDS patients had CD4 cell counts below 150/mm^3.

In a comparative study of European and African HIV-positive individuals Corinne Liesnard and colleagues (Erasme Hospital, Brussels) found that significantly more Europeans (70% versus 49%) had positive serology for *T. gondii*, indicating that Europeans are at higher risk for developing cerebral toxoplasmosis. Dr. Liesnard and others recommended that HIV-infected patients who have not yet been infected with *T. gondii* should carefully avoid potential sources of exposure, such as raw meat and crude vegetable consumption, gardening, and contact with cat litter. Dr. Michael Stein and colleagues (Rhode Island Hospital, Providence) found that HIV-positive homosexual men living in Rhode Island have a much lower *T. gondii* seroprevalence rate compared with that cited for other U.S. regions. This study of 82 consecutively seen HIV-positive persons also found that heterosexually infected persons had the lowest *T. gondii* seroprevalence compared with homosexual men and IVDUs.

Using a decision analysis model, Dr. David Rose (Mount Sinai School of Medicine, New York) recommended that, among patients with serologic evidence of latent toxoplasmosis, the benefits of long-term prophylaxis to prevent cerebral disease outweigh the risks (of therapy) unless the prophylactic regimen used is both toxic and ineffective. But, in clinical practice, no consensus on when to treat is evident: Some administer preventive therapy only to those who have experienced an episode of cerebral disease; others recommend prophylaxis based on CD4 cell count and *T. gondii* serology; and still others based prophylaxis on the likelihood of acquiring toxoplasmosis (by considering lifestyle or geographic location). Dr. Matheron's group (above), for example, suggested primary prophylaxis for toxoplasmosis among patients with CD4 cell counts under 100/mm^3.

MYCOBACTERIAL INFECTIONS

Mycobacterium Tuberculosis. Infection with *Mycobacterium tuberculosis* (the tubercle bacillus), generally distinguished as either pulmonary or extrapulmonary tuberculosis (TB), has been reported in increased incidence among HIV-infected populations in developing countries as well as in urban centers in developed countries. Dr. Peter Eriki (World Health Organization, Geneva) reported the WHO 1990 estimates: more than 2 million people are thought to be dually infected (with HIV and *M. tuberculosis*), and that more than 1.5 million of these are in sub-Saharan Africa. HIV seroprevalence rates of 20–30% are common among TB patients in many African countries. However, in Burundi, 55% of TB patients are HIV-positive; in Zaire, 38% of patients hospitalized with TB are HIV-positive. Comparable rates were found in Kampala, Uganda (66% of newly diagnosed TB patients are HIV infected) and in New York City Hospital (61.2%). In contrast, in India, only 1 of 292 TB cases (0.3%) was HIV-positive. Javier Romo (Mexico City General Hospital) and colleagues reported that in Mexico the annual prevalence of TB rose from 10% in 1986 to 63% in 1989. And in their current study of 200 AIDS patients, Dr. Romo's team found 86 (43.4%) to be positive for *M. tuberculosis*.

Based on data from Eleonora Quinhoes (Hospital Gaffrée a Guinle, Rio de Janeiro) and colleagues and Maxwell Mahari and colleagues (City Health, Harare, Zimbabwe) TB has emerged as a strong and accurate predictor of underlying HIV disease among hospitalized patients in Brazil and Zimbabwe. In the United States, the Centers for Disease Control (Atlanta) reported that through 1988, 4% of all AIDS cases (3,301 of 79,329) had docu-

mented TB. Four states (New York, Florida, New Jersey, and Texas) and two cities (San Francisco and Los Angeles) were found to account for almost 80% of these HIV/TB cases.

The association between HIV and TB is also apparent when the statistics are studied obversely: Anita Barry (Boston Department of Health & Hospitals/Boston University Schools of Medicine & Public Health) and colleagues, for example, reported that 51 of 188 persons with confirmed or suspected TB—inactive as well as active—were also HIV-positive. Other Conference reports presented data supporting the increasing trend in the number of TB cases in U.S. metropolitan areas. TB control programs in many countries are being seriously challenged by the HIV epidemic.

Clearly, effective programs to prevent, identify, and treat TB among people with HIV disease are needed in all countries. Infection with HIV appears to be a significant risk factor for progression of latent tuberculosis infection to clinically active tuberculosis. Additionally, immunosuppression due to HIV infection very likely increases new tuberculosis infection and consequent clinical tuberculosis.

Secondary Transmission. A major concern in public health and hospital infection control has been the possibility of secondary transmission of TB from patients with HIV disease to their household or occupational contacts and care providers. In a study comparing 58 HIV-negative/TB-positive patients with 42 HIV-positive patients with pulmonary TB, Ekungola Baende (Projet SIDA, Kinshasa, Zaire) and colleagues found no evidence of increased secondary transmission to 323 household contacts.

Clinical/Radiological Features of Dual Infection. The clinical pattern of active TB among patients with HIV disease is characterized by some interesting variations from the usual TB presentation. Helene Cerigo (New York VA Medical Center) and colleagues demonstrated that pulmonary abscesses are common at initial presentation and also may develop during treatment; the authors suggested prompt diagnosis and early drainage to improve treatment outcome. Jean Batungwanayo (Centre Hospitalier de Kigali, Rwanda) reported that pleural effusion among patients with TB in Rwanda is frequently associated with HIV infection. In an effort to characterize radiological features of HIV-associated TB, Samuel Gothua (Kenya Medical Research Institute, Nairobi) and coworkers compared antero-posterior chest radiographs from 191 TB patients, 58 of whom were also HIV-infected. No specific radiological *patterns* were found to be diagnostic for HIV infection in TB patients, although a number of features were suggestive of underlying HIV disease. Carmen S. Camara (Gaffrée & Guinle University Hospital, Rio de Janeiro) and colleagues pointed out that although significantly immunocompromised patients with HIV disease who develop TB may have unusual radiographic features and disseminated disease, normal chest x-rays do not rule out a diagnosis of TB—in particular, endobronchial TB.

Treatment. Standard treatment regimens effective in TB patients who do not have HIV disease may fail in the special dual-infection setting. Dr. Iris Davis and colleagues (New York Hospital-Cornell Medical Center) reported that standard three-drug therapy does not appear adequate for HIV-positive patients. Indeed, neurological involvement (specifically, meningitis) was associated with TB treatment failure: 3 of 3 patients with pulmonary or miliary TB treated with a standard 3-drug regimen developed neurological complications within 6 months of diagnosis. However, 4- and 5-drug therapy regimens showed at least partial clinical responsiveness.

Dr. Jeremy Sugarman (Duke University Medical Center, Durham, North Carolina) and associates reported that the standard treatment of TB in Tanzania (Thiazina, a tablet containing the sulfa drug thiacetazone and isoniazid, followed by streptomycin injections for 2 months) often leads to Stevens-Johnson syndrome in a disturbingly high proportion of patients. The authors noted that patients with HIV infection may have an increased sensitivity to certain sulfa-containing drugs and may be more prone to develop Stevens-Johnson

syndrome than other patients—a finding that will affect the management of TB in East Africa, where thiacetazone is often used in treatment. (Stevens-Johnson syndrome is a severe form of erythema multiforme. Vivid erythematous purpuric lesions usually appear suddenly in symmetrical distribution around the oral and anogenital mucous membranes, accompanied by constitutional symptoms such as malaise, headache, fever, arthralgia, and conjunctivitis.)

Relapse after treatment is also more common when TB occurs in patients with HIV disease; Dr. Laura Zamora and colleagues (Hospital Clinic, Barcelona) showed that relapses happened as long as 20-22 months after initial therapy and recommended that maintenance drug therapy be considered in patients who are likely to survive for longer periods. Prophylaxis to prevent active TB among HIV-infected patients who have evidence of previous exposure to TB is an established clinical practice in many countries. Dr. D. Wadhawan (University Teaching Hospital, Lusaka, Zambia) and colleagues demonstrated the efficacy of isoniazid prophylaxis for *M. tuberculosis* among adults patients with HIV in Zambia.

Mycobacterium Avium. Mycobacteria other than *M. tuberculosis* (nontuberculous mycobacteria) also cause significant morbidity and mortality in patients with HIV disease. Maria Rosario Araneta and colleagues (New York City Department of Health) reported finding *M. avium intracellulare* in 18 (25.7%) of 70 autopsy studies of IVDUs with AIDS (*M. xenopi* was found in 1.4% of cases and *M. gordoni* in 1.4%).

The correlation between nontuberculous mycobacteria isolation and clinical symptoms is imperfect (symptoms are nonspecific—such as fever, night sweats, or hepatosplenomegaly); some patients with disseminated *M. avium* complex infection (DMAC) may not even develop significant or limiting manifestations. In a retrospective case-control investigation of 97 HIV-positive patients, Robert Horsburgh (Emory University School of Medicine, Grady Memorial Hospital, Atlanta) and coworkers, showed that DMAC contributes significantly to premature mortality among all categories of patients with HIV disease. Of the 15 patients for whom data was presented, none had CD4 cell counts greater than $100/mm^3$. In an independent study, Dr. Joseph Havlik and coworkers (Emory University School of Medicine, Grady Memorial Hospital, Atlanta) showed that the frequency of DMAC increased as CD4 cell counts dropped below $100/mm^3$. This group was able to isolate *M. avium* from the blood of patients with HIV infection and fever—especially when anemia, weight loss, night sweats, or hepatosplenomegaly are also present; however, Constance Benson and colleagues (Rush-Presbyterian-St. Luke's Medical Center, Chicago) could not define any predictable pattern of isolation of *M. avium* from blood or bone marrow, nor of *M. avium* colonization of the respiratory or gastrointestinal tracts, that was clearly associated with development of DMAC.

Treatment for DMAC remains less than satisfactory, although some Conference reports offered positive findings. Given the lack of efficacy of isoniazid and traditional TB treatment regimens in DMAC, many investigators have used alternative drugs in combinations. Dr. Catherine Leport (Hôpital Bichat-Claude Bernard, Paris) reported that rifabutin has been used in open (compassionate use) trials, always in combination with 3 other drugs: isoniazid, ethambutol and clofazimine. In a French multicenter study, a quadruple regimen of rifampin, ethambutol, isoniazid, and clofazimine with or without clarithromycin has demonstrated the efficacy of clarithromycin against DMAC. John Pottage and colleagues (Rush-Presbyterian-St. Luke's Medical Center, Chicago) found, in a small preliminary study, that a multiple drug regimen (using IV amikacin and oral clofamizine, rifampin, ethambutol, and ciprofloxacin) produced favorable responses in 19 of 20 patients with a mean treatment time of 5.3 months.

As yet, there do not appear to be any indicators to predict the occurrence of widespread, significant DMAC. According to Dr. Frederick Siegal (Long Island Jewish Medical Center/Albert

Einstein College of Medicine, New York) and coworkers, rifabutin (ansamycin) may delay the onset of DMAC in AIDS patients with opportunistic infections. Many issues complicate treatment of DMAC in addition to defining the most active drug combinations, such as identifying which patients require treatment, and for how long. The presence of significant constitutional symptoms (especially fever, night sweats, or hepatosplenomegaly) in patients from whose blood *M. avium* has been isolated may suggest the need for therapy, but other microorganisms may be the cause of the same symptoms in these same patients.

MYCOPLASMA

Reports that a new strain of mycoplasma species, *Mycoplasma incognitus*, was associated with nephropathy among patients with HIV disease was surrounded by much controversy at the Conference. Dr. Shyh-Ching Lo and colleagues (Armed Forces Institute of Pathology, Washington, D.C.) demonstrated the presence of *M. incognitus* antigens (by staining with monoclonal antibodies) and the presence of mycoplasma-like structures (by electron microscopy) in renal tissue with nephropathy in 15 patients with AIDS-associated renal insufficiency. Further, Dr. Lo's team, showed that *M. incognitus* is not sensitive to erythromycin (a normally effective antimycoplasma agent), although it is susceptible *in vitro* to tetracycline, doxycycline, and ciprofloxacin. Dr. Luc Montagnier (Institut Pasteur, Paris) independently reported his team's finding of *M. incognitus* as well as other mycoplasma species in the blood and from tissue (and, although rarely, brain) biopsies of AIDS patients.

Many scientists responded to both Dr. Lo's and Dr. Montagnier's observations with skepticism, suggesting that the Mycoplasma species observed may have been contaminants—a common problem in working with cells in tissue culture. This debate occurred in the setting of a larger discussion about the possible role of mycoplasmas in potentiating the effects of HIV, and even of causing significant morbidity and even mortality among people without HIV, as well—a controversy that thus far, remains unresolved (see Chapter 2: PATHOGENESIS & IMMUNOLOGY).

BACTERIAL DISEASES

Bacterial Enteritis. Jean-Pierre Couland and coworkers (Hôpital Bichat-Claude Bernard, Paris) showed this year that nontyphoidal salmonella bacteremia can be prevented by AZT without antibiotics. Recurrence of this bacteremia is a well recognized complication in AIDS patients and, in the past, long-term antibiotics have been used to prevent relapse. Dr. Catherine Leport (Hôpital Bichat-Claude Bernard) pointed out in her plenary session that this interesting effect of AZT is related to the drug's well known activity against gram negative bacilli (which includes salmonella and all other enterobacteria).

Charlotte Kent (University of California, Berkeley) and coworkers found that campylobacter (helicobacter) enteritis is often associated with ingestion of undercooked eggs or chicken in HIV-infected persons, as it is in the non-HIV-infected; the authors recommended that people with HIV infection be carefully educated about safe food preparation to prevent not only bacterial enteritis but also toxoplasmosis (see above).

Cat Scratch Disease. Sylvana Tuur and colleagues (Armed Forces Institute of Pathology, Washington, D.C.) presented observations on 10 AIDS patients who experienced a systemic, severe, form of cat scratch disease. The authors emphasized the clinical pattern of hepatic involvement—which can be misdiagnosed as either granulomatous hepatitis or as peliosis hepatitis. In another report, Carol Kemper and associates (Stanford University School of Medicine and Santa Clara Valley Medical Center, San Jose, California) associated the pathogen of cat scratch disease with extra-cutaneous bacillary epithelioid angiomatosis in a histopathological study of an HIV-infected individual. In both reports, antibiotic therapy produced improvement.

Table 7.2. Infections Reported in Association with HIV

Complication	Causative Agent	Treatment	Incidence
Amebiasis	*Entamoeba histolytica, Entamoeba coli*	Diiodohydroxyquin, metronidazole	Amebiasis remains an occasional diagnosis among homosexual men who engage in unprotected anal intercourse with other men; it is an infrequent cause of diarrhea in persons with HIV disease.
Blastocystis	*Blastocystis hominis*	Iodoquinol, metronidazole, trimethoprim/sulfamethoxazole (TMP/SMX)	This parasite is frequently identified with other intestinal pathogens in the stools of patients with chronic severe diarrhea (persisting for 4 to 6 weeks); its pathogenic role in any specific case, however, is uncertain.
Blastomycosis	*Blastomyes dermatidis*	Ketoconazole, amphotericin B	Disseminated or dermatologic blastomycosis is endemic in parts of North America. To date its incidence among HIV-infected persons, although reported, has been rare.
Campylobacter *see* Helicobacter	—	—	—
Candidiasis	*Candida albicans, C. glabrata, C. tropicalis*	Clotrimazole troches, ketoconazole, nystatin, amphoterocin B, fluconazole, itraconazole	Oropharyngeal or esophageal candidiasis complicates the course of the majority of cases of HIV disease by the time AIDS is diagnosed. Thrush is a useful predictor of the short-term development of severe immunodeficiency in people with ARC.
Chagas' disease	*Trypanosoma cruzi*	None established; nifurtimox in acute phase, primaquine phosphate, benznidazole (in trials)	Only a few reports have shown *T. cruzi* to be associated with HIV disease.
Chancroid	*Haemophilus ducreyi*	Erythromycin, TMP/SMX, ceftriaxone	Chancroid, one of the genital ulcer diseases, is a common STD in countries where heterosexual HIV transmission is prevalent. *H. ducreyi* seroprevalence studies in Africa show that the highest rates are among those groups at risk for HIV infection—most notably, prostitutes and clients of prostitutes.
Chlamydia	*Chlamydia trachomatis*	Tetracycline, erythromycin	Chlamydial infections are becoming increasingly prevalent in industrialized countries, especially in the U.S., and are a common finding in HIV-infected patients. However, the incidence of chlamydial disease in people with HIV has not been reported to be increased.
Cladosporiosis	*Cladosporium*	Treatment undetermined	A single case of intracerebral cladosporiosis in an AIDS patient was reported in 1988.
CMV Infection	Cytomegalovirus (CMV)	Ganciclovir (DHPG), foscarnet, acyclovir	Disseminated CMV infection involving the GI tract, lungs, and heart is common in people with AIDS, as is CMV retinitis. At least 30% of persons with HIV disease have evidence of CMV infection.

Table 7.2. Infections Reported in Association with HIV *Continued*

Complication	Causative Agent	Treatment	Incidence
Coccidioidomycosis	*Coccidiodes immitis*	Amphotericin B, fluconazole	This fungal disease is endemic in Mexico, Central and South America, and parts of the southwestern U.S. Disseminated infection with *C. immitis* is particularly prevalent among HIV-infected persons in southern California and Arizona.
Cryptococcosis	*Cryptococcus neoformans*	Fluconazole, amphotericin B, amphotericin B/flucytosine, iatroconazole, ketoconazole	Cryptococcal meningitis and disseminated cryptococcosis are life-threatening and are seen in 5% to 13% of all persons with AIDS, often as the presenting illness.
Cryptosporidiosis	*Cryptosporidium*	Eflornithine, spiramycin, recombinant IL-2, somatostatin	Previously seen only in animal handlers, children in day-care centers, and travelers, intestinal cryptosporidiosis has been diagnosed in at least 20% of people with HIV disease. Cryptosporidium parasites cause diarrhea, which is often refractory to therapy.
Donovanosis (granuloma inguinale)	*Calymmatobacterium granulomatis*	Tetracycline, TMP/SMX	Some reports indicate that *C. granulomatis* has been isolated from genitoanal lesions among homosexual/bisexual men with HIV disease.
Enteritis	*C. albicans, Mycobacterium avium intracellulare*, herpes simplex virus (HSV), *Cryptosporidium, Isospora belli*, CMV, *Shigella, Strongyloides stercoralis, B. hominis, Aeromonas, Giardia lamblia, Clostridium difficile, E. histolytic, E. coli, Salmonella*. (See also specific infections.)	Treat according to pathogen	Gastrointestinal infections—found throughout the GI tract, from the oral cavity to the rectum—are common among people with symptomatic HIV disease; roughly 30% to 90% of people with HIV disease develop chronic diarrhea as a result of "HIV-related enteropathy." This enteropathy may involve many pathogens, HIV itself, and/or the collateral effects of drug therapy, dietary changes, nutritional deficiencies, and other medical problems.
Epithelioid angiomatosis	Cat scratch disease bacillus (possibly)	Erythromycin	Findings of fever and widespread cutaneous lesions (reddish, vascular papules) which resemble those observed in cat scratch disease have been reported in HIV-infected individuals, including persons with AIDS.
Folliculitis	*Staphylococcus aureus, Demodex folliculorum*	Penicillinase-resistant penicillin, rifampin, benzoyl peroxide wash, clindamycin, ultraviolet light (UVB) therapy	Pruritic eruptions and pruritic follliculitis are common in patients with HIV. Of all the HIV-associated folliculitides, $^1/_2$ to $^2/_3$ are attributed to *D. foliculorum* or are of unknown etiology.
Genital ulcer diseae (GUD)	*H. ducreyi*, HSV, *Treponema pallidum, C. trachomatis, C. granulomatis*	Erythromycin, TMP/SMX	GUD encompasses those STDs manifested by genital lesions or ulcers. GUD may be an important cofactor in the sexual transmission of HIV; epidemiological studies in Africa and in urban centers in the U.S. reveal a higher incidence of GUD among HIV-positive persons than among their HIV-negative counterparts.

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Table 7.2. Infections Reported in Association with HIV *Continued*

Complication	Causative Agent	Treatment	Incidence
Genital warts (condyloma acuminatum)	Human papilloma virus (HPV)	Podophyllin, cryotherapy, laser therapy, surgery	Genital papillomavirus infection, especially in women who engage in penile-vaginal intercourse, may be a cofactor in the sexual transmission of HIV. HIV infection may exacerbate HPV infection in men and women.
Giardiasis	*Giardia lamblia*	Quinacrine, metronidazole, furazolidone, pyrimethamine, sulfadiazine	Giardiasis, an intestinal disorder, is commonly seen among homosexual/bisexual men with HIV disease.
Gingivitis	Numerous pathogens	Treat according to pathogen	Because of poor response to conventional therapy and the severity of gingivitis (gum tissue necrosis and exposure of bone) in people with HIV disease, this condition is referred to as HIV-associated gingivitis.
Gonorrhea	*Neisseria gonorrhoeae*	Amoxicillin plus doxycycline, ceftriaxone plus doxycycline, tetracyclines, spectinomycin plus doxycycline, ciprofloxacin plus doxycycline, penicillin	The incidence of gonorrhea remains high in urban STD clinics; rates of rectal gonorrhea recently increased in some urban clinics among homosexual/bisexual males. Penicillin-resistant strains of *N. gonorrhoeae* may explain, in part, this observed increase.
Hairy leukoplakia	Epstein-Barr virus (EBV)	Acyclovir, ganciclovir	Hairy leukoplakia—so-named because of the "hairy" appearance of the oral mucosa—is found almost exclusively in people with HIV disease. Evidence of oral hairy leukoplakia in non-HIV infected populations (notably, transplant patients with immunodeficiency) has recently been reported.
Helicobacter infection	*Helicobacter pylori* (formerly *Campylobacter pylori*)	Erythromycin, norfloxacin, ciprofloxacin	This bacterial infection causes gastroenteritis; in HIV disease it is especially common among homosexual men.
Hepatitis	Hepatitis A virus; hepatitis B virus; hepatitis C virus; hepatitis D virus; hepatitis E virus	Primarily supportive (bed rest, maintenance of fluid and electrolyte balance)	Viral hepatitis remains an important co-infection in people with HIV disease. Hepatitis B and D are particularly common among HIV-infected homosexual/bisexual males, most of whom, some studies show, also have markers of past resolved or active hepatitis B. Among HIV-infected drug injectors and their sexual partners, hepatitis B (preventable by vaccine), C, and D are common.
Herpes	Herpes simplex virus, type 1 (HSV-1); herpes simplex virus, type 2 (HSV-2)	Acyclovir, foscarnet, vidarabine	Oral herpes is classically attributed to HSV-1, but close to 50% of all AIDS patients with oral lesions show HSV-2 infection; genital herpes is mainly associated with HSV-2. Dual infection with HSV-1 and HSV-2 is not uncommon in the sexually active population.
Herpes zoster (shingles)	Varicella-zoster virus	Acyclovir	Herpes zoster, or shingles, may have an unusual presentation in HIV disease; disseminated zoster in HIV-infected patients may be unresponsive to treatment with acyclovir.

Table 7.2. Infections Reported in Association with HIV *Continued*

Complication	Causative Agent	Treatment	Incidence
Histoplasmosis	*Histoplasma capsulatum*	Amphotericin B, fluconazole, ketonazole, itraconazole	Once seen primarily in endemic areas, (e.g., the central and southern U.S.), the incidence of disseminated histoplasmosis—a fungal disease—is increasing among people with AIDS.
Isosporiasis	*Isospora belli*	Furazolidone, pyrimethamine, sulfadiazine, TMP/SMX	This protozoal infection of the bowel is especially common in homosexual/bisexual men with HIV disease.
Leishmaniasis	*Leishmania donovani*	Neostibosan (IV)	This malaria-like disease is caused by a (visceral) protozoan and is transmitted to humans via sandflies. It is now rarely being diagnosed in persons with HIV disease from areas other than the tropics.
Leprosy	*Mycobacterium leprae*	Dapsone/rifampin, clofazimine, corticosteroids	HIV seropositivity in patients with leprosy may be disproportionately high in endemic areas, such as Haiti. Ongoing studies are examining the association between HIV infection and infection with *M. leprae.*
Lymphoid interstitial pneumonitis (LIP)	Unknown etiology	No established therapy; corticosteroids (experimental)	This chronic, progressive pulmonary disease occurs in 51% of children with AIDS; it is rare among adults.
Meningitis	*C. neoformans, Coccidioides, Listeria monocytogenes, M. tuberculosis, Toxoplasma gondii* (See also specific infections.)	Amphotericin B, fluconazole, ketoconazole	Meningitis is an inflammation of the membranes, or meninges, that surround the brain and spinal cord; cryptococcal meningitis is (after PCP) the second deadliest opportunistic infection in people with AIDS.
Molluscum contagiosum	Molluscum contagiosum virus	Cryotherapy and curettage	Although common in all HIV-infected populations with ARC or AIDS, molluscum contagiosum occurs in unusual body areas (e.g., face, neck) in adults.
Mycobacteremia (nontubercular)	*Mycobacterium avium-intracellulare* (MAI), *M. fortuitum, M. haemophilum, M. kansasii, M. leprae, M. ulcerans, M. xenopi*	Amikacin, ansamycin (rifabutin), ciprofloxacin, ethambutol, rifampin, clofazamine, cycloserine	Mycobacterial infections caused by MAI occur quite commonly among people with HIV disease—generally as a late complication of immunosuppression and usually as a disseminated infection. MAI is associated with serious pulmonary, cardiac, and gastrointestinal disease in people with AIDS. Other atypical mycobacteria (e.g., *M. xenopi)* have a higher incidence in people with HIV disease than in the general population, but are still rare.
Myocarditis	*Pneumonocystis carinii, M. tuberculosis, H. capsulatum, T. gondii, M. avium-intracellulare,* CMV EBV, *C. albicans, C. neoformans*	Treat according to pathogen	This condition often goes undiagnosed, but autopsy reports show that inflammation of the muscular walls of the heart is not uncommon in people with AIDS.

(Continues on next page)

Table 7.2. Infections Reported in Association with HIV *Continued*

Complication	Causative Agent	Treatment	Incidence
Nocardiosis	*Nocardia asteroides*	Minocyline/amikacin, minocyline/netilmicin/imipenem, TMP/SMX	Infections due to *Nocardia* are rare; less than 1% of persons with AIDS develop pulmonary or other manifestations.
Oral warts	Human papilloma viruses	Acyclovir; surgical removal	Oral warts and lesions commonly recur after removal in immunocompromised patients.
Pelvic inflammatory disease (PID)	*Neisseria gonorrhoeae, Chlamydia*	Spectinomycin, cephalosporin, ampicillin	The incidence of HIV infection among women with PID, especially those visiting urban STD clinics, is reported to be significant.
Periodontitis	*Actinomyces viscosus, Actinobacilus actinomycetemcomitans, Peptostreptococcus micros, Bacteroides intermedius, Wolinella, Candida, Fusobacterium*	Chlorexidine, povidone-iodine lavage	Periodontitis manifests in many people with HIV disease as severe necrosis and loss of periodontal attachment and loss of bone.
Pneumonia	*Pneumocystis carinii, Aspergillus, Legionella micdadei, Haemophilus influenzae, Streptococcus pneumonia, Staphylococcus aureus, H. capsulatum,* CMV, *Legionella pneumophila, M. avium intracellulare, M. tuberculosis* (See also specific infections.)	Amphotericin B, pentamidine (aerosol and IV), dapsone, dapsone/trimethoprim, eflornithine, TMP/SMX, trimetrexate, clindamycin/primaquine	Pneumonia caused by *P. carinii* (PCP) is a hallmark of AIDS and ultimately occurs in at least 80% of people with HIV infection. Because of its recurrence rate, morbidity, and risk of mortality, PCP prophylaxis and therapy is the focus of much clinical research. Preventing PCP will significantly reduce hospital costs, length of hospital stay, and lost time from jobs.
Retinitis	CMV, HSV, varicella zoster virus, *T. pallidum*	Ganciclovir (DHPG), foscarnet, acyclovir, penicillin	CMV retinitis is particularly common (roughly 30%) in persons with AIDS; left untreated, retinitis can cause blindness. Findings of syphilitic retinitis in HIV-infected persons have been reported recently.
Rhodococcus infection	*Rhodococcus equi*	Sulfa-based antibiotics	Rhodococcus infection has (on rare occasions) produced lung abscess in some severely immunocompromised patients.
Salmonellosis	*Salmonella*	TMP/SMX, ciprofloxacine, chloramphenicol, amoxicillin (ampicillin), ceftriaxone	Salmonella enterocolitis and enteric fever may be associated with HIV disease among homosexual men.
Sarcoidosis (lymphogranulomatosis)	Unknown etiology	Treatment undetermined	Black males with HIV infection seem to be at increased risk for sarcoidosis, according to a 1988 study of 700 HIV-positive military applicants.
Scabies	*Sarcopies scabiei*	Lindane, crotamiton, sulfur	This superficial parasitic STD, while not an opportunistic infection, is common among HIV-infected men.

Table 7.2. Infections Reported in Association with HIV *Continued*

Complication	Causative Agent	Treatment	Incidence
Seborrheic dermatitis	*Pityrosporum orbiculare, P. ovale*	Clotrimazole or ketoconazole in combination with a mild topical steroid	Although the causative agents are part of the natural skin flora, in immunocompromised patients, seborrheic dermatitis is extremely common: more than 35% of persons with ARC or AIDS have seborrheic dermatitis.
Sepsis	*Staphylococcus, Streptococcus, Pseudomonas aeruginosa, Salmonella, Clostridium perfingens, H. influenzae, Rhodoturula rubra, Pneumococcus*	Treat according to pathogen	Most cases of sepsis reported among AIDS patients and other immunocompromised hospital patients are attributable to nosocomial (hospital-acquired) infections. In many instances, sepsis results from immunosuppression caused by aggressive chemotherapy of Kaposi's sarcoma, lymphoma, or other neoplasms.
Syphilis	*Treponema pallidum*	Penicillin, ampicillin, amoxycillin/probenicid (experimental)	Primary or reactivated syphilis is being reported in growing numbers at urban STD clinics throughout the world. Some researchers are trying to determine whether a history of syphilis is a risk factor for acquisition of HIV. (Other factors, including use of crack, however, may also contribute to the increased incidence of syphilis.)
Toxoplasmosis	*Toxoplasma gondii*	Pyrimethamine/sulfadiazine, trimetrexate, TMP/SMX, clindamycin plus pyrimethamine, spiramycin	Toxoplasmosis of the brain and spinal cord (as opposed to disseminated toxoplasmosis) is a significant, life-threatening opportunistic infection in persons with AIDS. Current studies show that at least 3% of all AIDS patients develop CNS toxoplasmosis.
Tuberculosis	*Mycobacterium tuberculosis*	Ethambutol, isoniazide, pyrazinamide, rifampin, streptomycin	Although only extrapulmonary TB is an indicator disease for the CDC surveillance definition of AIDS, the incidence of pulmonary TB among HIV-infected persons has been rising in many countries, including the U.S.
Trichomoniasis	*Trichomonas vaginalis*	Metronidazole	Based on reports from STD clinics, this common STD is more often encountered in HIV-infected persons than in their HIV-negative counterparts.

SYSTEMIC FUNGAL DISEASES

Cryptococcosis. Infection with *Cryptococcus neoformans* remains the most common source of disseminated fungal disease in patients with HIV. (See also Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION for a discussion of cryptococcal infection in the central nervous system.) Research presented by Isabelle Lacomte (Tenon Hospital, Paris) and colleagues confirmed that systemic cryptococcal disease is associated with advanced immunosuppression and almost always occurs with CD4 cell counts equal to or less than $100/mm^3$. Although cryptococci are regularly suspected as pathogens, confirming their role in a specific situation is difficult because of diagnostic problems. Dr. Lacomte's team recommended repeated screening for cryptococcal antigen in serum as the best test for systemic disease. Due to the occurrence of false positives on cryptococcal antigen serum tests, Anita Noble (Santa Clara Valley Medical Center, San Jose) and coworkers investigated the effect of pronase treatment (pronase is added to specimen samples) on test outcomes. Based on 97 sera analyses and 42 cerebrospinal fluid (CSF) analyses (from 37 HIV-positive patients), the authors found pronase treatment to reduce the chance of false positives and of unclear or prozone-like (false negative) reactions.

False positive cryptococcal antigen tests in CSF also lead to mistaken diagnoses; Alejandra Gurtman and colleagues (Elmhurst Hospital Center, New York) found that a positive result for cryptococcal antigen in CSF, in the absence of serum antigen, often was falsely positive. They recommend treating on the basis of positive CSF cryptococcal antigen only if other clinical and laboratory data support the diagnosis of cryptococcal meningitis. Diagnosing pulmonary cryptococcosis is also difficult because specific clinical and radiographic patterns do not exist.

The major advance in the management of cryptococcosis has been the development of fluconazole, a drug recently shown to be much less toxic than established antifungal drugs (notably, amphotericin). Dr. Catherine Leport (Hôpital Bichat-Claude Bernard, Paris) pointed out that in an ongoing prospective randomized trial, the benefit of fluconazole long-term maintenance therapy was such that the trial was discontinued: patients receiving either ketoconazole or amphotericin were given the option to take fluconazole. The ease of administration and acceptability as outpatient therapy for maintenance also recommend fluconazole.

Cryptosporidiosis and Microsporidiosis. Cryptosporidiosis frustrates clinicians and patients alike. Cryptosporidial enteritis and intractable diarrhea, its most vexing manifestation, cause significant morbidity and complicate issues of nutrition, mobility, and performance status for people with HIV. In persons with AIDS, cryptosporidial infection can lead to death. Dr. Timothy Flanigan (Case Western Reserve University, Cleveland, Ohio) and colleagues reported that the ability to clear the causative agent of cryptosporidiosis may be related to a history of previous opportunistic infections and CD4 cell count. Dr. Flanigan's team noted that 7 of 8 patients in whom the infection cleared had not had a previous opportunistic infection, but 11 of 18 patients with uncontrolled cryptosporidiosis had a previous opportunistic infection. The CD4 cell count range for individuals who cleared infection was $41\text{-}533/mm^3$ compared with $22\text{-}106/mm^3$ for those who did not clear the infection.

Treatment studies this year produced only slight encouragement. As shown by Rosemary Soave (Cornell Medical Center, New York) and colleagues, oral diclazuril produces a dose-dependent improvement in diarrhea, but no cure, in doses up to 400 mg/day, without toxicity. Niels Hojlyng (Statens Seruminstitut, Copenhagen) and coworkers found some evidence of benefit, but also toxicity, in therapy with hyperimmune bovine colostrum in a study of 16 AIDS patients.

Microsporidia may also cause chronic diarrhea in people with advanced HIV disease. Eeftinck Schattenkerk and colleagues (Academic Medical Center, Amsterdam) reported data indicat-

ing that microsporidial enteritis—especially of the small bowel—is associated with significant diarrhea, and Jan Orenstein (George Washington University Medical Center, Washington, D.C.) and coworkers identified two new microsporidial species associated with such diarrhea.

Other Fungal Diseases. Oral and esophageal candidiasis continue to be a significant fungal infection among immunocompromised patients. Dr. Daniel Urbain and colleagues (St. Pierre University Hospital, Brussels) showed that fluconazole at 100 mg/day successfully treated patients with esophageal candidiasis in an average period of 19 days. And Dr. P. Lavilla and colleagues (Universidad Autonoma, Madrid) showed that weekly fluconazole (200 mg orally) is effective in reducing the incidence of relapse following the successful treatment of candida esophagitis.

Dr. Kenneth Pursell and colleagues (Memorial Sloan-Kettering Cancer Center, New York) demonstrated that while *Aspergillus* species commonly colonize in people with HIV, invasive disease is rare and is usually associated with cytotoxic therapy and neutropenia. Similarly, Dr. David Denning (Santa Clara Valley Medical Center, San Jose, California) and associates reported their observations on pulmonary aspergillosis: it occurs late in the course of HIV disease, often follows cytotoxic or steroid therapy, is associated with neutropenia, and generally responds to treatment.

Two interesting studies evaluated the patterns of coccidioidomycosis in HIV-infected patients; both were conducted in areas with a high incidence of coccidioidal disease. Niel Ampel and colleagues (Tucson VA Medical Center, University of Arizona College of Medicine) and Lawrence Cone and colleagues (Eisenhower Medical Center, Rancho Mirage, California) showed that active, severe coccidioidomycosis is associated with reduced CD4 cell counts of 185 to 200 cell/mm^3. Dr. Cone's team found that very low CD4 counts (less than 35/mm^3) correlate with extensive, life-threatening disease. However, even in endemic areas, positive coccidioidal serology alone does not predict the occurrence of active disease.

ORAL INFECTIONS AND LESIONS

Infections and lesions in the oral cavity are not uncommon in asymptomatic and symptomatic HIV-infected individuals. Dentists, oral surgeons, and other health care professionals must be able to recognize and treat HIV-associated oral complications, as well as HIV-related symptomology. (HIV-infected patients may seek care at a dental clinic—for acute pain with spontaneous oral bleeding or with extensive soft tissue involvement, for example—without being aware of their HIV status.) Oral manifestations associated with HIV infection, as reported at the Conference, include:

- candidasis (erythematous and pseudomembraneous)
- oral warts
- gingivitis
- periodontitis
- dental caries
- aphthous ulcers (recurrent)
- oral hairy leukoplakia (manifested as a "hairy" appearance of the oral mucosa)
- Kaposi's sarcoma lesions in the oral cavity
- lymphoid tumors in the oral cavity (non-Hodgkin's lymphoma involving tongue, gingiva, hard and soft palate)
- salivary gland disease (defined as enlarged salivary glands or presence of xerostomia—that is, dryness of the mouth due to insufficient secretions)

Dr. Zulema Casarigo (Hôpital Fernandez, Buenos Aires) and colleagues reported that in their study of 109 HIV-infected patients, 59 (54.2%) had one or more oral lesions. In contrast, among 194 HIV-negative subjects 20 (10.3%) had evidence of oral disease.

Therapy for oral infections is generally localized topical antifungal, antiviral, or antibiotic. For HIV-associated oral/oropharyngeal candidiasis, oral fluconazole capsules (daily dosage: 50–100 mg for 2 to 4 weeks) appear to effect a faster clinical response than topical therapy,

such as clotrimazole troches. In a study of 24 patients, Saralyn Lichter and coworkers (Johns Hopkins University School of Medicine, Baltimore) reported that oral fluconazole therapy is associated with a decreased relapse rate when compared with ciotrimazole troches. Dr. Jacques Reynes and coworkers (University of Montpellier, France), however, found long-term (3–14 months) fluconazole maintenance therapy to be associated with a relatively high relapse rate: 17 of 53 (32%) ARC/AIDS patients on 50 mg daily every other week had severe relapse, some with esophageal involvement. (See also "Other Fungal Diseases," above.)

CANCERS AND NEOPLASMS

Early in the AIDS epidemic, infectious disease specialists and epidemiologists noted that two types of cancers—Kaposi's sarcoma and lymphoma—in particular, were commonly associated with HIV infection. The connection between HIV infection and lymphoma (cancer of the lymphoid tissue) can conceivably, at least in part, be explained by HIV's insidious infiltration—and thorough disruption—of the lymph tissue and lymphatic system. A link with Kaposi's sarcoma (a connective tissue cancer, affecting endothelial cells that line the blood vessels), however, is somewhat puzzling. Basic science researchers have been looking for a specific etiologic agent (distinct from HIV) in Kaposi's sarcoma, as well as possible pathogenic mechanisms by which HIV could induce a cancerous state in endothelial cells. Although some epidemiological studies implicate a sexually transmitted infectious agent, other studies have demonstrated a relationship between HIV-induced immune changes (such as excess cytokine production) and incidence. (See Chapter 2: PATHOGENESIS & IMMUNOLOGY for a detailed discussion.)

KAPOSI'S SARCOMA

Prior to the AIDS epidemic, the incidence of Kaposi's sarcoma (KS) was restricted primarily to pockets in Africa, Mediterranean countries, and, in the United States, in older men of Jewish or Mediterranean descent. By the end of 1981, however, 96 cases of KS in the United States were reported to the Centers for Disease Control—most of which were in homosexual men who were later found to be HIV infected.

Epidemiology. Data from the United States, Canada, South America, and Europe reveal the incidence of KS to be greatest among male homosexuals. Dr. Elisabeth Couturier (WHO Collaborating Centre on AIDS, Paris) and colleagues stated that 86% of all KS cases reported from 12 European countries through 1989 occurred among homosexual/bisexual men. By comparison, only 5 1% of KS cases occurred among IVDUs; 1.9%, among homosexual IVDUs; 0.7%, among transfusion recipients; 0.4%, among hemophiliacs; and 2.1%, among persons infected via heterosexual contact.

Several reports have noted a recent downward trend in the annual rates of KS. Dr. Martha Guimaraes Dias (Gaffrée & Guinle University Hospital, Rio de Janeiro) and colleagues, for example, studied the annual rate of new KS cases among new AIDS cases in Brazil during the period 1983–89: They reported that a significant drop was evidenced from 1985 to 1986 and this drop was sustained through 1989. In Africa, however, new cases of HIV-associated KS continue to be a significant cause of morbidity among both children and adults. Dr. Edward Mbidde (Uganda Cancer Institute, Kampala) and associates reported that, based on their study of KS patients (evaluated from January 1988 through the end of 1989), HIV-associated KS is occurring at increasing rates in both female and male patients (who are exclusively heterosexual).

Immunogenetics. Studies continue to explore the possibility that a genetic component may play a role in the development of KS. Dr. Dean Mann and colleagues (National Cancer Institute, Bethesda, Maryland) reported that immunogenetic differences could be distinguished between HIV-positive individuals with KS and asymptomatic HIV-infected individ-

uals or HIV-seronegative individuals. In examining the frequency of HLA (major histocompatibility complex) antigens in 278 homosexual men, Dr. Mann's team found HLA-B35, C4, DR1, and DQ1 antigens in increased frequency among the KS group; HLA-DR3 was observed to be less common in the KS group (8%) compared to controls (27%). The authors concluded that HIV-disease outcome may be influenced by genes and/or gene products that are involved in regulation of the immune response.

Etiology. Findings reported by Dr. Alvin Friedman-Kien (New York University Medical Center) and colleagues suggested that KS occurring in homosexual men may indeed be caused by a sexually transmitted agent. Dr. Friedman-Kien's team was able to identify 7 HIV-seronegative homosexually active men with biopsy-confirmed KS; in 6 of these, detection of HIV by polymerase chain reaction (PCR), using HIV *gag* DNA sequences as probes, was also negative. The authors reported that the 7 cases had normal absolute CD4 cell counts. If a discrete etiologic agent is involved in KS, it is not clear why KS occurs with much greater frequency in HIV-infected persons. A report appearing since the Conference showed that the risk of Kaposi's is 20,000 times more likely in a person with HIV infection.

Dr. Lisa Jacobson and colleagues (Multicenter AIDS Cohort Study, Bethesda) reported their findings on 8 subjects who had developed KS out of 347 HIV seroconverters in the MACS, a prospective study of homosexual/bisexual men. Compared with matched control subjects (seroconverters who did not develop KS), men with KS had more male sexual partners prior to seroconversion. Furthermore, the subjects who developed KS also had, prior to seroconversion, significantly more anal/oral contacts in which they were the oral participant. This differential in oral (rimming) behavior between KS cases and controls did not persist after seroconversion. Dr. Jacobson's team concluded that their data support the hypothesis that a sexually transmitted cofactor for KS exists and that exposure to the cofactor likely occurs prior to HIV infection.

In their long-term prospective study of a cohort of HIV-infected homosexual males, Dr. Chris Archibald and colleagues (University of British Columbia, St. Paul's Hospital, Vancouver) examined risk behaviors engaged in prior to seroconversion among men who developed KS compared with those who developed opportunistic infections. At the time of enrollment in the study, the men who later developed KS had significantly more sex partners and had experienced more receptive anal sex, fisting, and use of inhaled nitrites than the group who developed opportunistic infections. Furthermore, they found the most powerful predictor for development of KS to be high numbers of sex partners residing in "original HIV epicenters" (San Francisco, Los Angeles, and New York City) during 1978–83. From these findings the authors postulated the existence of a cofactor that is transmitted sexually in the same manner as HIV, but with a much lower degree of efficiency. They added that the widely documented changes in sexual behavior would have had an earlier effect on the spread of the cofactor than on HIV, so that it is likely the cofactor would remain in the original HIV epicenters. They also hypothesized that, since nitrite inhalants are associated with increased participation in risky sexual behaviors, nitrite use enhances transmission of the cofactor. Finally, they believe that the reduction in nitrite use and in high-risk sexual practices can explain the recent widespread decline in the incidence of KS in Canada and the United States.

Dr. Warren Winkelstein (University of California, Berkeley) and colleagues studied two groups of AIDS patients: those who first progressed to KS and those who first progressed to PCP. Looking at the 24 months preceding the AIDS diagnosis, they compared behavioral risk factors, clinical findings, and biological markers of disease progression. Although risk factors for the two groups were found to be similar, the authors found that in the PCP group, patients were much more likely to have experienced constitutional symptoms, lymphoid mass depletion, and thrush 6 months prior to onset of PCP,

whereas in the KS group, patients were more likely to have had hairy leukoplakia. For both groups, CD4 and CD8 cell counts were similar at 24 months prior to diagnosis, but CD4 cell counts declined much more in the PCP group while CD8 cell counts actually increased among the KS group as time to AIDS lessened. Levels of antibody to p24 were consistently higher in KS patients while p24 *antigen* levels were consistently higher in the PCP group. These differences suggest that KS may be independent of HIV-induced immunodeficiency.

Treatment. Several research groups reported their preliminary findings on a variety of treatment strategies, including anti-tumor drugs, alpha interferon, and granulocyte-mononuclear colony stimulating factor (GM-CSF). Many of the strategies included the use of AZT.

Dr. G. Guermonprez and colleagues (Hôpital Pitie-Salpêtriére, Paris) reported that bleomycin as a single chemotherapeutic agent (5 mg/day given 3 days every 2 weeks) was effective in producing a complete or partial clinical response in 50% of the studied patients with five or more lesions. Of 50 patients in the trial, 4 had to be taken off the drug due to adverse effects, which included skin rash, acrosyndrome, fever, and anemia; hematologic toxicity did not prevent concomitant use of AZT.

Dr. Gustavo Ganzales-Canali and coworkers (Hôpital Saint-Antoine, Paris) found a favorable response in 25 of 30 KS patients treated with a combination of alpha interferon and AZT (4 patients discontinued treatment due to persistent myalgia and leukopenia). Dr. Susan Krown and colleagues (Memorial Sloan-Kettering Cancer Center, New York) found alpha interferon, AZT, and GM-CSF to be effective in an initial clinical trial. They concluded that the addition of a low dose of GM-CSF (5 μg/kg daily) prevented neutropenia, which would have been induced by the combination of alpha interferon (5 million units daily) and AZT (200 mg every 4 hours).

LYMPHOMAS

Incidence. At the Conference, several speakers commented on the growing incidence of B cell lymphomas (especially non-Hodgkin's lymphoma) among patients with HIV disease, perhaps attributable to the prolonged survival times afforded by antiviral agents such as AZT. Dr. Sergio Roithamann and coworkers (Laennec Hospital, Paris) reported that, based on the French Registry of HIV-Associated Tumors for the period January 1987–May 1989, 160 lymphomas were recorded. When the incidence of tumors was examined according to HIV risk category, a history of IV drug use was associated with Hodgkin's disease more frequently than with non-Hodgkin's lymphoma. When examined according to clinical status, Hodgkin's disease and Burkitt's lymphoma (a B cell lymphoma of particularly high frequency in Central Africa) tended to occur earlier in the course of HIV infection than non-Hodgkin's lymphoma. Dr. Robert Yarchoan (National Cancer Institute, Bethesda, Maryland) pointed out that B cell lymphomas occur most in patients who have had CD4 cell counts below $50/mm^3$ for more than 1 year.

Treatment. In Dr. Roithamann's study (above), the frequency of complete remissions in Hodgkin's disease was found to be comparable to that observed in the absence of HIV disease, but is very low in HIV-associated non-Hodgkin's lymphoma. Other presenters confirmed that both Hodgkin's disease and non-Hodgkin's lymphoma tend to be high-grade when diagnosed, aggressive in progression, and refractory (resistant) to ordinarily successful chemotherapeutic combinations. Furthermore, several reports documented that attempts to control tumor growth with chemotherapy often result in precipitating significant opportunistic infections—the fungal, protozoal, and viral diseases usually associated with HIV and the bacterial processes common among patients with neutropenia.

Dr. Rafael Rubio (Madrid) and colleagues found that in their study of 27 HIV-infected patients with Hodgkin's disease, 24 were in advanced clinical stages (CDC Stage III/IV) at diagnosis; 14 of 23 patients developed AIDS after their diagnosis. The authors reported that response to therapy was generally poor and median survival was calculated to be 15 months.

Both the formal presentations and informal discussions at the Conference emphasized the absence of durable remissions during and after therapy and the large number of patients with tumor progression after treatment. An experimental treatment trial investigating the efficacy of multiagent chemotherapy with or without granulocyte-mononuclear colony stimulating factor (GM-CSF) is ongoing. Dr. Lawrence Kaplan and associates (San Francisco General Hospital) reported that no difference in p24 antigen levels was detectable in non-Hodgkin's lymphoma patients receiving CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) with or without GM-CSF. Although the authors noted that p24 levels were significantly reduced following administration of chemotherapy, they suggested that this effect was probably associated with the cytotoxic nature of the chemotherapeutic agents and not a direct effect on HIV replication.

At a satellite conference on the neurological and neuropsychological aspects of HIV infection, Dr. Mark Rosenblum and colleagues (Northwestern University School of Medicine, Chicago) reported some rather encouraging findings on primary brain lymphomas in HIV-infected patients. Dr. Rosenblum's team noted clinical improvement and stasis of tumor progression in 22 of 29 patients receiving radiation therapy. (See also Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION.)

OTHER CANCERS

Although cancers other than KS and lymphoma have been noted in HIV-infected individuals, the incidence of such cancers in this population does not appear to be higher than that in the general population. Dr. Charles Rabkin and colleagues (National Cancer Institute, Bethesda, Maryland) reported data from a cancer surveillance program that examined cancer trends among single, never-married men for 9 U.S. regions. Results showed that tumors other than Kaposi's sarcoma, non-Hodgkin's lymphoma, and possibly anal/anorectal carcinoma did not increase in incidence during the early HIV epidemic years (1979–87).

Among women, Dr. Sten Vermund (now at National Institute of Allergy & Infectious Diseases, Bethesda) and colleagues found that symptomatic HIV-positive women who are also infected with human papilloma virus (HPV) are at high risk for cervical cancer. Although asymptomatic HIV-positive women are at a somewhat lower risk, this risk is, nevertheless, significantly higher than that for HIV-negative women. Dr. Wolfgang Friedman (Rudolf Virchow Hospital, Berlin) and coworkers also noted an association between cervical neoplasia and HIV infection among 84 women with a history of IV drug use. Cervical dysplasia/neoplasia was seen in 41% of HIV-infected women, compared to 9% of HIV-negative women. Clinical manifestations attributable to HPV infection were observed twice as often in HIV-positive women as in HIV-negative women.

NUTRITION

A variety of nutritional issues and complications confront persons with HIV infection. Many of the nutritional complications, which increase with advancing HIV disease, continue to be addressed by health care providers, nutritionists, and dieticians, but it is clear that much work remains to be done to expand the range of useful and effective options for nutritional intervention. Complications affecting or influenced by nutritional status in HIV disease (which may either directly or indirectly contribute to decline of the patient's immune function) include, but are not limited to:

- cachexia (wasting syndrome with general lack of nutrition)
- nausea and vomiting
- chronic diarrhea
- nutrient malabsorption
- opportunistic infections of the gastrointestinal tract
- abnormal liver and kidney function
- adverse effects of individual medications or their combined effects
- impaired chewing and swallowing
- impaired or altered ability to smell and taste
- physically or psychologically induced anorexia

As pointed out by several Conference presentations, any of these complications, or combinations of them, can further impair immunological function of HIV-infected individuals as they are capable of exacerbating nutrient deficiencies. For many patients chronic diarrhea is one of the most debilitating aspects of HIV-related disease. If a specific etiologic agent, such as one of the bacteria or parasites that cause opportunistic infections in the gastrointestinal tract can be identified, management may be achievable by antibiotics or antiparasitic drugs. Unfortunately, in most cases, control of chronic diarrhea in the HIV-infected patient remains an elusive therapeutic goal.

The majority of discussions revolved around early nutritional assessment and intervention, when appropriate, such as supplemental nutrients. Barriers to optimal nutritional management of HIV-infected individuals were also discussed by researchers and clinicians. The absence of simple, accurate, reproducible, and cost-effective tests of micronutrient sufficiency is a limiting problem. Most clinicians have access only to gross and insensitive measurements, such as total body weight or skinfold caliper-estimated body fat percentage. Tests for specific vitamin levels are often expensive, and marginal results still require interpretation. Choosing appropriate interventions and evaluating their efficacy is difficult, and therapy for some aspects of HIV-related malnutrition is limited or unsuccessful. Nevertheless, the potential for real benefit from careful nutritional management demands that researchers and clinicians better define reasonable assessment techniques and apply available therapies to improve the quality of life for people with HIV disease.

HIV AND THE GASTROINTESTINAL TRACT

Several basic science research teams have been trying to ascertain whether direct infection of gastrointestinal cells by HIV can mediate some of the gastrointestinal abnormalities seen in HIV-infected patients. *In vitro* experiments showing that human tumor and normal cell lines derived from the gastrointestinal tract are infectible by HIV require confirmation; some scientists argue that such HIV-infected cell lines are actually an *in vitro* artifact.

A singular study provided evidence that HIV affects the digestive system early in the disease process. Satya Dandekar (University of California, Davis) reported his team's findings from absorption studies of 9 HIV-infected persons with diarrhea as well as findings from 9 rhesus macaques infected with simian immunodeficiency virus (SIV). Among the HIV-infected individuals, 5 of 7 patients were found to malabsorb dietary fat; depressed activity of the digestive enzymes sucrase and lactase and structural changes in cells called enterocytes were also noted. HIV RNA was detected (by *in situ* hybridization of intestinal jejunal biopsies) in enterocytes of 1 ARC patient with severe malabsorption and in mononuclear cells of the intestines (specifically in the lamina propia and epithelium) in 2 ARC and 2 AIDS patients. Similar, more pronounced changes were found in the SIV-infected macaques; HIV RNA was detected in 6 of 8 animals. The authors proposed that direct infection of intestinal mucosal cells by HIV is a relatively early event in HIV-infected individuals, and that HIV affects the development, maturation, and function of enterocytes, which results in malabsorption of nutrients. Although these findings are preliminary and need to be confirmed, they hold important implications regarding early nutritional assessment of and therapy for the HIV-infected patient.

CACHEXIA

Several research groups are exploring the causes of HIV-associated wasting syndrome and the effects of HIV on the digestive system. Some groups are investigating whether factors other than malabsorption and decreased intake play a role. Timothy Seaton and colleagues (New York Medical College, Valhalla), for example, reported that cachexia appears to be caused by a complex interplay of malabsorption, poor diet, and altered metabolism. In their study comparing asymptomatic HIV-infected persons and ARC/AIDS patients, protein and calorie intake was found to be sufficient in both groups. Interestingly protein and calorie intake did not correlate with anthropometric measurements (such as body weight), leading the researchers to conclude that malabsorption and altered metabolism were more important contributing factors in the development of cachexia.

Jean-Louis Vilde and colleagues (Hôpital Bichat-Claude Bernard, Paris) provided evidence that altered metabolism contributes to cachexia. In their study, 9 ARC and 20 AIDS malnourished clinically stable patients (without fever or uncontrolled opportunistic infections) were compared with uninfected normal-weight and anorectic controls. Analysis showed resting energy expenditure, relative to lean body mass, to be greater for HIV patients; the authors offered that an increase in resting energy expenditure may be partially responsible for the progressive wasting of HIV-infected patients.

NUTRITIONAL STATUS AND IMMUNOLOGICAL FUNCTION

Some investigators have suggested that deteriorating nutritional status reflects deteriorating immunological function. Dr. Stephen Blatt and colleagues (Wilford Hall USAF Medical Center, Texas) showed that patients with the greatest CD4 deficit generally have the greatest degree of lipid abnormalities. (Lipid abnormalities have previously been associated with non-HIV related cachexia, such as parasitic infections and cancer). Dr. Blatt's team examined serum hypertriglycerides and serum HDL-cholesterol in their study of 482 patients at various stages of HIV infection. Patients with progressive HIV disease (CD4 cell counts below $400/mm^3$), and especially those with counts below $200/mm^3$, had significantly increased levels of serum triglycerides with decreased levels of total and HDL-cholesterol. Dr. Carl Grunfeld and coworkers (UCSF and Columbia University College of Physicians & Surgeons, New York) noted that HIV-infected (non-AIDS) patients and AIDS patients with high serum triglycerides were more likely to have high levels of the cytokine alpha interferon than were HIV-negative persons. More significantly, Dr. Grunfeld's group noted a relationship between alpha interferon levels and triglyceride clearance in these patients. The authors concluded that since alpha interferon has been previously shown to modulate lipid metabolism *in vitro* and *in vivo*, the observed increased serum triglycerides in HIV-infected patients may be directly related to alpha interferon-mediated decreased triglyceride clearance.

Although causality between nutritional status and immunological function is difficult to demonstrate, most nutrition researchers support the premise that improving the patient's nutritional status may ameliorate or slow the rate of decline of immunological function.

ASSESSMENT OF NUTRITIONAL STATUS

To assess the nutritional status of individuals with HIV infection—and evaluate the potential benefits of nutritional intervention—various parameters can be measured. Monitoring variables such as the following may aid correction of specific nutrient deficiencies:

- body weight
- body mass index
- percent desirable body weight
- blood chemistries (serum albumin, for example)
- trace element/vitamin concentration

A 16-month nutritional assessment of two groups of men in early stages of HIV infection (19 asymptomatic or early ARC and 7 advanced disease), conducted by Ann Coulston and colleagues (Stanford University Medical Center, California), found decreases in body weight, percent body weight, and body mass index to be efficient, early indicators of decreased nutritional status. Surprisingly, no changes in laboratory markers of nutritional status (such as total protein or serum albumin) preceded malnutrition or symptoms of advancing disease. A decrease in body weight appeared to be the earliest indicator. The authors offered that nutritional intervention (to add calories, protein, and vitamins and minerals to their diets) at the first sign of weight loss might forestall the malnutrition that commonly accompanies progression to AIDS. Other research teams do not agree and have argued that by the time weight loss is seen in many patients, the opportunity to intervene and restore the patient to optimal nutritional status may be lost.

Although trace element and vitamin deficiencies have been observed in HIV-infected patients, the prevalence and significance of such deficits are not known. John Malcolm and coworkers (Royal Newcastle Hospital, Australia) reported that low serum zinc levels were found in 28 of 29 ARC or AIDS patients. Other abnormalities observed included low levels of B_{12}, and B_1 (thiamine) and both high and low levels of vitamin A. Anna Riera and associates (Hospital de la Santa Creu i Sant Pau, Barcelona) found low levels of vitamin B_{12}, possibly caused by ileal malabsorption, in 10 (16.7%) of 60 HIV-infected patients. M. Baum and colleagues (University of Miami School of Medicine) found a similar incidence of vitamin B_{12} deficiency (approximately 20%) in a group of 100 HIV-infected males with progressive generalized lymphadenopathy (CDC stage III).

NUTRITIONAL INTERVENTION

Susan Johnston and colleagues (Hospital Germans Trias i Pujol, Barcelona) reported that increases in weight, body mass index, upper arm circumference, and triceps skinfold thickness were observed in 12 of 16 AIDS patients who had received a high calorie/protein diet for more than 3 months. In 8 of the 16, increases were noted in total proteins, serum albumin, prealbumin, and transferrin. (A high calorie/protein diet was maintained with either liquid oral supplements and home total enteral nutrition or total enteral support alone.) These findings support the premise that early intervention may be an effective way to maintain or improve nutritional status. The study sought to demonstrate, by using patients who already experienced 5% to 10% weight loss and poor appetite, that early nutritional intervention helps prevent the usual progressive deterioration in nutritional status of AIDS patients. The authors suggested that improving nutritional status may increase cell-mediated immunity and thereby help reduce susceptibility to opportunistic infections. Indeed, decreased values of parameters studied correlated with disease progression, indicating that more aggressive nutritional therapy may be required at certain stages.

Appetite stimulation may also help to improve nutrient intake. In a preliminary report on the effectiveness of megastrol acetate, Fernando Viteri (University of California, Berkeley) and colleagues concluded that this appetite stimulant (administered at 800 mg/day) helped produce gains in weight, adiposity, and lean body mass in 11 ARC patients who had previously experienced a weight loss of at least 5% of total body weight.

Nutritional supplementation with parenteral (intravenous) nutrition may also be an important adjunct in management of patients in which oral supplementation does not appear to help. Paul Cimoch and coworkers (Center for Special Immunology, Ft. Lauderdale, Florida) demonstrated that supplemental parenteral nutrition, used to augment oral feeding, was effective in some cases of protein-calorie malnutrition. Improved functional ability and quality of life (no significant infectious or metabolic complications) was noted in 9 AIDS patients receiving supplemental parental nutrition.

CARE DELIVERY

The complexities of clinically managing a single patient with HIV-related disease are magnified several-fold when seeking to devise strategies to deliver care to whole patient populations. Furthermore, studies assessing specific aspects of HIV care delivery—treatment utilization and financing, for example—have limited generalizability. Inconsistencies in diagnoses, treatment, care practices, and even terminology exist not only among institutions but even within an institution; this is further compounded when attempting to extrapolate from various regions and countries. Lack of standardization in data collection and reporting also contribute to the extreme difficulties of analyzing HIV treatment, care costs, and related outcomes.

Access to care and medications—an issue of considerable import and controversy—has been shown to vary by continent and country and, in countries without national health plans, by an individual's economic situation. Several studies discussed below demonstrate that the absence of—or lack of access to—ambulatory care, community-based support services, and non-hospital long-term care options continues to promote unnecessary hospital utilization.

Nevertheless some trends regarding the organization, financing, and delivery of care are emerging. Models of care delivery now emphasize the need for comprehensive care strategies, whereby a patient's long-term needs are also addressed. Integrated hospital, ambulatory, and home services or long-term care facilities, it is argued, will ensure proper care for HIV-infected patients. The patterns of care and service delivery for people with HIV disease continue to evolve as care providers become more knowledgeable and as policy decisions regarding cost containment are implemented.

HOSPITAL-BASED CARE & ASSOCIATED COSTS

Surveys assessing utilization of hospitals and specific services by patients with HIV disease may prove useful in procuring the resources needed for their care. Several research teams reported recent declines in hospital use by HIV-infected persons (in comparison with the early years of the epidemic). Although HIV-related hospital use increased in the United States, Mary Moien (National Center for Health Statistics, Hyattsville, Maryland) reported that the rate of increase was slower for 1986-88 than for 1984-86, based on data from the National Hospital Discharge Survey. Based on 20,000 HIV-related hospital discharges for 1988-89, Gabriel Bez and associates (Ministry of Health, France) reported an increase in ambulatory services and a decrease in the average length of hospital stay. A decrease was also noted in the average direct medical cost of each hospital stay. This trend in decreased hospital use was noted in some but not all industrialized countries. Two reports from Italy and Spain, for example, showed the number of HIV admissions to continue to increase through 1989. According to several Conference presentations, AIDS patients require considerably more nursing care than patient with other diagnoses. Furthermore, significant differences in the utilization of services needed by AIDS patients are apparent; see Table 7.3.

Average Length of Stay. Mary Moien (cited above) also pointed out that the (U.S.) national average length of hospital stay has been on the decline: from 17.1 days (in 1985) to 14.1 days (in 1988). Better management of HIV disease along with improved surveillance, prophylaxis for infectious diseases, and more effective treatment of opportunistic infections may account for shorter hospital stays. A study based on claims for in-patient hospital care for participants in a Medicaid home-care program (March 1987 through January 1989) revealed that hospital and acute care utilization was lower than that observed in state, regional, and nationwide surveys. Cheryl Merzel (Rutgers University, New Brunswick, New Jersey) and coworkers found the average length of stay (12.7 days) to be shorter than that reported for persons with AIDS in the northeastern United States. While cautious in comparing their data with those from other studies, the authors offered that the relative decreased utilization may reflect the Medicaid (home care) program's effects.

Table 7.3. Comparative Use of Hospital Services

Based on a study of 111 AIDS patients and (matched) general medical patients admitted to a hospital during 1981–89, Philippe Vanhems and colleagues (Hôpital Cantonal Universitaire, Geneva) reported their findings of differences in hospital utilization.

Procedure	AIDS Patients (# procedures/100 admissions)	Control Patients (# procedures/100 admissions)
Brain CAT scans	64	19
Bronchoalveolar lavage	31	7
GI endoscopy	29	5
CSF examination	46	9
Lymphocyte typing	3[illegible]	0
Blood cultures	340	50

Anne Shields and colleagues (Washington State Office on HIV/AIDS, Seattle) reviewed hospital discharge data for HIV-related hospital admissions for 1985–88. They reported a decline in average length of stay (13.2 days in 1985, 11.3 days in 1986, and 9.7 days in 1987 and 1988) for all HIV-related hospitalizations over the 4-year period. Although average charges fell during the study period, an increase was noted from 1987 to 1988. The authors attributed this to an increase in per diem charges—an indicator that reduced admission charges may eventually be offset by continuing increases in per diem hospital rates. The authors concluded that as the average length of stay for HIV admissions stabilizes, further declines in hospital-based costs for HIV care will only be achieved by avoiding hospitalization.

Yamil Kouri (Harvard Institute for International Development, Cambridge, Massachusetts) and coworkers also reported that in Puerto Rico the average length of stay declined 47% from 1987 to 1988: from 21.5 days to 11.3 days. Furthermore, HIV-related in-patient care was also noted to decline dramatically, paralleling a considerable growth in utilization of alternative forms of care—notably, ambulatory and home care.

Few countries other than the United States reported average length of stay data. Figures from other countries that were presented may, therefore, not be representative of the entire country. Indeed, independent studies from Italy showed widely divergent figures. Nicoletta Orchi (Regional Epidemiology Unit, Rome) and coworkers reported, that based on data from 8 Latium Region hospitals through the end of 1989, the average length of stay for a patient with AIDS was 36 days. The authors did note that hospital stays in Rome are significantly longer than those outside the metropolitan area. Giovanna Gavazzeni and associates (Ospedali Riuniti, Bergamo) reported that the average length of stay for AIDS patients has steadily declined since 1985 and for the year 1989 was 14.2 days. Stefano Bertozzi (Massachusetts Institute of Technology, Cambridge) and colleagues found that, in a rural Western Zaire hospital, the average length of stay differed significantly between patients with an HIV-related diagnosis (28 days) and non-HIV-related admissions (17 days) during January-October 1989.

"AIDS Hospitals" in the United States. Richard Selik and colleague (Centers for Disease Control, Atlanta) presented information that supports the argument that most AIDS care has been concentrated in relatively few hospitals. In characterizing hospitals in which more than 100 AIDS cases were initially diagnosed, the authors found that such hospitals represented less than 3% of the 7,102 hospitals in the American Hospital Association registry. Of 117,781 persons with AIDS reported to the CDC as of December 31, 1989, more than half (56%) were diagnosed at such hospitals. Five states (New York, California, New Jersey, Texas, and Florida) accounted for 64% of persons with AIDS and 65% of "AIDS hospitals." When comparing hospitals with respect to ownership, more "AIDS hospitals" were publicly owned (38%) than the hospitals with lower AIDS caseloads (33%).

Based on data from more than 10,000 AIDS hospitalizations (from a sample of short-stay, nonspecialty hospitals during 1987–88), Judy Ball (Agency for Health Care Policy and Research, Rockville, Maryland) and colleagues found a strong causal link between the size of a hospital's AIDS caseload and effectiveness in treating AIDS cases. Statistical analyses revealed that a 1% increase in AIDS patient volume led to a 0.2-2.4% improvement in risk-adjusted mortality. Worse outcomes were found among hospitals with higher levels of minority AIDS discharges and those located in urban areas.

Hospital Discharge Delays. Nancy Hunter-Young (Clinical Partners, Boston) and colleagues reviewed data from 30 hospital admissions for AIDS and HIV infection at a large Boston teaching hospital. The 30 admissions reviewed totaled 970 patient days. Of the 970 patient days of hospital care, slightly more than 164 (17%) were deemed potentially manageable in an out-of-hospital setting. Difficulty coordinating out-of-hospital care accounted for 83% of hospital discharge delays. (Delays in scheduling tests and delays associated with research protocols were two other factors cited in the study, accounting for 14% and 3% of delays, respectively.) The elements complicating coordination of out-of-hospital care included:

- awaiting sub-acute facility placement (22%)
- awaiting psychiatric facility placement (23%)
- awaiting hospice placement (5%)
- difficulty obtaining insurance information (12%)
- resistance to care plan by patient or family (33%)

The average charge per day hospitalized was calculated to be $1,006, and the delayed days totaled $165,487. Mindy Widman and coworkers (University of Medicine & Dentistry of New Jersey School of Osteopathic Medicine, Camden) studied charts of patients discharged from 9 hospitals in New Jersey and Philadelphia between March 1988 and January 1990 with AIDS/ARC diagnoses or AIDS indicator disease diagnoses. They found that discharge delays were attributable to service shortage, discrimination or fear on the part of providers, and patient ineligibility for the service desired. Statistically significant correlations were observed in 79% of cases between discharge delay and patient race, profession of discharge planner (social worker or nurse, for example), and insurance.

In their survey of hospital discharge planners (in hospitals serving the greatest number of AIDS patients in metropolitan Chicago), Reggi Marder and coworkers (University of Illinois, Chicago) reported that patients stayed in hospitals an average of 8 days beyond their acute care needs. Together these findings underscore the need for effective discharge planning and planning for nonhospital long-term care. Difficulties in placing persons with AIDS in care facilities have obvious financial and quality of life costs.

OUTPATIENT SERVICES & ASSOCIATED COSTS

Numerous presentations provided evidence of increased use of out-of-hospital services by HIV-infected patients and persons with AIDS—a finding which utilization and costing studies show translates into significant reductions in medical care costs. One such study was conducted by Richard Liekweg and colleagues (Duke University Medical Center, Durham, North Carolina) in which they noted that total and per diem charges for treating patients with HIV-related complications varies according to the locus of care; see Table 7.4.

Virginia Weslowski and colleagues (National Public Health and Hospital Institute, Washington, D.C.) found that hospitals responding to the U.S. Hospital AIDS Survey reported an increase in the average number of outpatient visits per hospital. A study of service utilization and charges for ARC patients attending outpatient clinics of four public and two nonprofit community hospitals in California, conducted by Dr. James Kahn (University of California, San Francisco) and coworkers, revealed that most medical charges are associated with outpatient care. Based on data for January to December 1987, the average

Table 7.4. Inpatient versus Outpatient Costs

Total and per diem charges for treating patients with HIV-related complications have been shown to vary according to the locus of care. Richard Liekweg and colleagues (Duke University Medical Center, Durham, North Carolina) presented data based on 22 HIV-infected patients with different complications of HIV disease treated as strictly outpatients or as inpatient/outpatients compared with data from HIV-infected patients treated as strictly inpatients (controls).

Diagnosis	Inpatient	Inpatient/ Outpatient	Outpatient
CMV disease	$649 x 14 days = $9,086	$502 x 21 days = $10,542	$203 x 21 days = $4,263
PCP	$1,067 x 24 days = $25,608	$700 x 20 days = $14,000	$261 x 18 days = $4,698
Cryptococcal meningitis	$771 x 24 days = $18,504	$473 x 49 days = $23,177	not apllicable
Wasting syndrome	$678 x 24 days = $16,272	$431 x 37 days = $15,947	$149 x 29 days = $4,321

number of outpatient visits per patient was 0.95 per month, with inpatient admissions reported as uncommon. Average total charges were $375 per month: $202 for outpatient services and $148 for inpatient services. Components of outpatient charges included laboratory (31%), pharmacy (23%), clinic fee (23%), and radiology (16%). Variation in outpatient charges among hospitals was minimal.

A study examining 1987 hospital discharge data from New Jersey hospitals, found that Medicaid patients (that is, patients with partial medical insurance provided by the U.S. government) had the highest average number of hospital admissions, total days of hospitalization, and total hospital costs. Molly Coyle (Johns Hopkins University, Baltimore) and colleagues offered that these findings suggest that federal policy must change to improve access to ambulatory care (currently Medicaid provides greater reimbursement for in-hospital services).

EARLY HIV INTERVENTION

Accumulated clinical experience has demonstrated rather clearly that early intervention (AZT therapy in asymptomatic or mildly symptomatic HIV-infected persons, for example) can mean a slowing of the progression of the disease process. Although attempts are being made to make early intervention accessible and affordable, several studies have concluded that asymptomatic persons and patients with early stage HIV disease are not taking advantage of early intervention strategies. Early HIV intervention includes "miscellaneous" care needs, such as regular physical examinations and laboratory tests as well as prophylactic treatments.

Still in the initial stages are estimations of the ambulatory needs of patients who are not acutely ill. Ellen Rautenberg and colleague (New York City Department of Health) reported that among HIV-positive STD clinic attendees (a largely asymptomatic population), service needs—primarily laboratory tests—and associated resource requirements are high. Of approximately 6,880 HIV-positive persons visiting New York City STD clinics each year, 1,376 (20%) are estimated to have CD4 cell counts below 200/mm^3 and need immediate PCP prophylaxis, with most also needing AZT; 2,064 (30%) have CD4 cell counts of 200–500/mm^3 and therefore should be assessed for early AZT use. Approximately 50% (3,440) have CD4 cell counts above 500/mm^3, but should nevertheless be monitored every 6 months. The authors emphasized that although this population is not acutely ill, they estimated that at least 20% are already severely immunocompromised.

A report by David Stanton (Johns Hopkins University, Baltimore) and coworkers reported their experience with a new comprehensive initial evaluation center for patients with HIV infection. A review of the first 364 patients seen between December 1988 and December 1989 revealed several medical and social service needs, including: primary medical care, housing, drug treatment, mental health services, and assistance obtaining entitlements (financial aid). Demographic analyses showed that an increasing percentage of patients seeking evaluation and services were black (65%) or women (27%).

Limited Access to Care. Many persons with early stage HIV disease, some of whom may not be aware they are seropositive, do not receive adequate medical care, for a variety of reasons. Chief among these, worldwide, is limited access to primary care, which means that early HIV disease is often left untreated. This is especially true for low income and other disenfranchised people who have historically had limited access to preventive medical care. In Africa, for example, serious economic constraints and limited hospital beds and supplies mean that most countries are only able to render patient care to a fraction of the number of AIDS patients. H.M. Ntaba (Ministry of Health, Malawi) told Conference participants that the majority of AIDS patients in the developing world have no access to inpatient care. In the United States, limited access to care in some cities has been said to be a consequence of overburdened doctors; as little as 20% of all doctors may be treating 80% of all AIDS patients.

As pointed out by Dr. Debbie Fleetwood and colleagues (Johns Hopkins University, Baltimore), the first HIV-related medical care is generally provided in a hospital emergency room. This emergency room care is typically a crisis response to the symptoms of an advanced opportunistic infection—long after antiviral therapy or primary prophylaxis for opportunistic infections would have been helpful.

Options for follow-up care after the "crisis" are typically limited. Financial constraints may limit access to the medications needed for the continuation of treatment or initiation of secondary prophylaxis. Other barriers to care include lack of patient familiarity with and distrust of "the system" for care provision and, on the part of the care provider, lack of AIDS-related knowledge and skill. These factors, compounded by the lack of resources for patient education and support, contribute to poor patient compliance and a high probability of continued use of the emergency facilities for future medical care.

AZT and Prophylactic Drug Use. Much data relating to early therapeutic intervention is based on homosexual/bisexual cohort studies, which makes it difficult to tell whether the same trends exist for the rest of the HIV-infected population. Data from the MACS and San Francisco Men's Health Study (prospective U.S. studies of homosexual/bisexual men) reveal that infected subjects are not taking advantage of therapies approved for early stage disease. William Lang (Children's Hospital of San Francisco) and associates, for example, reported that based on the percentage of persons using AZT during 1987–89 and aerosol pentamidine in 1989 (in two cohorts of homosexual/bisexual men), a significant number of eligible men are not taking these prophylactic measures that could reduce HIV morbidity.

One research group led by Dr. Neil Graham (Johns Hopkins University School of Public Health, Baltimore), noting undertreatment with AZT in their study of a pre-AIDS group, speculated that the price tag of AZT may be a primary reason why eligible individuals are forgoing AZT treatment. Other factors cited by Dr. Graham's team and independently by Malcolm Hooker and colleagues (St. Stephen's Clinic, London) included fear of adverse drug effects and social stigma. Quite unexpectedly, neither team has witnessed a major movement toward AZT therapy among well-informed asymptomatic individuals.

Economic Impact of Early Intervention. Peter Arno (Montefiore Medical Center/Einstein College of Medicine, New York) and Jesse Green (New York University Medical Center) reported results of their cost modeling system, which projects the cost of making early intervention widely available in the United States. The projected cost of providing services

and drugs to people with HIV who could benefit from them was $1.85 billion per year ($0.6 billion for monitoring and $1.25 billion for treatment). Pharmaceuticals constituted 79% of the total projected estimate. The authors concluded that policy changes and shifts in resource allocation are needed to promote the integration of prevention education and treatment services; enhance primary care delivery systems, particularly in inner cities; and reduce the prices of drugs needed for antiviral therapy and opportunistic infection prophylaxis. They also pointed out that policies designed to further reduce the price of pharmaceuticals remains an important strategy for assuring access to early intervention. In an analysis of drug budget expenses in a low AIDS incidence area, Lil Miedzinski and coworkers (University of Alberta, Edmonton) noted that the recent (1989) price reduction in AZT offers only a partial alleviation of their provincial health care budget burden.

Several investigators reported that PCP prophylaxis and AZT therapy is cost effective. Dr. Mark Smith and colleagues (Johns Hopkins University, Maryland) reported that based on hospital charges for patients with PCP episodes, aerosol pentamidine—despite its considerable cost—may be expected to produce savings of $97,000 per 100 patients per year. Even using a model with a high PCP breakthrough rate (17%, compared to actual incidence of 9.5%), aerosol pentamidine is estimated to be cost effective.

Using published natural history data and randomized trial results, Kenneth Freedberg (Boston City Hospital) and coworkers compared the cost effectiveness of AZT and PCP prophylaxis (alone or together) with no treatment. (Costs included those associated with medication, monitoring complications, and treatment.) Comparing AZT therapy with no treatment for patients with CD4 cell counts between 200/mm^3 and 500/mm^3, AZT was found to increase life expectancy by 1.94 years at an added cost of $24,700 (the cost-effectiveness ratio was $12,800 per year of life saved). AZT and PCP prophylaxis further increased life expectancy by 0.05 years for an added $2,100 (the cost-effectiveness ratio was $44,300 per year of life saved). Trimethoprim/sulfamethoxazole was found to be more cost effective than aerosol pentamidine. From these results the authors noted that AZT and PCP prophylaxis may be reasonable for (as yet unidentified) selected subgroups of patients and not all patients with CD4 cell counts in the range of 200-500/mm^3.

CHANGING PATTERNS OF CARE

The patterns of care and service delivery for people with HIV disease are changing as care providers become more knowledgeable and as policy decisions regarding cost containment are implemented. Kathryn Coltin and colleagues (Harvard Community Health Plan, Boston) examined the effects of changing care patterns in an effort to identify key factors that influence the annual cost of caring for a patient with AIDS. Medical records and claims data were reviewed for all (225) patients in a health maintenance organization (HMO) who were treated for AIDS during a 5-year period ending September 30, 1989. For each succeeding annual cohort of patients, increases were noted in the following areas: number of patients treated, months of treatment for each patient, treatment contacts, total per patient care costs, and average length of survival. Costs-per-patient-month declined steadily throughout the 5-year period.

- Between 1985 and 1986, there was a marked shift from inpatient care to home care.
- Between 1986 and 1987, a shift from inpatient to outpatient care was noted.
- Between 1987 and 1989, inpatient days decreased even further; outpatient contacts increased, although the proportion of face-to-face visits declined; and mean survival almost tripled.

The authors attributed these changes to multiple factors:

- improved symptom recognition by patients
- early diagnosis and treatment of opportunistic infections by primary care physicians

- emphasis on prophylactic treatment
- delivery of care via telephone
- emphasis on early discharge planning

Finally, the authors pointed out that the traditional fee-for-service model would not have achieved the same cost savings.

Physician-Patient Relationship. The changing nature of the physician-patient relationship is evident in the attitudes of both patients and physicians. Previous studies have shown that physicians' attitudes toward homosexuality and drug use, for example, are important factors affecting their ability to provide appropriate medical care to people with HIV infection (see Chapter 9: WORKPLACE ISSUES).

Several Conference studies addressed aspects of patient preferences and expectations regarding the manner in which they are treated by physicians. These presentations support anecdotal observations that many AIDS patients, especially homosexual/bisexual men, want a high degree of autonomy and a share in decision making relating to treatment and care. Donna Barnes and colleagues (University of California, San Francisco) conducted a series of focus groups for homosexual/bisexual men and IVDUs with HIV disease in Los Angeles and Seattle. Most patients wanted to play an active and empowered role in decisions about their care, as was evident from the three major themes that emerged: patients believed that many people with HIV disease knew as much or more about HIV disease than most physicians; patients with HIV often seek alternative therapies to medical care, the search for better care is felt to be empowering and therapeutic in itself; and patients wanted a caring, personal relationship with their physician.

Similar findings were reported by Bryan Macguire and colleagues (University of California, San Francisco), who conducted a survey of 174 patients with HIV disease attending an ambulatory care clinic. These patients were predominantly homosexual, white, and middle class. Of the patients surveyed, 94% felt that they should participate fully in discussions about their medical treatment; only 27% felt that HIV disease was too complex for them to be involved in such decision making. Most patients wanted as much information as possible from their physician whether it was good news or bad. Although few significant differences in opinion emerged related to the demographic variables, it was noted that patients over 40 years of age had slightly more passive attitudes.

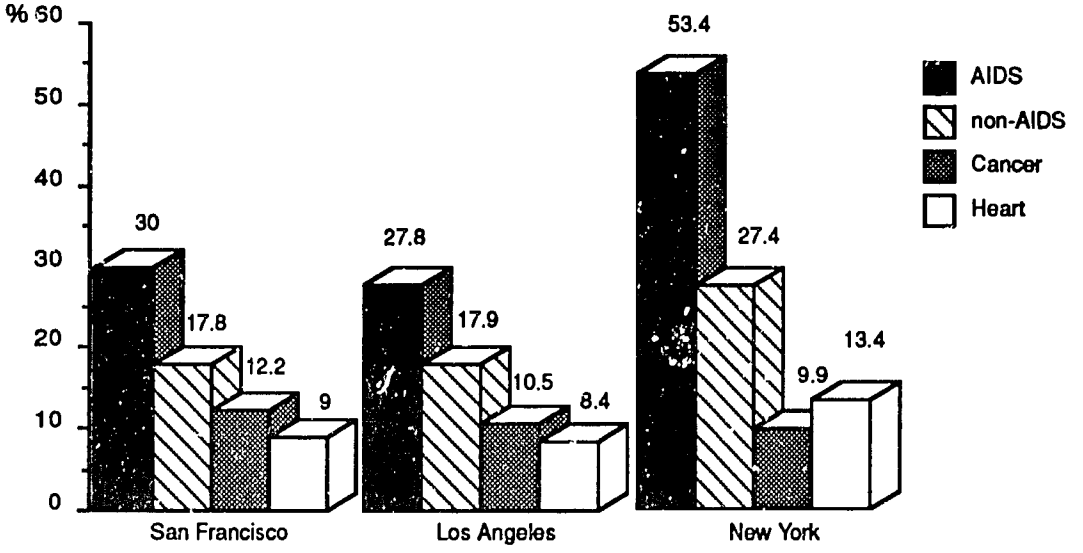
Few studies have sought to investigate the preferences of patients with HIV disease who are poor and/or of a minority group. Many care providers believe that similar preferences exist for people in these groups, but do not get expressed unless trust in the care provider can be established and patient education about the disease process can be provided.

Clinical Research Issues. The clinical trial design has been at issue at the last two International Conferences. Peter Armitage (Oxford University, U.K.) discussed developments in trial methodology that are particularly useful in AIDS research, where progress needs to be made quickly. These developments include:

- designs that enable more than one question to be asked at the same time (so-called factorial designs)
- use of early indications of health changes as surrogate measures, even though survival remains the measure of overriding concern
- flexibility in the options open to patients—such as offering a choice between several randomized compartments
- flexibility in the definition of treatment regimens
- interim analyses during the accumulation of trial data, which may lead to early termination of a trial (However it was pointed out that over-stating differences in early responses may backfire, as such differences may be reversed after longer periods of follow up.)

Figure 7.4. U.S. Public Sector Financing of AIDS Care

Data from three U.S. cities with high AIDS incidence show that more hospitalized AIDS patients are covered by Medicaid than patients with other diagnoses. (Based on a presentation by Dr. Jesse Green, New York University Medical Center.)



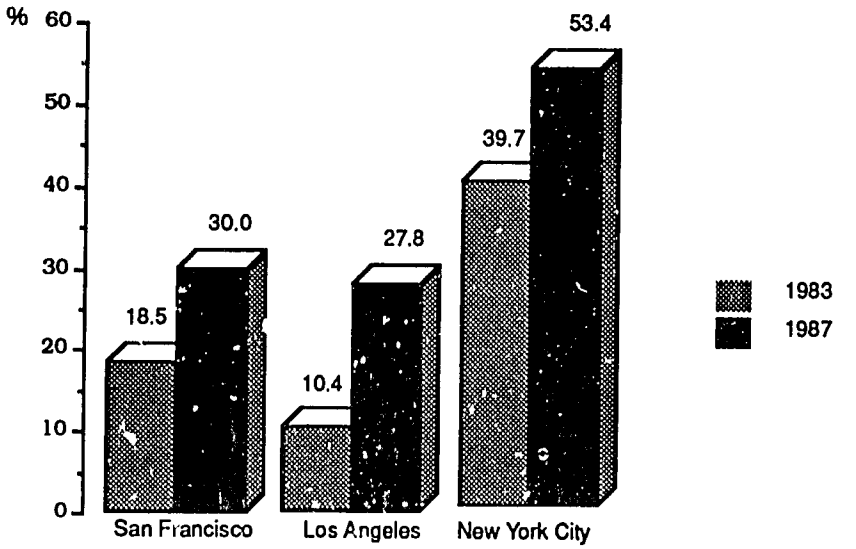
Community-based clinical trials, open (compassionate use) trials, and "parallel track" community-based research were the subject of both formal and informal Conference discussions. ("Parallel track" refers to the U.S. Public Health Service proposal whereby promising HIV-related investigational drugs are to be made more widely available while controlled clinical trials, essential to establishing safety and efficacy, go forward.) The Public Health Service has acknowledged that the proposal was developed with significant input from community advocates, industry representatives, researchers, and interested members of the public. The impetus for parallel track trials arose from the growing number of people with AIDS and HIV infection unable to take standard therapy, or for whom standard therapy is no longer effective, and who cannot participate in ongoing controlled clinical trials. Community-based clinical trials, it is hoped, will complement and not compete with clinical research at academic centers. Many advocates for HIV-infected people also hope the effort will improve access to new therapies for minorities and women—populations that have traditionally had limited access to university-based research programs.

Despite the excitement created by the policy changes, many at the Conference were still skeptical about the effort over the long term. From the discussion sessions, it was evident that many clinicians feel that the bureaucratic nature of the paperwork and the increased appointment time required to see a patient who is participating in a research protocol usurps too much of their time. The lack of information provided to the physician along with the lack of timely feedback on clinical trial results to the physician and the patient were also cited as additional factors creating barriers to involvement with community-based research.

Dr. Elly Katabira (Mulago Hospital, Uganda) reported that, in developing countries, very few clinical trials have been conducted although many HIV-infected people are willing (in fact, desperate) to participate. The foreseeable problems associated with clinical trials in developing countries are significant and need to be carefully addressed. Although availability, access to, and cost of drugs have been discussed as a major limiting obstacle to implementation of clinical trials, other aspects include:

Figure 7.5. U.S. Public Sector Financing of AIDS Over Time

Dr. Jesse Green (New York University Medical Center) also presented data from New York City, Los Angeles, and San Francisco that show a higher number of hospitalized AIDS patients were covered by Medicaid in 1987 compared with 1983 The number of patients covered by private insurance for the same years declined.



- *Study Population.* Subsumed in this category are numerous design problems: The target population may not be recruitable and therefore accessible populations may be the only study option. Constraints such as distance from research centers or inadequate transportation means that selection criteria must be relaxed in order to obtain the necessary number of participants. Inadequate or improper basic medical work-ups may lead to inclusion of unsuitable subjects. Relaxation of selection criteria means that interpretation of data will be very difficult.
- *Personnel.* Fewer doctors are available with research interest and experience and with clinical trial training. Also limited in number are pharmacologists, toxicologists, laboratory technicians with modern technology training, AIDS counsellors, and statisticians.
- *Equipment.* Both the quantity and degree of modernization of facilities and equipment for clinical trials are limiting factors. Insufficient in number are the hospitals, research centers, and clinics; equipment and reagents needed for basic investigations (such as that needed for ELISA); and equipment and materials needed for immunological studies (such as that needed for CD4 cell tests).
- *Financial Support.* Monies from local governments, ministries, institutions, drug companies, donor agencies, and foreign countries need to be raised.
- *Ethical Issues.* Local research guidelines may be ignored; inability to afford experimental drugs by governments or individuals; and political and/or authority interference are all ethical concerns.

COSTING STUDIES

The total cost associated with the treatment of people with HIV disease is rising as the number of people with HIV disease increases. Because health care costs in general are increasing and because various treatments are being initiated at earlier points in the HIV-disease process, the total cost of medical care is also increasing. A survey of the reports attempting to estimate AIDS-associated costs reveal wide variances among patients, facilities, and geographic regions. Costing studies seeking to estimate average daily/annual/lifetime hospital charges, for example, are limited by several factors, including lack of coherent and evaluable

data on seroprevalence, morbidity, survival times, service utilization, and cost per unit of service. Comparisons are further complicated by fluctuating monetary values as records for various years are analyzed. Also, qualifiers are needed for different patient populations—pediatric versus adult patients, for example. For reasons of data availability, the discussion below is limited to reports from the United States. Because there is no national health plan, a significant issue with respect to health care and health care costs in the United States is that of public versus private sector financing.

Sources of Payment. Samuel Matheny and colleagues (Health Resources and Services Administration, Rockville, Maryland) reported preliminary data from an analysis of 29,000 patient records in 21 U.S. cities. Although wide variations existed in principal source of payment for particular services, several trends were noted:

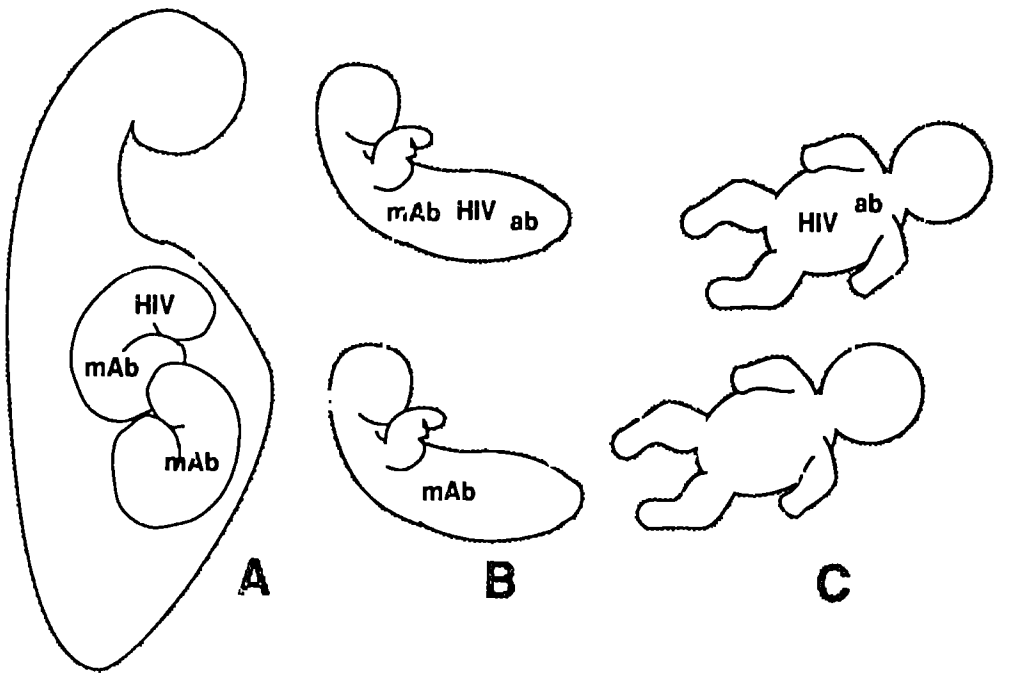
- The federal Medicaid program was the major source of payment for subacute skilled nursing (64% of all patients).
- State funds were the primary source of payment for drug therapy (35.5%) and dental services (41%).
- Private insurance was not a major source of payment for any one service.
- Services with no resources for payment cited (and requiring further investigation) were residential care (58%), home care (15%), and inpatient care (11%).

The public sector's role in providing HIV care and treatment appears to be increasing according to several presentations at the Conference. Jesse Green (New York University Medical Center) and Peter Arno (Montefiore Medical Center/Einstein College of Medicine, New York) reported their data examining payor distribution for hospital-based care comparing HIV-related treatment with that for other diseases. Discharge data from hospitals in New York, San Francisco, and Los Angeles for the 5-year period 1983–87 revealed that hospitalized patients with HIV infection were much more likely to have Medicaid coverage than were patients treated for other types of disease, even when adjustments for demographic differences were made; see Figure 7.4. Over the course of the 5-year period, for all three cities in the study, the proportion of hospitalizations covered by Medicaid increased while hospitalizations covered by private insurance declined; see Figure 7.5. Medicaid's disproportionate share of HIV treatment costs seems to imply that HIV disease is strongly associated with poverty. However, the authors noted that patient demographics alone do not fully account for Medicaid's disproportionate share of treatment costs. Other possible explanations cited were loss of employer-based group health insurance, impoverishment due to longer survival and high medical costs, avoidance of patients with HIV by some insurance companies, and liberalized Medicaid eligibility.

Based on hospital claims for persons with AIDS from all Empire Blue Cross & Blue Shield subscribers (a private U.S. insurance company) during the past 8 years, Robert Padgug and colleague (Empire Blue Cross & Blue Shield, New York) found that due to increased non-institutional utilization, earlier and more intensive treatment, and improved survival, lifetime costs associated with HIV infection are steadily increasing and may approach $150,000 per patient.

Differential Access to Care. Certain sectors of the population are more likely to be eligible for publicly funded care and treatment than others, especially during the early stages of HIV disease. Elmer Smith and colleagues (Health Care Financing Administration, Baltimore) reviewed eligibility requirements for Medicare and Medicaid and compared these programs' requirements with HIV incidence, prevalence, and risk category to assess the likely availability of health care in the early phases of HIV disease. They found that low-income HIV-infected pregnant women, infants, and children have the best chance of being covered by Medicaid at all stages of HIV disease. Low-income male IV drug users and homosexual/bisexual males with advanced ARC or AIDS are likely to be covered by

Figure 7.6. Maternal Transmission of HIV Antibodies



The origin of HIV antibodies in infants born to seropositive mothers cannot be established until approximately 15 months of age. (A) Antibodies to the virus may be passed from an infected mother to her fetus with or without the virus itself. (B) At birth, the neonate may possess only maternal antibodies (mAb) or, if HIV has been transmitted, both neonatal antibodies (ab) and maternal antibodies may be present. (C) By about 15 months of age, the child will have cleared any maternally derived antibodies. Therefore, detection of antibodies prior to 15 months may or may not reflect presence of the virus (true HIV infection).

Medicaid and, possibly, Medicare (if made eligible by significant Social Security paycheck deductions over the years). Low-income IV drug users and homosexual/bisexual males in the *early* stages of HIV disease are typically ineligible for either Medicaid or Medicare. Health care costs for uninsured, low-income, or single people and childless couples in the early stages of HIV disease are also not likely to be funded by the public sector under the current federal programs.

PEDIATRIC HIV INFECTION

The incidence of infection in the pediatric population is steadily rising at an alarming rate—in the United States alone, at least 1,500–2,000 children are estimated to have contracted HIV perinatally in the 1-year period from 1988 to 1989. In Africa, where the incidence of HIV infection in the general population is very high, the pediatric HIV crisis is even more acute.

Pediatric HIV infection may occur via intrauterine transmission, blood transfusion, breastfeeding, needle contamination, or sexual contact. In most parts of the world, including Africa, HIV transmission to children is largely vertical; that is, from an HIV-positive mother. However, in Eastern Europe and Asia, the majority of pediatric AIDS cases are attributable to contaminated blood and blood products. Perinatal transmission and epidemiological data on HIV infection in children are discussed in depth in Chapter 3: EPIDEMIOLOGY.

CLINICAL ASSESSMENT

Pediatric HIV infection poses unique problems in terms of diagnosis, disease progression, and treatment. Children born to HIV-positive mothers cannot be accurately diagnosed (by conventional tests that identify IgG antibodies to HIV) until about 15 months of age. Antibodies to the virus detected prior to that time may be of maternal origin, and therefore may not be reliably indicative of true infection or disease. These maternally derived antibodies are completely cleared from the child's circulation by about age 15 months; thereafter, the presence of anti-HIV antibodies reflects actual HIV infection of the child (see Figure 7.6). Thus the Centers for Disease Control (Atlanta) have established specific criteria for a diagnosis of pediatric AIDS: children less than 15 months old with detectable antibodies to HIV are classified as indeterminate, while older children with detectable antibody levels are classified according to whether they are symptomatic or asymptomatic. Children older than 15 months of age that no longer test positive for HIV antibodies are classified as seroreverters (see Appendix B: CLASSIFICATION OF PEDIATRIC HIV INFECTION).

Because of the diagnostic difficulties wrought by transient maternal antibodies in children less than age 15 months, a diagnosis of HIV infection is made presumptively, on the basis of their clinical symptoms, many of which are apparent by age 6 months. Investigators at the Conference documented numerous HIV-related clinical symptoms that should raise the suspicion of HIV infection in children under age 15 months and suggest follow-up by primary care givers. These include:

- oral candidiasis
- wasting syndrome
- *Pneumocystis carinii* pneumonia (PCP)
- salmonella
- lymphocytic interstitial pneumonia (LIP)
- lymphadenopathy
- anemia
- hepatomegaly (enlarged liver)
- splenomegaly (enlarged spleen)
- neurocognitive defects
- growth retardation
- intestinal abnormalities
- regression through developmental milestones
- chronic or recurrent diarrhea
- herpes zoster
- parotid gland enlargement
- mucus membrane lesions
- persistent fever of unknown origin
- recurring viral or bacterial infections

As can be seen from this list, many of the complications of HIV infection manifested in adults are also observed in infected children. Opportunistic infections in adults are thought to result from reactivation of latent infections normally kept in check by an active immune system. In pediatric patients, the same infectious microorganisms cause disease following a primary infection. Accordingly, countries in which endemic diseases abound, such as Africa, have a much higher incidence of opportunistic infections than elsewhere. While the issue of whether or not immunization regimens should be discontinued in HIV-infected children has raised some debate, it is thought that in countries such as Africa, where traditional childhood illnesses are life-threatening, such vaccination programs should continue. Dr. Francoise Dabis (INSERM, Universite de Bordeaux, France) and colleagues presented data indicating that to date, no adverse reactions following immunization regimens have been noted in HIV- infected children studied in Africa.

CLINICAL MANIFESTATIONS

Pulmonary Involvement. Acute respiratory illnesses, notably *Pneumocystis carinii* pneumonia (PCP) and lymphocytic interstitial pneumonia (LIP), have a higher incidence in pediatric AIDS patients than in their adult counterparts. While LIP is a chronic condition

associated with other clinical symptoms such as lymphadenopathy, hepatosplenomegaly, and recurrent pneumonia, the mortality associated with this disease is fairly low. In contrast, PCP is associated with an extremely high mortality rate.

Early diagnosis of HIV infection is especially important for the success of prophylactic therapies in controlling or even averting some of these complications. At present, children with a CD4 count of less than 500 cells/mm^3 are considered at risk for PCP, and some physicians are beginning to treat these children prophylactically with antimicrobial therapy (although as yet no formal guidelines have been set for prophylactic treatment of PCP in children). However, use of the CD4 count alone as an assessment of risk for PCP is being reevaluated by several researchers. Dr. Andrea Kovacs (Los Angeles County & University of Southern California Medical Center) reported data that suggests that while a CD4 count of less than 500 cells/mm^3 is associated with PCP, the association is stronger in children who were infected via transfusion than via perinatal infection. Among children under age 1 who were perinatally infected and have PCP, 67% had a CD4 cell count above 500/mm^3, and 33% had a CD4 count above 1000/mm^3. Dr. Kovacs concluded by pointing out that CD4 counts alone are not reliable predictors of risk for opportunistic infections such as PCP, and that current recommendations for PCP prophylaxis should be reevaluated for children less than 1 year old. A second report, presented by Margaret Bash and colleagues (Walter Reed Army Medical Center, Washington, D.C.) similarly suggests that pediatric patients, particularly those infected perinatally, may have advanced disease in the absence of abnormal CD4: CD8 ratios (a marker used with some confidence in adult patients). Thus these criteria alone may not identify children at risk for opportunistic infections, particularly PCP.

Other Complications. Although pulmonary disease is associated with a high mortality rate in HIV infected children, it is by no means the only serious complication of HIV disease progression. Malnutrition and acute diarrhea are common and, in many cases, fatal conditions for HIV-infected children in many countries, including those in Africa, according to reports by Dr. Philippe Lepage (Centre Hospitalier de Kigali, Rwanda) and coworkers and Adel Malek (University Teaching Hospital, Lusaka, Zambia). In the latter study, 623 hospitalized children with suspected immunodeficiency were examined and 198 were seropositive. Of the HIV-positive children, 97% had acute gastroenteritis, and 86.8% suffered severe malnutrition. The survival rate among these children was very low: 41% died during the 3-year study; 19% died during their first hospitalization.

Common childhood illnesses are also found with higher frequency in HIV-positive children as compared with their HIV-negative counterparts, according to a study by Dr. Jeff Roberts (Projet San Francisco, Kigali, Rwanda) and coworkers. HIV-positive and HIV-negative children were compared over a 5-month period for the number of outpatient office visits, symptoms, laboratory tests ordered, and medications prescribed. While no significant difference was seen in the number of tests performed or positive results obtained for malaria or stool pathogens, specific medications such as oral rehydration therapy (for diarrhea), vitamins, and Bactrim (a broad spectrum antibiotic) were prescribed significantly more frequently for HIV-positive children.

DISEASE PROGRESSION AND PROGNOSIS

In general, the severity and multiplicity of HIV-associated clinical symptoms is directly related to the level of immunosuppression in HIV-infected children. Pediatric AIDS cases appear to fall into two groups in terms of their disease progression. Infants who show clinical symptoms within the first year of life develop an acute, rapidly progressing form of the disease, and death usually occurs within 7 to 10 months from diagnosis. Children who do not develop clinical symptoms until later in life (after 2 years of age) generally have a chronic and more prolonged disease course that more closely resembles the progression of disease in adults.

A study by Dr. Dominique Costagliola and associates (INSERM, Universite Paris) underscores this difference in disease progression: these authors determined that the mean incubation time in children who developed clinical symptoms as neonates was only 18 months, whereas the remainder of pediatric cases examined developed AIDS in a mean time of 6.4 years. (This was in contrast to a mean time-to-disease of 9.9 years in adult homosexuals studied.) Dr. Kiran Shah and coworkers (Lincoln Hospital, New York Medical College, Valhalla) documented longer survival times in perinatally infected children who have delayed manifestation of disease; in their study, 11 children were seen for initial examination between ages 5 and 11. Interestingly, the clinical presentation in this group was not always abnormal; only 3 of the 11 children had a nonspecific infection, while the others were examined on the basis of presumed risk because of infected siblings or parents. A 9-year-old in this group remains asymptomatic and immunologically normal. These studies suggest that greater numbers of children may be surviving for longer periods of time without overt symptoms of disease. Thus, the authors concluded, children born at risk require long-term follow up with a sensitivity to a clinical patterns differing from those manifested by neonates with HIV disease.

Researchers and epidemiologists are searching for clues to explain these differences in disease course and prognosis. It is unclear whether differences in HIV transmission mode (perinatal, sexual, or via transfusion) have any effect on disease progression in pediatric patients. Results from one study of a small cohort of 25 children in Brazil, presented by Norma P.M. Rubini (University of Rio de Janeiro) and associates, suggest that children who are perinatally infected with HIV have a shorter incubation and survival time than those infected via blood transfusion. As suggested by other researchers, further study may reveal that the immature immune system in a fetus or newborn may be more susceptible to the crippling effects of HIV than that of an older child who encounters the virus through blood transfusion (although these authors did not state whether the two groups were age-matched).

Markers traditionally recognized as being predictive for disease progression in adult populations are being systematically studied in the pediatric population. A study of 281 children and adolescents (younger than age 18) with hemophilia revealed that, as in their adult counterparts, CD4 cell percentages and beta-2-microglobulin levels may be of prognostic value for risk of progression to AIDS. Deoraj Caussy and coworkers (National Cancer Institute Multicenter Study of AIDS in Hemophiliacs, Bethesda, Maryland) presented data showing that AIDS incidence is strongly associated with CD4 cell percentages and (independently) with beta-2-microglobulin.

TREATMENT AND CLINICAL TRIALS

Until recent years, HIV infection was predominantly an adult disease, with relatively few cases of pediatric infection. For this reason, most of what we know about the efficacy and safety of drugs used for the treatment of HIV infection is based on clinical trials performed on adults. With the rapid advance of the disease into the pediatric population, this information is no longer sufficient.

AZT. A number of clinical trials of AZT at modified dosage regimens are currently underway in the treatment of pediatric HIV-infected patients. Thus far, AZT treatment in this population has been associated with clinical improvement of many of the nonspecific symptoms, a reduction in the number of viral and bacterial infections, increased appetite, and a general improvement in the sense of well-being. The results of these clinical trials were reported by several investigators. Dr. Charles Mitchell and associates (University of Miami School of Medicine) noted that the AZT was well tolerated by pediatric patients, with fewer adverse effects than are seen in adult patients. Similarly, a

study reported by Dr. Maria Isabel De Jose (Hospital Infantil la Paz, Madrid) indicates that AZT has a low toxicity and is associated with fewer incidents of opportunistic infections in pediatric patients. All of the children studied showed clinical improvement during the first 6 months of treatment, although it is unclear if the improvements will be long-lasting. Dr. Rodolphe Garraffo and colleagues (Wellcome, Inc., Paris) also reported that AZT therapy is generally well tolerated in children. In their study of 22 children receiving AZT, 6 required interruption of therapy (3 of whom stopped therapy altogether), and 8 were put on a lower dose regimen.

Dr. Edward Connor (University of Medicine & Dentistry of New Jersey & Children's Hospital, Newark) reporting for the Zidovudine Study Group, summarized data from a Phase II study of 88 children in nine U.S. clinical trial centers: AZT treatment was found to be associated with a transient rise in CD4 levels, which decreased then stabilized, by week 36, to levels slightly above those at the initiation of treatment. The greatest increases in CD4 counts were observed in children with the most severe depletion of lymphocytes at the onset of treatment.

Ross McKinney (Duke University Medical Center, Durham, North Carolina) reported studies of other immunological parameters, clinical symptoms, and virological findings in children treated with AZT in this same Phase II trial. Results indicated that children with severe ARC and AIDS responded to AZT treatment with a decrease in nonspecific symptoms such as diarrhea, loss of appetite and lethargy. These changes were accompanied by a shift in the concentrations of serum antibodies towards normal levels, as well as a transient increase in CD4 levels. Viral p24 antigen decreased to undetectable levels in 12 of 30 patients, and levels were markedly reduced in the other children. For ethical reasons, there were no matched control groups that did not receive treatment in these studies—primarily because most investigators believe that the results of AZT clinical trials to date indicate a longer survival time and increased comfort in patients receiving therapy.

Dr. Paola Marchisio (University of Milan) and colleagues reported that CD4 cell counts, and to a lesser extent neopterin levels, may serve as markers of AZT efficacy, based on their study's findings on 6 "responders" and 3 "non-responders." Children who had CD4 cell counts below 100/mm^3 prior to initiation of therapy, and at 3- and 6-month intervals, either progressed to more advanced disease while on therapy or had to discontinue therapy due to adverse effects. Although an early decrease in serum neopterin was noted to reflect AZT efficacy in this population, the correlation was not as significant as that with CD4 cell counts.

ddI. Dideoxyinosine (ddI) is another drug with potent antiviral activity that is currently being evaluated in Phase I and Phase II clinical trials in pediatric patients. A report by Dr. Karina Butler (National Cancer Institute, Bethesda, Maryland) indicated that a 24-week evaluation of symptomatic HIV-infected children treated with ddI found no evidence of neurotoxicity and was associated with

- a decrease in the size of enlarged lymph nodes, spleen, and liver
- an increased CD4 count and CD4: CD8 ratio
- a decrease in the levels of viral p24 antigen

Clinical improvement was observed in children who had not received any previous therapies, as well as in children who had become intolerant of or refractory to AZT. While the number of children studied was small (43 patients aged 8 months to 18 years; median age, 5 years), these results are promising, and offer particular clinical benefit to HIV-infected children who are unable to take AZT.

The effects of ddI on cognitive functioning was also evaluated by Pamela Wolters and coworkers (National Cancer Institute, Bethesda). These researchers measured various parameters of neurological and psychological function in the 43 children evaluated in the previously cited study. Their results indicated that ddI treatment improved the

neurodevelopmental functioning of some children, and at least maintained the cognitive function of over half the patients. (See Chapter 8: NEUROLOGICAL ASPECTS OF HIV INFECTION for a discussion of neuropediatrics.)

Soluble CD4. Phase I trials are beginning on soluble CD4, another potentially therapeutic agent that blocks the attachment of the virus to susceptible cells. Peggy Weintrub (University of California, San Francisco) reported that in a 10-week study of 7 children receiving soluble CD4 therapy, the drug was well tolerated, with no apparent adverse clinical or immunological side effects. Longer Phase II trials are necessary before it will be known whether soluble CD4 provides any clinical benefit.

PREVENTION

While increased education and behavioral changes can influence whether or not adults become infected with HIV, children do not have the opportunity to actively avoid viral infection. Studies are currently being initiated to determine if drug regimens given to HIV seropositive mothers during pregnancy can prevent or reduce the risk of HIV infection in their unborn children.

In a Phase I clinical trial, AZT was given to 10 HIV-positive women during the last trimester of pregnancy (1200 mg/day) to determine the safety and clearance of the drug in the mother and fetus. The results from one of these patients were reported by Paula Schuman (Wayne State University of Medicine, Detroit). AZT freely crosses the placenta, and similar levels of the drug were seen in mother and child after birth. Viral p24 antigens and HIV DNA (as measured by polymerase chain reaction, PCR) were negative in both mother and child immediately following birth, and CD4 levels in the mother were slightly increased. The HIV status of this child and others in the study are not yet known.

In a separate report, William Crombleholme (University of California, San Francisco) and coworkers described their treatment of an HIV-positive mother who elected to continue her AZT therapy throughout her pregnancy. She received 500 mg/day AZT; at birth her child was negative for the virus by PCR analysis and by blood culture. The child was placed in a Phase I AZT trial at 11 days after birth, and became HIV-positive by lymphocyte culture analysis at 53 days postpartum. By age 5 month, the child was symptomatic, with fever, enlarged spleen and liver, and a reduced CD4 count. Thus in this case, at the dosage given, AZT did not prevent vertical transmission.

The viral load in the HIV-infected mother, the dosage of a given therapeutic agent, and the duration of treatment all likely contribute to the potential efficacy of a given drug in preventing perinatal HIV infection or delaying the onset of clinical symptoms. Expanded clinical trials and the availability of newer, more effective antiviral agents will, it is hoped, prevent or control this disease in future unborn children at risk.

CHAPTER 8

Page 231 **BACKGROUND**
232 **NEUROPATHOGENESIS**
232 Cell Tropisms
235 Animal Models
236 **NEUROIMMUNOLOGY**
236 Crossing the Blood-Brain Barrier
237 CNS Immunological Changes
238 Virus Detection in the CSF
239 **NEUROLOGICAL MANIFESTATIONS OF HIV INFECTION**
239 HIV-Related Dementia
241 Autoimmune Neurological Disease
243 Opportunistic Infections of the CNS
246 Progressive Multifocal Leukoencephalopathy
247 Primary Brain Tumors
248 HIV Encephalitis
248 **NEUROLOGICAL DIAGNOSES**
249 Neuro-Imaging Tests
251 Electrophysiological Testing
252 Oculomotor Testing
253 **PSYCHIATRY**
254 Psychopharmacologic Issues
255 Suicide
256 **NEUROPSYCHOLOGY**
256 Manifestations of Neuropsychological Dysfunction
256 Prevalence of Neuropsychological Deficits
258 Neuropsychological Testing & Evaluation
258 Neuropsychological Dysfunction & Disease Stage
259 Progression of Neuropsychological Dysfunction
260 Drug Use and Other Confounding Factors
260 AZT Therapy and Neuropsychological Performance
261 Rehabilitation of the Cognitively Impaired
261 **NEUROPEDIATRICS**
261 Neurological/Neuropsychological Manifestations
262 Prevalence of Pediatric Neuropsychological Dysfunction
263 HIV-Infected Children with Hemophilia
263 Effect of Antiviral Therapy
263 Deficits in Uninfected Children of Seropositive Women

NEUROLOGICAL ASPECTS OF HIV INFECTION

BACKGROUND

Approximately 50% of HIV-infected individuals will exhibit neurological or neuropsychological dysfunction at some time during the course of their disease. Seen at various stages of HIV disease are organic brain disease, such as encephalopathy, and peripheral nervous system disorders, such as motor and sensory neuropathy. Also frequently diagnosed in HIV-infected patients are neurocognitive disorders, including deficits of memory and attention, personality changes and mood disorders. The underlying causes of HIV-related neurological disease are not yet understood, but basic and clinical scientists are exploring a number of possible mechanisms by which HIV infection can cause such profound neurological and neuropsychological dysfunction.

The damage and destruction of the nervous system seen in many HIV-infected persons, and some of the possible mechanisms by which this damage may occur, were the focus of a number of presentations at both the VI International AIDS Conference and at a satellite meeting on Neurological and Neuropsychological Complications of HIV Infection (June 16–19, 1990, Monterey, California). Participants also discussed some of the other neurological aspects of HIV infection, as well as current advances in the diagnosis and treatment of neurological dysfunction. This chapter includes presentations from both conferences.

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NEUROPATHOGENESIS

While the primary, and most adverse pathogenic outcome of HIV infection is profound immunosuppression, the virus also appears to handicap the neurological function of many infected individuals. In the early years of the AIDS epidemic, clinically evident neurological disorders were noted in some patients who were later determined to have had AIDS, and specific neuropathologies were found on postmortem examination of the brain. Since that time, numerous reports have documented the presence of HIV in both the brain and spinal cord in patients with or without clinically evident neurological disease. However, it remains an open question whether HIV *directly* causes neurological disease and whether all the neurological and neuropsychological deficits observed in HIV-infected patients can be attributed solely to HIV. Researchers are currently trying to resolve the following questions:

- Does HIV enter the nervous system in all cases of HIV infection?
- How does HIV gain entry to the nervous system? Are there multiple means of viral entry? Does the mode of entry affect the degree or type of neurological dysfunction?
- Do "neurotropic" HIV strains, which prefer the cells of the nervous system, exist?
- Does HIV *directly* infect and destroy elements of the nervous system, such as nerve cells (neurons) and supportive cells (glia)?
- Does the presence of HIV in the brain activate other pre-existing "dormant" infections, and/or does HIV induce immunological changes in the nervous system that create a permissive environment for opportunistic infections in the brain?
- Does the amount of virus in the central nervous system correlate with the extent of neurological damage observed?

HIV may induce its neuropathological effects directly by destroying cells in the nervous system following viral infection. Destruction of key cells in the nervous system may also result from the release of potentially toxic viral products, such as soluble gp120. Alternatively, indirect mechanisms may contribute to the damaging effects of HIV on the nervous system. Any disturbance in the biochemical environment of the central nervous system (CNS, which includes the brain and spinal cord) could affect the functional state of virtually every resident cell type. HIV-induced immunosuppression, for exqmple, creates a permissive environment for the unbridled growth of opportunistic infectious agents in the CNS. The supporting cells in the brain and spinal cord (glial cells, such as astrocytes, oligodendrocytes and microglia), which are critical to proper functioning of neurons (the cells responsible for transmission of neural impulses), may also be sensitive to changes in the biochemical environment. The presence of HIV in the brain and spinal cord could create subtle microenvironmental changes that, in turn, could affect normal neurotransmitter release or binding, resulting in neurological dysfunction.

By determining which cells of the nervous system are susceptible to HIV infection—and ultimately those which are indeed infected *in vivo*—scientists may be able to uncover the mechanism(s) by which HIV impairs neurological function.

CELL TROPISMS

The identification of cell type(s) infected by HIV in the brain remains a vigorously debated topic. Although *in vitro* experiments suggest that HIV is capable of infecting a variety of cell types residing in the CNS, the *in vivo* relevance of these results is uncertain. Several studies using cell cultures consisting of neurons, astrocytes, and other glial cells demonstrate that such cells can be directly infected by HIV. But, as many researchers caution, it is not clear whether *in vitro* studies using cell lines derived from human nerve cell tumors (neuroblastomas), glial cell tumors (gliomas) or those derived from animal CNS tissue accurately reflect the susceptibility of corresponding cell types in the intact human brain and spinal cord to viral infection.

Figure 8.1. Model of Neuropathogenesis

Hypotheses that seek to explain the observed neurological and neuropsychological dysfunction in HIV-infected persons include both direct and indirect pathogenic mechanisms.

Direct

Direct mechanisms include those in which HIV infects and damages various CNS elements; such *potential targets* include:

- endothelial cells that line brain capillaries (and constitute the blood-brain barrier);
- glial cells (the supportive cells in the CNS)
- microglia (small phagocytic cells that also play a supportive role)
- macrophages

Various CNS cell types may also be directly affected by products produced and released by HIV, such as free gp120 (known to be toxic to neurons *in vitro*)

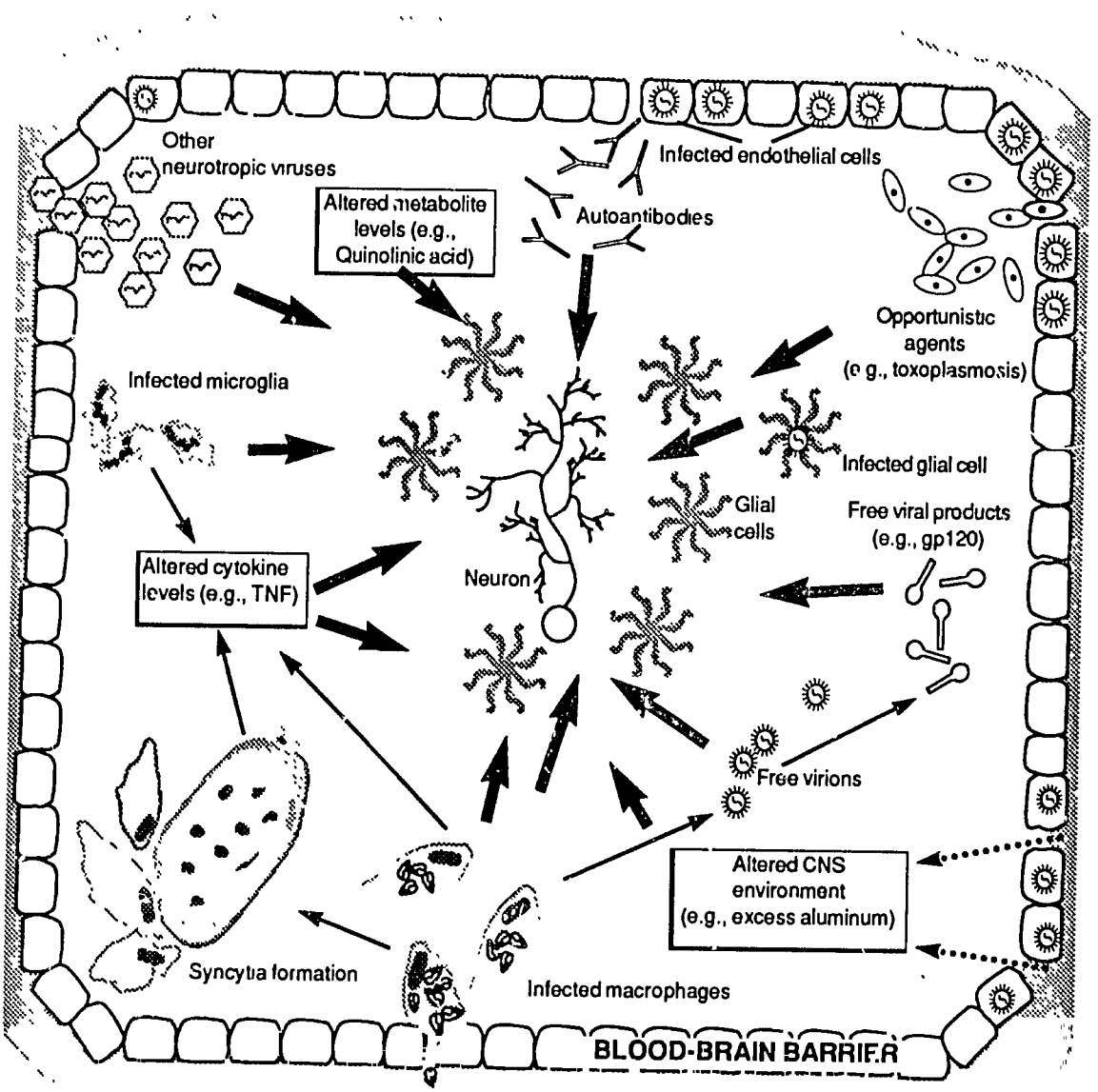
(*Note*: Few, if any, models suggest that neurons can be directly infected by HIV.)

Indirect

Indirect mechanisms include:

- Infection of endothelial cells may compromise the integrity of the blood-brain barrier, possibly altering the delicately balanced microenvironment of the brain. For example, aluminum, normally rigorously excluded from the CNS, may be able to pass through a "leaky" blood-brain barrier in HIV-infected persons. Aluminum, even in trace amounts, is a potent neurotoxin.
- Certain autoantibodies may be neurotropic and may target important CNS proteins for destruction, resulting in profound neuropathogenic effects.
- Altered cytokine and metabolite levels—which may impair the normal functioning of glial and neural cells—may result from altered production by infected microglia, macrophages, or syncytia.

Finally, by either direct or indirect mechanisms, HIV may create a permissive environment in the brain for the establishment of opportunistic infections or other neurotropic viral infections.



Postmortem studies performed on brains from patients who have died of AIDS suggest that HIV infects at least three types of cells in the CNS:

- monocytes/macrophages
- microglial cells
- endothelial cells that line the capillaries of the CNS (more rarely)

Considerable disagreement persists among scientists regarding the potential infection of endothelial cells by HIV; not all scientists are convinced that these cells are actually infected in the patient. Glial cells (astrocytes and oligodendrocytes) continue to be studied for their susceptibility to HIV infection. Interestingly, no studies have demonstrated that CD4 cells (T lymphocytes bearing the CD4 receptor molecule) residing in the CNS are infected by HIV.

Monocytes/Macrophages. A substantial body of evidence now implicates infected macrophages as the primary conduit by which HIV enters the brain and spinal cord. In fact, many researchers want to abandon the term "neurotropic" as a descriptor of certain strains of HIV, and replace it with "macrophage-tropic." While it is presumed that an intact blood-brain barrier does not allow free virus to pass directly from the systemic circulation to the brain or spinal cord, macrophages harboring HIV may be able to traverse this barrier, allowing HIV access to the CNS (see "Blood-Brain Barrier," below).

In situ DNA hybridization studies have demonstrated that macrophages in the CNS of HIV-infected individuals carry specific HIV genes. Researchers would like to know whether such macrophages serve as the only reservoir of HIV within the CNS, or if the virus goes on to infect other cell types in the brain and spinal cord. Are macrophage-tropic strains of HIV in the CNS capable of expanding their cell tropism? If so, does this result in global neurological dysfunction? Or is the observed infection of microglia and endothelial cells relatively inconsequential?

Microglia. Microglia (small, nonneural cells that form some of the supporting structure of the nervous system) are phagocytic cells (literally, particle eating) whose chief function is to remove damaged elements from the CNS. At the Conference, Dr. Henry Dorn and colleagues (National Institutes of Health, Bethesda, Maryland) reported that several laboratory strains of HIV, derived from infected monocyte/macrophage cells, can replicate in cultures of human brain cells. Replication was found to occur primarily in the microglial cell populations. These researchers noted that other HIV strains, derived from infected T lymphocytes, do not replicate in brain cell cultures, suggesting that isolates from macrophages and T cells may be different. Once infected with HIV, the microglia formed giant cell masses with multiple nuclei. These multinucleated giant cells, or syncytia, are frequently observed both *in vivo* and *in vitro* as a pathological consequence of HIV infection; indeed, syncytia formation is considered to be a characteristic cytopathic effect of HIV (see Chapter 2: PATHOGENESIS & IMMUNOLOGY). Dr. Dorn's team found that whereas HIV-infected macrophages persist in culture with few apparent ill effects, virus buds from infected microglial cells, and 90% of the cells die within 3 weeks of infection. The infection and destruction of microglial cells in culture appears to be a CD4-dependent process, since addition of antibodies to the CD4 molecule blocks these events. (The cell surface molecule, CD4, found on a subset of T lymphocytes, macrophages, and other immune system cells, is thought to be the primary, but perhaps not exclusive, receptor by which HIV attaches to and infects a host cell.)

These observations suggest that direct infection of microglial cells by HIV, as well as the cell damage and death associated with such infection, may play an important role in neuropathogenesis. Dr. Dorn and colleagues commented in a post-Conference report (published in August) that indirect mechanisms likely contribute to neuropathogenesis: some of the substances released by infected or dying microglial cells, including tumor necrosis factor and toxic oxygen intermediates known as "free radicals," may result in the nervous tissue pathology observed in brains of HIV-infected individuals with neurological involvement.

ANIMAL MODELS

One approach that may yield an understanding of the pathogenesis of HIV-associated neurological disease is the development of animal models that closely mimic the neurological effects of HIV disease in humans. Simians have been the favorite animal model for these studies because of their evolutionary relationship to humans and the existence of simian immunodeficiency virus (SIV), which is closely related to HIV. In addition to simians, other less closely related species including the cat, rat, rabbit, and mouse have also been studied for their potential as models for examining HIV's effects on nervous function. If HIV can establish infection in the nervous tissue of these species, and if the damaging effects of infection are comparable to those seen in humans, these species may serve as valuable models for studying the ravaging effects of HIV on the human brain.

Simians. Dr. Elaine Jordan and coworkers (National Institutes of Health, Bethesda, Maryland) characterized some of the neurological effects seen in rhesus monkeys infected with SIV so as to evaluate these animals as a potential model for neurologic disease progression in humans. On examining the brains of infected monkeys using magnetic resonance imaging (MRI), approximately 40% of infected animals were found to have tissue loss, or atrophy, in the cerebral cortex (for a description of the MRI technique, see "Neuro-Imaging," below). Other signs of neurologic pathology were also documented in these animals, including brainstem lesions, dilation of the ventricles (fluid-filled spaces in the brain), and loss of myelin (the fatty substance that sheaths and insulates nerve cells, helping to speed the rate at which electrical signals travel down the nerve). These observations are remarkably similar to those seen in HIV-infected humans. Direct examination of tissue samples from SIV-infected monkeys revealed glial cell aggregates, multinucleated giant cells, vacuolar myelopathy (the appearance of vacuoles, or spaces within the spinal cord), peripheral neuritis (inflammation of peripheral nerves), and myositis (inflammation of muscle). These pathological consequences of SIV infection closely parallel those seen in humans infected with HIV.

In SIV-infected animals, virus can be readily isolated from the brain, spinal cord, peripheral nerve and cerebrospinal fluid (CSF, the fluid that bathes the brain and spinal cord). In addition, electron microscopic analysis shows virus budding from infected cells in the CNS. As in HIV-infected humans, the predominant infected cell type in the CNS of SIV-infected monkeys is the monocyte/macrophage.

In an independent study of SIV-infected rhesus monkeys, Dr. Bruno Hurtrel and colleagues (Institut Pasteur, Paris) reported findings similar to those of Dr. Jordan's group. In addition, Dr. Hurtrel's group found that SIV could be readily isolated from the CNS of SIV-infected animals early in infection (a finding paralleled in early-stage HIV infection in humans). Early in the disease process, many of the SIV-infected cells within the CNS are located close to blood vessels, suggesting that a major route for SIV entry into the nervous system may be by means of infected cells (macrophages and/or lymphocytes) traveling from the systemic circulation.

Animal models may be particularly useful in examining the *mechanisms* by which HIV damages the brain. Dr. Melvyn Hayes (National Institute of Mental Health, Bethesda) and coworkers reported large differences in the levels of the naturally occurring neurotoxic agent quinolinic acid in the CSF of SIV-infected versus uninfected macaques. Normal simian CSF levels of quinolinic acid are on the order of 20 nanomoles per liter; in contrast, animals with simian AIDS had CSF quinolinic acid levels of at least 300 nanomoles per liter. These increased levels were noted soon after infection by SIV and remained elevated for the duration of infection. Dr. Elaine Jordan and coworkers, in the study cited above, observed elevated CSF levels of quinolinic acid in SIV-infected animals with neurological lesions and cortical atrophy. Thus, chronically elevated levels of quinolinic acid in the CNS may be linked with, or result in, nervous system damage in infected primates.

Overall, the remarkable similarities observed in the nervous systems of SIV-infected monkeys and HIV-infected humans suggest that SIV and simian AIDS may be an extremely valuable model of HIV-related neurological disease. However, it is important to keep in mind that SIV infection in monkeys and HIV infection in humans, although quite similar, are not identical. One study emphasizing this point was presented by Dr. Leroy Sharer and collaborators (New Jersey Medical School, Newark) who found that in more than 200 SIV-infected macaques, only 1 developed vacuolar pathology of the spinal cord. In sharp contrast, vacuolar changes in the spinal cord are a common consequence of HIV infection in humans, particularly in later disease stages. Furthermore, the vacuolar changes described in the affected macaque were quite distinct from the myelopathy of the lateral and dorsal columns typically seen in HIV-infected individuals.

Rabbits. Dr. Rita Anand and colleagues (Food and Drug Administration, Bethesda, Maryland) reported on their attempts to develop an *in vivo* rabbit model for studying HIV neuropathogenesis. They inoculated rabbits with a strain of HIV isolated from a human patient diagnosed with AIDS dementia. The rabbits developed nervous tremor 30 days after inoculation and by 90 days were extremely agitated. Analysis using the polymerase chain reaction (PCR, a sensitive gene amplification technique by which tiny amounts of HIV DNA can be detected; see Chapter 4: DIAGNOSTIC TESTS FOR HIV) showed that the virus had clearly established itself in the rabbits' nervous system. Interestingly, HIV isolated from infected individuals who did not evidence neurological difficulties did not establish infection in the rabbit CNS, even after 250 days. Such results suggest that particular strains of HIV may have a predilection for traveling to and establishing residence in the central nervous system, whereas other HIV strains may be less neurotropic.

Rats. Dr. Anand and coworkers also examined the effects of HIV on neural cells in the rat. As with the previous study, virus was isolated from human patients with and without symptoms of neurological disease. Virus was then added to cultures of cells taken from the rat cerebellum. HIV isolates from individuals with neurological disorders had dramatic effects on these cultured cells; the cells became granulated in appearance, their branching processes were disrupted, and cellular networks that had already formed in culture fell apart. PCR findings showed that all cell types were infected, many of which were actively expressing virus. Strains of HIV taken from other patients had less severe effects, again suggesting that different isolates of the virus may differ in their neurotropism. In addition, the data showed that HIV can directly infect and destroy neural cells in the rat; the ability of HIV to infect neural cells in humans, however, remains the subject of considerable debate.

NEUROIMMUNOLOGY

Once it was determined that HIV is capable of entering and damaging the nervous system of infected individuals, attention focused on the resultant effects. Researchers are now beginning to detect some of the immunological changes in the brain and spinal cord that occur as a direct or indirect result of infection by HIV.

CROSSING THE BLOOD-BRAIN BARRIER

For many years, the central nervous system was believed to be an immunologically "privileged" organ system; the brain and spinal cord were not considered to be under the same immune surveillance as other body organs such as the liver, heart, kidneys, and skin. Immune components such as white blood cells and antibodies patrol these peripheral organs by way of the circulatory and lymphatic systems, effectively eliminating most bacterial and viral infections in the process. The brain and spinal cord were considered separate from this conventional immune surveillance; instead, the CNS was believed to be patrolled by its own set of immune cells and soluble factors, distinct from those circulating through

the rest of the body. In effect, two immune systems were thought to exist, each functioning independently of the other. The anatomical structure critical to the integrity of this separation is the blood-brain barrier, a tight seal between the peripheral circulation and the structures of the CNS. The seal is formed by endothelial cells, which make up the walls of the blood capillaries.

Recently, this concept of the CNS as independent from the systemic circulatory system has been challenged; many researchers are questioning the privileged status of the CNS and suggesting that significant cross-communication occurs between the systemic circulation and the fluid bathing the tissues of the brain and spinal cord. This debate has profound implications for persons with HIV infection of the CNS. If cells and other substances can readily pass between the systemic circulation and the CNS, then HIV is likely to gain entry to the brain and spinal cord via this route. Most researchers presenting results at the conferences now seem to favor this theory.

The precise way in which HIV gains access to the CNS remains unknown. HIV-specific antibodies can be detected in the CSF of infected individuals almost immediately after seroconversion (the time at which these antibodies can first be detected in the blood), suggesting that HIV enters the nervous system relatively soon after infection. Free virus circulating in the bloodstream may cross the blood-brain barrier. Alternatively, HIV may be "carried" into the brain and spinal cord covertly via infected cells. The two most likely cellular vectors for shuttling the virus into the CNS are macrophages and T lymphocytes, both of which harbor the virus in the blood of infected individuals.

Dr. Sidney Houff (National Institutes of Health, Bethesda, Maryland) emphasized that most evidence points to infected macrophages as the principal means by which HIV invades the CNS. *In situ* hybridization studies indicate that HIV-infected macrophages are found in the brain, and other studies reveal that certain macrophage-related parameters are elevated in the CSF early in the course of infection. Two of these parameters, neopterin and beta-2-microglobulin (markers of macrophage activation) are known to be elevated in the serum of HIV-infected individuals. Thus, increased levels of neopterin and beta-2-microglobulin in the CSF may be an indication of HIV-infected macrophages in the CNS.

CNS IMMUNOLOGICAL CHANGES

Invasion of the CNS by HIV is typically accompanied by certain immunological changes: the levels of certain antibodies in the CSF are often significantly elevated (specifically, those of the immunoglobulin G, or IgG antibody class), and an increased number of cells in the CSF (pleocytosis) is often observed.

Intrathecal Antibody Titers. Levels of antibodies located in the CNS (termed *intrathecal* antibodies) have been monitored as a means of gauging HIV activity within the brain and spinal cord. Increased intrathecal antibody levels are thought to correlate with productive HIV infection in the CNS; presumably increased levels are the result of increased antibody production by B cells within the CNS in response to antigenic challenge. In presenting a review of the literature, Dr. Houff pointed out that from the time of initial infection to the first sign of neurological manifestation, CSF antibody titers increase dramatically. A difference in titers is observed when examined according to HIV disease stage: Persons with ARC have significantly elevated CSF IgG levels compared with asymptomatic HIV-infected individuals, and antibody levels within the CSF generally remain quite high until very late in the course of infection. Furthermore, not only are intrathecal IgG antibody levels elevated in 89% of HIV-infected persons, but other antibody classes also appear to be influenced by HIV infection. Levels of IgA antibodies in the CSF are above normal in 42% of persons infected with HIV. Similarly, IgM levels are elevated in 37% of the HIV-infected population.

These findings suggest that the nervous system is mounting its own immunological attack against HIV. Dr. Houff reported that antibodies to the major HIV antigens are usually found, with most being directed toward the HIV envelope glycoproteins, gp160 and gp120. Surprisingly, analysis of intrathecal antibodies shows that HIV-specific antibodies constitute very little of the total CNS antibody pool. This means that the humoral response to HIV within the CNS is largely ineffective. The specificity, or antigenic target, of the majority of CSF antibodies remains an open question. Such lack of HIV specificity in these antibodies is in contrast to numerous previous studies indicating that asymptomatic HIV-infected individuals (with early HIV infection) have, in their blood, high titers of HIV-specific antibodies capable of neutralizing HIV *in vitro*. The reason for this fundamental difference between serum antibody specificity and CSF antibody specificity is unknown, but may be related to the as yet undefined differences between "neurotropic" and other strains of HIV.

Thus, while evidence of immunological changes in the nervous system of HIV-infected individuals has been found, this immune response is unique—unlike any occurring in the rest of the body. As reiterated by several Conference speakers, the antibody profiles seen in the CSF do not correspond to what one finds in the serum of the same individual; an HIV-infected patient may have elevated serum IgM and relatively normal serum titers of other antibody classes, and yet have elevated IgG levels and normal IgM levels in the CNS.

Other CSF-Immune Parameters. The CSF of HIV-infected individuals has been studied in an attempt to discern parameters that would be indicative or predictive of neurological disease. Such parameters could be extremely helpful in the diagnosis, prognosis, and therapy of the infected individual. Dr. Diane Griffin (Johns Hopkins University, Baltimore) and colleagues, studying immune function in HIV-infected and uninfected populations, tried to associate markers of immune status with the presence of neurological disease. They found that early in the course of HIV infection (prior to the onset of neurological symptoms), CSF levels of soluble CD8 and gamma-interferon are significantly elevated, suggesting that T lymphocytes in the CNS are activated in the early stages of HIV infection.

Macrophage activation markers (such as neopterin and beta-2-microglobulin, see above) are also elevated very early in the disease course in many individuals. As disease progresses, particularly in individuals suffering from neurological disorders such as sensory neuropathy, myelopathy, and dementia, this pattern changes, and only levels of the metabolite neopterin are markedly elevated. This shift in profile implies that macrophages are the predominant activated cell population in the CNS in the later stages of HIV disease; it also suggests that neopterin may be a reliable marker for the development of neurological disease in HIV-infected persons.

VIRUS DETECTION IN THE CSF

With the availability of PCR, a highly sensitive gene amplification technique capable of detecting minute amounts of HIV proviral DNA sequences, the presence of HIV in the CNS can be directly demonstrated by examining only a tiny volume of CSF. Using PCR analysis of CSF samples taken from HIV-infected individuals, Dr. P. Schmid and colleagues (West Los Angeles Veterans Administration Medical Center) reported preliminary data showing that HIV-seropositive individuals indeed have HIV DNA in their CSF, and that higher viral loads in the CSF are well correlated with the severity of neurological disease. If such is the case, PCR can likely be used in the early detection of HIV-related neurological disease; furthermore, quantitative PCR may prove useful in monitoring the extent and progression of neurological disease.

Similarly, Dr. Sunil Shaunak (Duke University Medical Center, Durham, North Carolina) and colleagues found that 20 of 22 patients with neurological abnormalities had detectable HIV proviral DNA sequences in samples of CSF examined by PCR analysis. Of 9 patients

without neurological abnormalities, 6 were PCR-negative, one was PCR-positive, and 2 gave indeterminate results. The authors also noted that HIV-infected but asymptomatic patients (with neither neurological nor systemic manifestations) were as likely to be PCR-positive (10 of 15) as were those with ARC or AIDS (11 of 16). Whether detection of HIV DNA sequences in the CSF of asymptomatic but HIV-seropositive patients is predictive of ensuing neurological involvement remains to be determined.

Taken together, these studies demonstrate that the detection of HIV DNA in CSF samples correlates well with clinically evident neurological disease. However, PCR technology is not yet able to accurately quantify the viral load in the CSF; thus at the present time, it is not possible to correlate the levels of HIV DNA with the degree of neurological disease.

NEUROLOGICAL MANIFESTATIONS OF HIV INFECTION

HIV-RELATED DEMENTIA

One of the more common, and most debilitating, neurological effects of HIV infection is the dementia frequently found in association with AIDS. Dr. Richard Price (University of Minnesota, Minneapolis), who coined the term "AIDS dementia complex" (ADC), discussed some of the neurological aspects associated with this form of dementia. ADC is generally defined as a clinical syndrome of subcortical dementia (meaning it affects primarily those areas "below" the cerebral cortex), and is characterized by deficits in cognition, behavior, and motor performance. These manifestations can range from mild (impaired concentration, apathy, slowed rapid movements) to severe (global dementia, mutism, paraplegia).

Estimates of the prevalence of HIV-related dementia among AIDS patients vary widely: ranging from 10% to 50% of those diagnosed with AIDS. In HIV-infected patients with no other symptoms, HIV-associated dementia is very rare, but the potential for developing dementia increases rapidly with the onset of other HIV-related symptoms. Exceptions have been noted, and in fact, patients lacking any systemic symptoms have presented with progressive ADC.

Not surprisingly, the natural history of ADC differs dramatically from individual to individual. Some persons show signs of mild cognitive impairment associated with the early stages of ADC, and these signs remain stable for long periods of time. Others exhibit a slowly progressive decline in cognitive, behavioral, and motor performance. Still others show a relatively rapid decline into global dementia. The mechanisms responsible for such wide variations among individuals are not understood.

Pathology. On neuropathological examination, a number of abnormalities are seen in the CNS of individuals with ADC. Gliosis (abnormal proliferation of glial cells) and pallor of the white matter are found in many cases. Other pathologies associated with late-stage complications include syncytia (multinucleated giant cells) and vacuolar myelopathy.

Detection methods that reveal viral DNA or RNA, HIV proteins, or viral particles show the distribution of productive HIV infection in neurologically impaired persons to be nonuniform throughout the brain and spinal cord. Areas deep in the brain such as the globus pallidus and other basal ganglia appear to be preferentially infected. In instances when HIV is localized in higher brain regions such as the cerebral cortex, the white matter is infected to a greater degree than the gray matter. Why HIV shows a predilection to infect certain areas of the brain and not others is unknown. One possibility, put forth by Dr. Price, is that the regional distribution of infection is a function of the mode of viral entry into the CNS. Certain brain regions may be more easily accessible to infected cells crossing the blood-brain barrier, and thus are more susceptible to infection.

Typically, productive HIV infection in the brain is less widespread than would be anticipated on the basis of the severity of clinical disease. Nonetheless, severe and widely disseminated infection is generally seen in the later stages of ADC. With the advent of more sensitive methods such as PCR for the detection of HIV, the brain tissue of persons with HIV-associated dementia has come under close scrutiny. As Dr. Price noted, various reports indicate that HIV can be detected in the brains of all individuals with ADC, and that the extent of the proviral distribution correlates well with the severity of the dementia. The finding of HIV in the CNS of all ADC patients suggests a possible pathogenic relationship between higher viral loads and more severe neurological disease.

Potential Pathogenic Mechanisms. In addition to the possibility of a direct neuropathological effect of HIV on the brains of individuals with ADC, a number of more indirect pathogenic mechanisms may exist.

Corticosteroid Levels. One possibility, suggested by Dr. Elyse Singer and collaborators (West Los Angeles Veterans Administration Medical Center), is that excess corticosteroid levels may have neuropathological consequences in HIV-infected persons. They found that CSF cortisol values are significantly higher in persons diagnosed with ADC when compared with those of non-neurologically impaired HIV-infected individuals. In addition, these levels drop significantly after the initiation of AZT therapy. How such elevated steroid levels result in CNS damage is unclear, but possibilities include:

- damage of cortisol-sensitive cells in the hippocampus (an area of the brain intimately linked with memory)
- enhancement of viral replication (steroids promote viral replication *in vitro*)
- an increase in the susceptibility of the brain to naturally occurring toxic substances such as quinolinic acid

Cytokine Levels. HIV infection in the systemic circulation results in dramatic effects on cytokine levels in the serum and in body organs. HIV may also disrupt the delicate cytokine balance in the brain, which, in turn, could contribute to or exacerbate the HIV-associated neuropathology. Cytokines, whose levels are normally tightly regulated, may profoundly damage the cells of the nervous system if present in too small or too large an amount. Dr. Lynn Pulliam and colleagues (University of California, San Francisco), examined the effects of various soluble factors on aggregates of human brain cells in their attempts to establish a human model of ADC. They found that cultured HIV-infected macrophages produce a soluble factor that causes vacuoles and atrophy within the cellular aggregates. These degenerative changes are reminiscent of those seen in the brains of persons with ADC, suggesting that a soluble factor released by infected macrophages may play a role in the pathogenesis observed in ADC.

Aluminum Deposits. Dr. Daniel Perl and coworkers (Mount Sinai Medical Center, New York) presented data suggesting another intriguing pathogenic mechanism. In their neuropathological study of 39 AIDS cases, 13 (33%) were found to have prominent vascular mineralization (deposition of minerals in blood vessels) of the basal ganglia. Unlike other examples of mineralizations (such as those composed predominantly of calcium, which are often seen in the brains of the elderly), the mineralizations found in these AIDS patients were extraordinarily high in aluminum. Even in small doses, aluminum is a potent neurotoxin. The buildup of this mineral in the brains of HIV-infected persons may result in severe neurological dysfunction. The way in which aluminum enters the brain of HIV-infected persons is unknown; one possibility is that HIV infection of endothelial cells lining the blood vessels of the brain compromises the integrity of the blood-brain barrier, allowing passage of aluminum, normally excluded from the brain, into the CNS.

Quinolinic Acid Levels. High levels of the naturally occurring neurotoxic agent quinolinic acid have been reported in the CSF of HIV-infected individuals, and higher levels have been

found in the brains of cognitively impaired individuals than in unimpaired individuals. New data presented by Dr. Alex Martin (National Institute of Mental Health, Bethesda, Maryland) and colleagues showed that in a group of HIV-infected individuals examined over a 6-month period, increasing levels of CSF quinolinic acid levels were strongly associated with a progressive slowing in tests of reaction time.

AUTOIMMUNE NEUROLOGICAL DISEASE

In a number of individuals, HIV infection results in neurological disease that can be linked to an autoimmune disorder. The immune system, aimed at clearing HIV from the CNS, may also be activated to attack elements of the infected individual's own nervous system. This process seems to be the result of a breakdown in the self/non-self distinction; the ability to distinguish between self and foreign molecules is critical to proper immune functioning (see Chapter 2: PATHOGENESIS & IMMUNOLOGY). Typically, only elements recognized as foreign are attacked and destroyed by a healthy immune system. However, the excessive production of antibodies seen in early stages of HIV disease seems to allow the generation of some antibodies directed against self antigens.

Dr. Grazia Liuzzi (University of Bari, Italy) and coworkers discovered that HIV-infected individuals with neurological disorders commonly had CSF antibodies directed against a normal, ubiquitous (self) protein called myelin basic protein. This protein is an important component of the myelin sheaths surrounding nerve fibers; loss of myelin basic protein is frequently associated with demyelination—a typical finding in many HIV-infected persons.

Aberrations in the cellular immune response mediated by cytotoxic T lymphocytes (CTLs) or through antibody-dependent cellular cytotoxicity (ADCC) may also represent potential autoimmune reactions leading to neuropathogenesis. In a normal immune response, CTLs kill only virally infected cells, or tumor cells bearing foreign markers, and antibodies that "coat" infected cells target them for destruction by natural killer (NK) cells. If "self cells" are targeted instead, or are marked by antibodies to attract NK cells, extensive host tissue damage can result.

Neuropathies. Several neurological disorders observed in HIV-infected persons are likely the result of autoimmune-mediated damage. The most common of these is neuropathy, a general term denoting a disturbance in the function of peripheral nerves. A significant percentage of the HIV-infected population suffer from neuropathy, which is most often manifested as a sensory neuropathy characterized by pain in the soles of the feet, decreased ankle and knee reflexes, and progressive weakness in the legs. As HIV disease progresses, such sensory neuropathies become increasingly common and are seen in the majority of individuals diagnosed with AIDS. One study, presented by Dr. Elyse Singer and coworkers (West Los Angeles Veterans Administration Medical Center) found peripheral sensory neuropathy to be the most common manifestation of HIV-related neurological disease. In this study, neuropathy was seen in about 20% of asymptomatic individuals, in about 80% of individuals with ARC or AIDS, and in all individuals showing any form of neurological dysfunction.

Pathological examination of tissue from HIV-infected persons with neuropathy reveals a significant infiltration of mononuclear cells into the damaged regions. These mononuclear cells may represent an immune component that has lost the ability to discriminate self from non-self. Dr. Sidney Houff (National Institutes of Health, Bethesda, Maryland) reported that many of these infiltrating mononuclear cells display the CD8 molecule, a marker of CTLs, on their surface. If these CD8-bearing cells are in fact CTLs, it is possible that these cells are participating in an autoimmune response.

Macrophages are also believed to play an important role in the genesis of peripheral neuropathies. In a study of AIDS patients with neuropathy, Dr. John Griffin and colleagues (Johns Hopkins University, Baltimore) found an abnormally high number of macrophages

in the areas surrounding the damaged nerves. Furthermore, the number of infiltrating macrophages correlated with the number of fibers undergoing degeneration, suggesting that such cells were actively involved in the tissue destruction. Many of these macrophages were producing interleukin-1 (IL-1), a cytokine made by activated macrophages. Thus, it appears that activated macrophages are prevalent in the damaged peripheral nerves of individuals with neuropathy, and it seems highly probable that these macrophages contribute to the pathogenesis of peripheral nerve disease in HIV-infected persons.

A second neuropathological disorder seen in a number of HIV-infected individuals and likely to be of autoimmune etiology is an inflammatory demyelinating polyneuropathy (IDP), also referred to as Guillain-Barré syndrome. Unlike the sensory neuropathy described above, most cases of IDP occurs in otherwise asymptomatic HIV-infected individuals and are characterized by an ascending muscle weakness that generally begins in the legs and moves upward to the arms and face.

In patients with this form of neuropathy, tissue examination also reveals macrophages attached to the myelin sheath of nerve fibers undergoing the demyelination process characteristic of this disease. In addition to the macrophage component, autoantibodies (those directed toward self antigens) also appear to be involved in IDP. Plasmapheresis, an immunomodulatory technique in which circulating antibodies (including autoantibodies) are filtered from the patient's blood, generally results in dramatic improvement in clinical symptoms.

Vacuolar Myelopathy. Diseases of the spinal cord are fairly common in HIV-infected individuals, particularly in later disease stages. Vacuolar myelopathy, a nonspecific degeneration in the spinal cord (as evidenced by the presence of cavities), is the most prevalent form of spinal cord dysfunction. Current estimates indicate that approximately 30% of all AIDS patients suffer from vacuolar myelopathy.

According to Dr. Yao-Tseng Chen and colleagues (Cornell University Medical College, New York), it is difficult to attribute a direct role to HIV in the pathogenesis of vacuolar myelopathy; in their study, both immunohistochemistry and PCR were used to examine the spinal cords of patients with myelopathy. Only rarely was HIV found in the spinal cord, suggesting that some indirect pathogenic mechanism, perhaps involving an autoimmune component, was responsible for the myelopathy.

Myopathy. Autoimmune disorders associated with HIV infection are likely to affect other tissues as well as cause damage to the nervous system. One disorder commonly found in HIV-infected individuals, particularly in the later stages of disease, is myopathy—a functional disturbance of muscle. Persons with myopathy generally have progressive muscle weakness, myalgia (muscle pain), and distal sensory neuropathy.

As reported by Dr. Marinos Dalakas (National Institutes of Health, Bethesda, Maryland), histopathological examination failed to reveal the presence of virus in the muscle of HIV-infected persons diagnosed with myopathy. However, Dr. Dalakas' team found that HIV could be isolated from macrophages in close proximity to the muscle. In addition, many CD8-bearing cells were found near areas of muscle damage, suggesting that CTLs may also contribute to myopathy.

Numerous studies have reported that many HIV-infected persons treated with the antiviral drug AZT (azidothymidine, or zidovudine) evidence myopathy; when AZT therapy is discontinued, a subset of these individuals show a dramatic improvement in muscle function. These findings suggested that AZT might play a role in the development of myopathy, and that this form of myopathy may be distinct from that induced by HIV. To determine whether AZT-related myopathy could be distinguished from myopathy more directly related to HIV infection, Dr. Dalakas examined AZT and non-AZT-treated infected patients who were matched with respect to other disease characteristics. No clinical differences were noted

Table 8.1. Most Reported Neurological/Neuropsychological Manifestations of HIV Infection

NEUROLOGICAL	NEUROPSYCHOLOGICAL
Ataxia (failure of muscular coordination)	Anxiety
Blurred vision	Attention deficit
Dementia	Concentration deficit
Encephalopathy (functional disturbances of the brain)	Confusion
Facial paralysis	Forgetfulness
Gait abnormalities	Memory deficit
Hearing loss	Personality changes
Hemiparesis	
Myelopathy (functional disturbances of the spinal cord)	
Neuropathy	
Seizures	
Spastic paralysis	
Speech disturbances	
Urinary incontinence	

among the patients in the two groups, and on muscle biopsy, the tissue looked quite similar. However, more detailed examination (by electron microscopy) revealed that myopathic muscle of AZT-treated persons exhibited a large number of ragged, red fibers which were generally not seen in the non-AZT-treated group. This type of fiber is believed to indicate damage to a particular cellular organelle, the mitochondrion. In these AZT-treated individuals, analysis of enzymes specific to mitochondria indicated that the biochemistry of these organelles was altered in the muscle cells. AZT may cause this mitochondrial damage by inhibiting the function of a vital enzyme, mitochondrial DNA polymerase, located within these structures.

Despite these provocative findings, the ability of AZT to induce myopathy remains controversial. Work presented by Dr. David Simpson and collaborators (Mount Sinai Hospital, New York) suggests that the pathology associated with AZT treatment is not as clear-cut as commonly believed. In the group of myopathic patients examined in their study, 15 had never received AZT. Of the 20 patients who had received AZT, the therapy was withdrawn in 10, and 8 showed no improvement of their myopathy. Furthermore, these researchers reported mitochondrial abnormalities in the muscle of both AZT-treated and non-AZT-treated persons with myopathy. Dr. Simpson also presented data suggesting that steroid therapy may be an effective means of countering myopathy in HIV-infected individuals. In a preliminary study, 8 out of 9 patients receiving the steroid prednisone exhibited marked improvement in muscle function.

OPPORTUNISTIC INFECTIONS OF THE CNS

Because of their immunocompromised status, many HIV-infected individuals experience a wide spectrum of diseases caused by infectious agents that are usually innocuous in individuals with normal immune function. A number of these "opportunistic infections" appear to preferentially infect and damage the nervous system. Two of the most commonly seen CNS infections in HIV-infected persons are cryptococcosis and toxoplasmosis.

Cryptococcosis. The most common opportunistic infection seen in CNS-associated disease in HIV-infected persons, the fungal disease cryptococcosis is documented in about 5% of AIDS patients. The fungus responsible for this disease, *Cryptococcus neoformans*, preferentially infects the tissues of the brain and the surrounding meninges (membranes), commonly resulting in lesions and meningitis. Even with early treatment, cryptococcal infec-

tion is life-threatening in many patients. Clinically, patients with cryptococcal infection complain of generalized symptoms such as headache, fever, and stiff neck; radiological findings are generally inconclusive. A definitive diagnosis can be established only on CSF analysis, in which the fungus or fungal antigens can be identified.

Response to therapy has been poor in most HIV-infected persons with cryptococcal infection; the standard primary therapy using amphotericin B, alone or in combination with flucytosine, has met with a 20–50% success rate. Such treatment commonly bears severe toxic effects, damaging the bone marrow and kidneys. Toxicity concerns are particularly relevant in the treatment of cryptococcosis, since long-term maintenance therapy is usually needed to prevent relapse.

Recently, a family of novel antifungal agents, the triazoles, have been introduced in the treatment of cryptococcal infections. Triazoles, including such agents as fluconazole, itraconazole and ketoconazole, appear to be significantly less toxic than other antifungal drugs. Evidence also suggests that triazoles may be more effective in the treatment of cryptococcosis. Data presented by Dr. Howard Aronow and collaborators (Keith Medical Group, Los Angeles) suggest that daily oral doses of ketoconazole may be quite effective as a prophylactic against cryptococcal infection. In their study, 210 HIV-infected men were given oral ketoconazole; a control group of 91 HIV-infected men were not given the agent. None of the ketoconazole-treated group developed cryptococcosis, whereas 11 of the control patients developed the disease. In addition, only 5 of the 210 treated men experienced toxicities that necessitated cessation of the therapy. Other treatment studies confirmed the efficacy and safety of fluconazole. Dr. M. Mullen and colleagues (Cabrini Medical Center, New York City) reported on two different dosing regimens and concluded that fluconazole given intravenously or orally is effective in inducing remission, although it rarely produces a microbiological cure. In their study of 16 patients with acute cryptococcal meningitis, toxicity was reported to be infrequent and mild when it did occur. However, as pointed out in this and other reports, successful therapy may require many weeks—commonly more than 40 weeks.

Toxoplasmosis. The protozoan parasite *Toxoplasma gondii*, the causative agent of toxoplasmosis, is ubiquitous in our environment, and is found in the feces of many domestic cats. Estimates suggest that more than 50% of the U.S. population have antibodies to *T. gondii*, and among individuals living in close proximity to cats, this figure may be greater than 90%.

Toxoplasmosis is often manifested first as an encephalitis associated with fever. Retinitis and psychomotor disturbances are also common complications. In certain instances, disease progression can be extremely rapid. Diagnosis is best obtained through brain biopsy. Toxoplasmosis often results in multiple CNS lesions, which may be detected by radiological methods, such as magnetic resonance imaging (MRI). Approximately 95% of patients with cerebral toxoplasmosis have serum antibodies to toxoplasma.

Because of the debilitating damage and rapid progression associated with toxoplasma infection, physicians urge early diagnosis and therapy. The standard therapy for toxoplasmosis combines the drugs pyrimethamine and sulfadiazine. Work presented by Dr. Bernhard Ruf and coworkers (Free University, Berlin) suggests that this combination is effective both as a primary prophylactic (to prevent initial infection) and as a secondary prophylactic (to prevent relapse). In their study of 47 HIV-infected patients receiving pyrimethamine plus sulfadiazine, only 1 developed toxoplasmosis. In addition, of 35 individuals receiving this combination as a secondary prophylactic, only 3 suffered a relapse. In contrast, 6 of 7 patients who did not receive secondary prophylaxis developed toxoplasmosis.

The severe toxic effects associated with the previously recommended regimen, pyrimethamine/sulfadoxine (Fansidar), present a major problem. According to Dr. Jan de Gans and colleagues (Academic Medical Center, Amsterdam), nearly 40% of patients receiving the combination therapy exhibit rather severe toxic effects, prompting a search for alternative

therapies. Dr. de Gans presented results using an alternative regimen; induction therapy consists of the standard pyrimethamine/sulfadiazine treatment with folinic acid to counter the bone marrow toxicity of the pyrimethamine. In the maintenance phase of therapy the sulfadiazine is removed, and the patient is administered only pyrimethamine and folinic acid. Preliminary results suggest that this combination, given orally in high dosages, is effective in the prevention of toxoplasmosis in AIDS patients.

Sulfonamides such as sulfadiazine are also effective in the treatment of *Pneumocystis carinii* pneumonia (PCP), a respiratory infection seen in many AIDS patients. However, using a sulfonamide as a prophylactic against toxoplasmosis severely limits its efficacy if it is used later to treat PCP. Dr. Ruf and colleagues tried an alternative therapy using pyrimethamine in conjunction with clindamycin to treat toxoplasmosis; their preliminary results were promising; 19 of 22 patients with toxoplasmosis showed clinical improvement after the institution of therapy.

Sulfa sensitivity in some patients may preclude administering sulfadiazine, but as reported by Melinda Tenant-Flowers and colleagues (St. Vincent's Hospital, Sydney, Australia), progressively graduated doses of sulfadiazine over several days may induce tolerance, allowing treatment with as much as 2 to 4 grams per day. Co-trimoxazole, which is available in many countries where sulfadiazine-pyrimethamine is not yet sold, may also have an important role in treating selected patients with CNS toxoplasmosis. An interesting study presented by Peter Nicholas and coworkers (Elmhurst Hospital, New York) showed that CNS toxoplasmosis was more frequent among patients receiving aerosol pentamidine as prophylaxis for PCP than among those who took co-trimoxazole. Andrea Canessa (University of Genoa) and associates found co-trimoxazole to produce both clinical and radiologic improvement in 18 of 25 AIDS patients with cerebral toxoplasmosis; responders had a mean survival of about 8 months, almost four times that of nonresponders.

Neurosyphilis. Syphilis is becoming an increasingly significant problem in the United States. In 1943, the U.S. incidence of syphilis was 76 cases per 100,000 population; by 1956, the number had been reduced to 4 per 100,000. However, by the 1980s, the frequency of syphilitic infections was again on the rise, particularly in urban areas. In 1986 the national incidence rate was 10.9 cases per 100,000, and by 1987 this value climbed to 14.7. In high-risk populations the prevalence of syphilis can be alarmingly high: according to Dr. Joseph Berger and colleagues (University of Miami, Florida), the incidence of syphilis in 1985 in males age 20–24 in Broward County, Florida was 4[illegible]6 per 100,000, and in blacks in the same area the rate was 730 per 100,000.

In both secondary and tertiary syphilis, CNS involvement is not uncommon. Many HIV-infected persons suffer from a concomitant syphilitic infection, and in the population studied by Dr. Berger and colleagues, approximately 1.5% of all AIDS patients have concomitant neurosyphilis. Epidemiological evidence suggests that the clinical profile of neurosyphilis differs among HIV-infected and HIV-seronegative individuals. Dr. Berger reported that patients with both AIDS and neurosyphilis are more likely to experience rash and ophthalmological problems than those with neurosyphilis alone. Cranial nerve abnormalities are more common in the AIDS group, as is an elevated CSF white blood cell count and decreased CSF glucose. Unlike the neurosyphilitic profile in non-AIDS patients, neurosyphilis in AIDS patients is typically syphilitic meningitis or meningovascular syphilis.

The epidemiological linkage between HIV infection and syphilis has led some researchers to posit that HIV infection can act as a risk factor for infection by *Treponema pallidum*, the causative agent of syphilis, or vice versa. However, no firm data has been presented to support this hypothesis. A second theory postulates an interaction between HIV infection and syphilis, such that one infection bolsters the effect of the other (described as acting as cofactors). This type of interaction would be expected to be especially acute in the nervous system of dually infected individuals, toward which both HIV and *T. pallidum* gravitate.

Dr. Marcia Updike and coworkers (Johns Hopkins Hospital, Baltimore) examined whether individuals co-infected with syphilis and HIV exhibit more neurological and/or neuropsychological abnormalities than individuals infected with HIV alone. Their results failed to find any relationship between co-infection and a greater frequency of neurological or neuropsychological dysfunction.

CMV Encephalitis. Co-infection with cytomegalovirus (CMV), a member of the herpesvirus family, is seen in 85-95% of all AIDS patients, according to Dr. Sabine Habicht and coworkers (Free University, Berlin). Active CMV infection, indicated by the presence of CMV lesions, is seen in nearly half of all AIDS patients on autopsy.

CMV enters the body and disseminates to a number of organs, including the central nervous system, where the virus causes substantial damage. In CMV/HIV co-infected individuals, polyradiculomyelitis (a disease of the nerve roots and spinal canal), microglial nodules, syncytia formation, demyelination, and lesions are commonly observed; it is not clear whether these manifestations are due to the effects of CMV infection, or HIV infection, or both.

CMV-induced encephalitis has been described in many HIV-infected individuals, although the exact incidence is under debate. Dr. Habicht reported that CMV encephalitis occurs in 5-33% of AIDS patients; in her view a rather rare finding. However, a number of Conference participants strongly questioned this statement, and argued that in their experience, CMV encephalitis is a much more common occurrence in AIDS. Dr. Habicht insisted that the clinical symptoms seen in this disease are far too nonspecific to establish a definitive diagnosis. Her conservative estimate may be related to the criteria she uses for a positive diagnosis, which requires documenting evidence of the virus itself in the CNS, or culturing CMV from the patient's CSF.

The efficacy of various therapeutic approaches in the treatment of CMV infection in HIV-infected persons has stirred significant controversy. Ganciclovir, an antiviral agent used in the treatment of systemic CMV infection, has been reported by some groups to have no beneficial effects, whereas other researchers report considerable success using this drug. Dr. Tarvez Tucker and colleagues (University Hospitals of Cleveland, Ohio) and Dr. Bruce Cohen and colleagues (Northwestern University Medical School, Chicago) presented data demonstrating considerable success using ganciclovir in the treatment of a CMV polyradiculopathy. In one case, the patient later developed retinitis, an inflammation of the retina, which also responded to ganciclovir. Foscarnet, a second antiviral agent used in the treatment of CMV, has also met with mixed results. Dr. Habicht cautioned that although these drugs are effective in certain instances, the decision to use a specific anti-CMV therapy should be made carefully, since these agents can be extraordinarily toxic.

PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

Progressive multifocal leukoencephalopathy (PML) is a rare demyelinating disease of the CNS, usually seen only in immunocompromised persons. The frequency of PML in AIDS patients ranges between 2% and 5%. Although radiological findings may suggest the development of PML, a definitive diagnosis requires brain biopsy. The prognosis for individuals with PML is generally quite poor; survival times average 6 months from diagnosis. To date, no effective treatment exists. The antiviral agent ribavirin, which was initially touted as potentially useful in the treatment of PML, has proven uniformly unsuccessful.

The cause of PML is still debated by some, but many researchers believe that the disease results from infection of CNS cells by a papovavirus (JC virus). This virus has been detected in the brain and bone marrow of many patients with PML. Dr. Thomas Weber and colleagues (University of Göttingen, FRG) used PCR analysis of cerebrospinal fluid to try to identify JC virus DNA sequences in HIV-infected individuals; 3 of 11 individuals tested

were found to have DNA sequences corresponding to specific JC virus sequences while experiencing symptoms suggestive of PML. Although these results are promising, more evidence is needed to establish the value of PCR for detecting JC virus in cerebrospinal fluid.

PRIMARY BRAIN TUMORS

The incidence of primary brain tumors increased precipitously in the 1980s. This increase is thought to be due largely to the HIV epidemic, since some HIV-infected individuals develop primary brain tumors during the course of their disease. In HIV-infected persons, most of these malignancies are lymphomas—cancers of lymphocytes (white blood cells) that reside in the brain. Dr. Mark Rosenblum and colleagues (Northwestern University Medical School, Chicago) pointed out that in the United States the incidence of primary brain lymphoma in AIDS patients has been estimated at between 2% and 5%, and projections suggest that primary CNS lymphoma will be the most common form of brain tumor by 1992. (Some researchers attribute the increases to a treatment effect: therapy may lengthen survival time and therefore enhance the likelihood of developing brain lymphomas.) In addition, secondary (metastatic) lymphomas of the CNS are not uncommon in the HIV-infected population. In a study of 276 AIDS patients conducted by Dr. Dirk Schurmann and colleagues (Rudolf Virchow University Hospital, Berlin), 23 (8%) were found to have primary or secondary lymphoma of the brain.

The vast majority of lymphomas in HIV-infected persons are classified as non-Hodgkins lymphomas; highly malignant tumors composed of B lymphocytes, the antibody-producing cells of the immune system. The cause underlying these B-cell lymphomas is unknown, but evidence suggests that secondary infection by a so-far unidentified virus other than HIV may trigger the development of these tumors. (Epstein-Barr virus has been implicated by several investigators.) The prognosis for individuals with CNS lymphoma is typically poor, since such malignancies tend to metastasize rapidly. As highlighted by Dr. Schurmann, the alarming rate at which these lymphomas spread mandates the early prophylactic use of chemotherapeutic agents. Early diagnosis of lymphoma is extremely important; yet the diagnosis of CNS lymphoma is difficult. The results of radiological imaging tests, such as magnetic resonance imaging (MRI) or computerized tomography (CT), are suggestive but by no means conclusive. Definitive diagnosis entails biopsy of brain tissue from individuals suspected of having lymphoma.

Recently, radiological methods have been employed to increase the efficiency and frequency of success of brain biopsy. Such techniques make use of the CT or MRI image to direct the biopsy probe to the affected region of the brain. Several studies reviewed at the conferences demonstrated the effectiveness of image-guided stereotactic biopsy as a diagnostic tool, not only for the detection of lymphoma, but for the detection of other CNS disorders. Dr. Robert Levy and collaborators (Northwestern University Medical School, Chicago) presented their results from a study of stereotactic biopsy in 30 AIDS patients. In 93% (28) of these patients, biopsy allowed an accurate diagnosis, which included lymphoma (in 9 individuals), PML (in 7), toxoplasmosis (in 7), and HIV encephalopathy (in 3). Similarly, Dr. Jacqueline Mikol (Hôpital Lariboisiere, Paris) and colleagues found image-guided biopsy allowed a definitive diagnosis in 25 of 30 individuals, and a highly probable diagnosis in 3 of the remaining 5 patients. Once again, diagnoses included lymphoma, PML, and toxoplasmosis. These results offer encouragement that stereotactic biopsy may allow earlier diagnosis and intervention in the treatment of CNS lymphomas as well as other CNS diseases.

Primary CNS lymphomas appear to respond to radiation therapy. Dr. Mark Rosenblum reported that his team found that 22 of 29 patients diagnosed with primary CNS lymphomas exhibited marked improvement following radiation treatment. In addition, among 26 patients who had primary CNS lymphoma but had not received radiation, autopsy revealed that 20 (77%) died of tumor progression. In contrast, no patients being treated with radiation died of tumor progression (most died of opportunistic infections).

HIV ENCEPHALITIS

A nonspecific encephalitis (inflammation of the brain) is seen in a number of HIV-infected individuals. This disease, now known as HIV encephalitis, was historically one of the first disorders noted in HIV disease. According to Dr. Juan Artigas and coworkers (Auguste-Viktoria Hospital, Berlin), HIV encephalitis is seen on autopsy in 36% of all HIV-infected individuals. This encephalitis produces chronic progressive damage to the brain, particularly to subcortical structures. Some of the pathological changes associated with HIV encephalitis include white matter lesions (including demyelination), glial cell loss, astrogliosis, and microcalcification.

The mechanisms of this damage are unknown, but both direct infection and indirect processes are thought likely. As highlighted by Dr. Artigas, infiltrating cells such as macrophages likely cause a significant amount of the damage. These cells may destroy integral components of the nervous system through the release of various cytokines. Disruption of the blood-brain barrier, possibly mediated by infection of endothelial cells lining the blood vessels, may also perturb the microenvironment of the CNS and result in significant damage (see "Blood-Brain Barrier," above).

The virus itself may directly inflict some of the pathological consequences of HIV encephalitis. Dr. Clayton Wiley (University of California School of Medicine, San Diego) discussed several of these possible effects: HIV proteins, such as the *env* or *gag* proteins (soluble gp120 or p24), may be directly toxic to CNS cells. The replication of HIV within the CNS may also result in significant damage. "Silent infection" of CNS cells such as neurons and glia (although so far neurons have not been unequivocally shown to be susceptible to HIV infection), may also account for some of HIV's neuropathogenic effects.

Lesions of the brain in HIV encephalitis have generally been associated with the subcortical white matter. However, a study reported by Dr. F. Scaravilli (The National Hospital, London) suggests that lesions in the cortical gray matter are more common than previously believed and may account for some of the neurological dysfunction seen in HIV infection. Other cortical damage is also frequently evident; according to Dr. Wiley, cortical atrophy is seen in approximately 75% of all individuals with HIV encephalopathy. Both expansion of the ventricles and widening of the sulci (the furrows in the cerebral cortex) contribute to this atrophy. A comparison between the brains of HIV encephalitic patients and asymptomatic HIV-infected individuals shows a marked decrease in hippocampal size in persons with encephalopathy. Areas of both the frontal and parietal cortex are of diminished thickness in persons with HIV encephalitis. Although the precise nature of this thinning is unknown, a selective loss of large neuronal cells is evident.

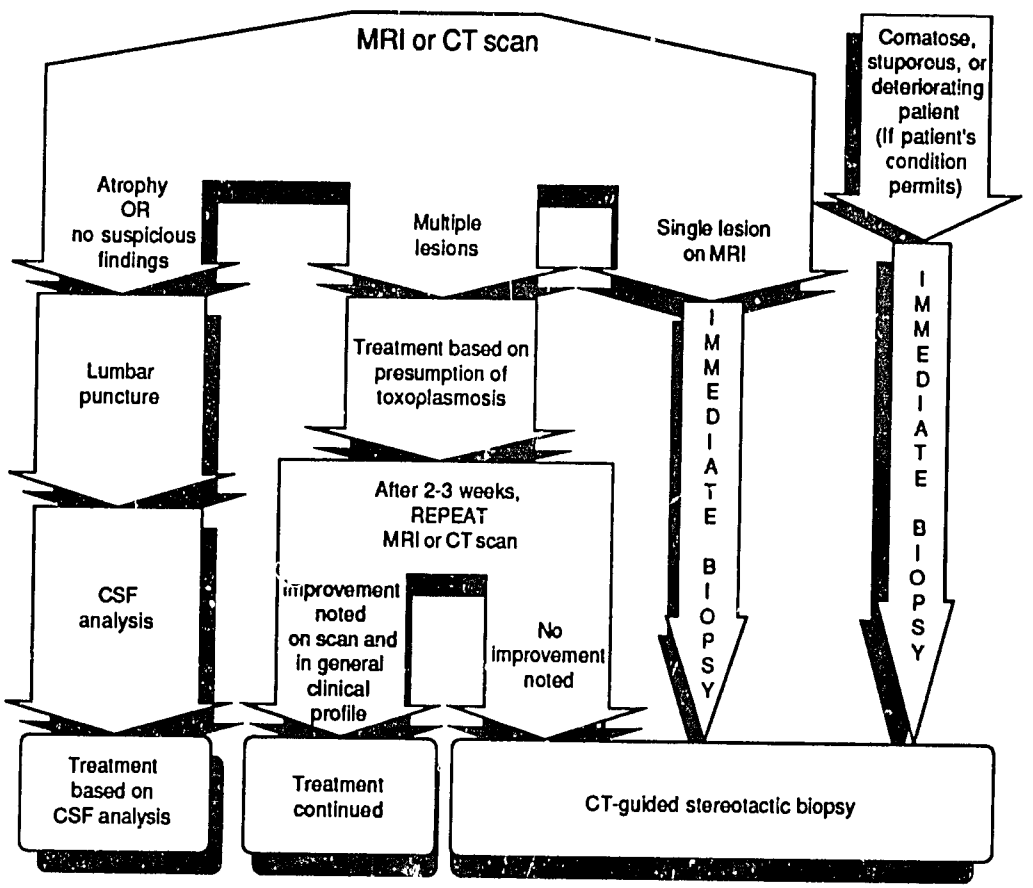
Finally, a new technique has allowed the number of synapses in particular areas of the brain to be counted (a synapse represents the connection between two nerve cells). HIV-positive individuals examined by this technique appear to have fewer synapses than their seronegative controls, and persons with HIV encephalitis have fewer frontal cortex synapses than those counted in asymptomatic individuals.

NEUROLOGICAL DIAGNOSES

Techniques used to diagnose neurological disease in the HIV-infected patient include CSF examinations, imaging (neuroradiological testing), electrophysiological testing, and oculomotor performance testing. CSF examinations are generally only diagnostic for certain opportunistic infections, such as cryptococcosis or neurosyphilis (see above). For patients with clinically evident neurological illness, diagnostic algorithms (see Figure 8.2) are generally followed.

Figure 8.2. Diagnostic Algorithm for Neurological Illness in HIV-Infected Patients

This algorithm for diagnosis HIV-related neurological disease, developed by Dr. Robert Levy and colleagues (Northwestern University Medical School, Chicago), was based on a study of more than 200 HIV-infected patients. Patients were initially given a general medical examination; if they were found to show symptoms of neurological illness, either CT or MRI imaging tests were performed. If radiological findings were relatively normal, lumbar punctures were done and the patients were treated on the basis of cerebrospinal fluid analysis. If one or more lesions were seen radiologically, and the patients were neurologically stable, they were treated for toxoplasmosis. After 2 to 3 weeks, a second imaging test was performed. If the findings from MRI or CT and the general clinical profile showed improvement, therapy was continued. If improvement was not noted, the patients underwent a CT-guided stereotactic biopsy. If the initial MRI showed a single lesion, or the patient was comatose, stuporous, or deteriorating, biopsy was done immediately.

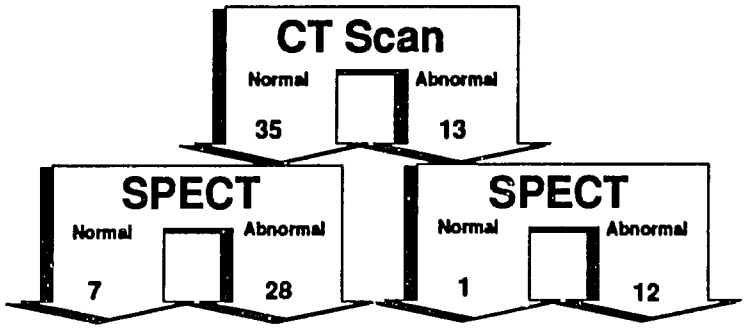


NEURO-IMAGING TESTS

Recently, a number of imaging techniques have been further improved to provide better profiles of the brain in cases of neurological dysfunction. Imaging techniques are noninvasive and facilitate the localization of specific regions of brain damage. Often these methods can also serve as diagnostic tools, outlining the areas of injury in the brain and differentiating the particular disease process at work. Since HIV-infected persons can suffer from a number of CNS disorders, neuro-imaging has been especially useful in narrowing the diagnostic possibilities. In some situations, neuro-imaging can also be used to assess the efficacy of therapeutic intervention.

Computed Tomography (CT). Perhaps the most widely used diagnostic tool for detection of lesions within the CNS of living patients is CT scanning. Briefly, CT scans provide com-

Figure 8.3. Single Photon Emission Computed Tomography (SPECT)



Preliminary findings reveal that SPECT, an imaging technique that measures cerebral blood flow, may signal impending neurological dysfunction. CT scanning and SPECT were performed on the same group of patients and the results were compared. SPECT identified 28 CT-normal HIV-infected individuals as having focal blood flow deficits. Follow-up studies will determine if such deficits are indeed reflective of impending clinical neurological dysfunction. (Based on a presentation by Dr. Karl Einhaüpl, University of Munich.)

puter-generated images of the brain, based on measuring the degree of x-ray absorption in different brain regions. To enhance the contrast between tissues, and to more effectively visualize blood vessels, tumors, and abscesses, radio-opaque compounds such as iodine are injected intravenously. The resolution of the CT scan is limited; according to neuroradiologists, detection of diffuse lesions, such as those seen in CMV encephalitis and PML, is inadequate by CT imaging alone.

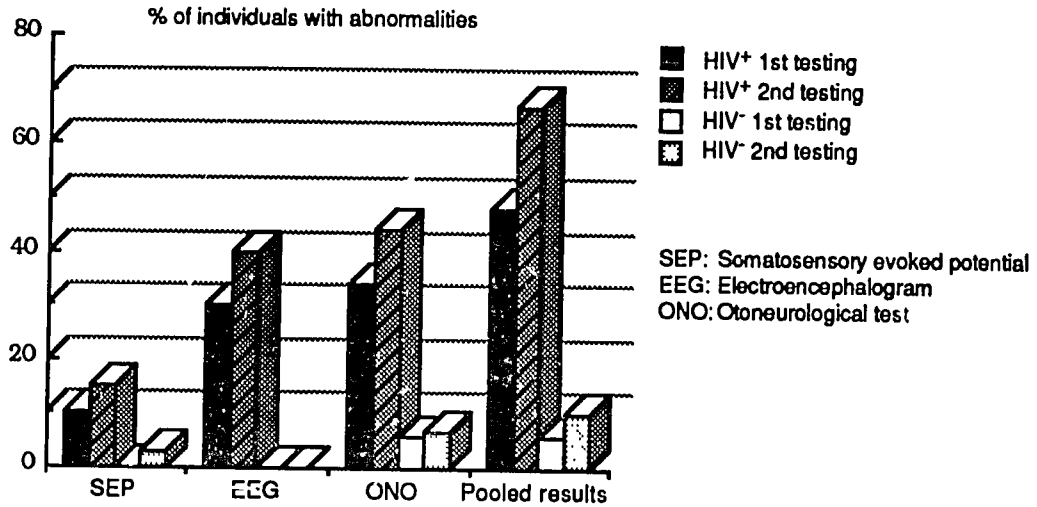
Magnetic Resonance Imaging (MRI). This imaging technique measures the distribution of water molecules in the brain. When placed in a magnetic field, certain atomic nuclei (in particular hydrogen nuclei, as are found in every water molecule) resonate, or vibrate, emitting a radio-frequency signal. Changes in the distribution of water in brain tissues can result from conditions such as edema (swelling) and local cell death, providing evidence of brain abnormalities.

Evaluating CT and MRI. MRI is considered more sensitive than CT, because of the greater contrast inherent to the technique, and serial MRI studies are considered relatively safe, because x-rays are not used. However, in some circumstances, such as the detection of calcification or the discrimination between a lesion and surrounding edema, CT scanning is more precise. Thus, CT and MRI are considered complimentary imaging techniques. However, neither CT nor MRI can directly identify a particular disease process—no HIV-associated CNS disorder has characteristic radiographic lesions exclusive to that disease.

In many instances, CT and MRI images are used to define the need for a biopsy in a particular patient. Drs. Samuel Ciricillo and Mark Rosenblum (University of California, San Francisco) examined the utility of these imaging methods in determining the need for biopsy. The identification of a single mass lesion using CT, or multiple lesions using either CT or MRI, did not aid in differential diagnosis. However, a single lesion on MRI was found to be indicative of lymphoma in 13 of the 18 cases examined. Such a result suggests that early biopsy may be prudent in cases of single-lesion MRI findings, so that radiation treatment can be initiated immediately in the event of a positive biopsy. In addition to their diagnostic capabilities, CT and MRI images have been used to guide biopsy probes to regions of damage (see "Primary Brain Tumors, above").

Single Photon Emission Computed Tomography (SPECT). In addition to CT and MRI, which enable the visualization of discrete CNS lesions, another imaging technique may prove useful for the early detection of HIV-associated neurologic disease. Single photon

Figure 8.4. Electrophysiological Tests to Detect Early Neurological Dysfunction



Three tests used to assess electrophysiological function were administered to a group of asymptomatic HIV-infected homosexual men on their first visit (HIV+ 1st) and on 6- to 9-month follow-up (HIV+ 2nd) visits; results are compared with those for a population of uninfected homosexual men over a similar period (HIV- 1st and 2nd). The percentage of individuals showing abnormalities on the three tests and the pooled data for the three tests are shown. Note that the HIV-infected group is much more likely to exhibit electrophysiological dysfunction and that progression of these abnormalities is demonstrated with time. (Based on data presented by Dr. Igor Koralnik, University Hospital, Geneva.)

emission computed tomography (SPECT) measures cerebral blood flow in different areas of the brain. Dr. Karl Einhaüpl (University of Munich) presented results using this technique in a study of 61 HIV-infected individuals at all stages of disease. In individuals without symptoms of neurological disease, nearly half (46%) were found to have focal blood flow deficits using SPECT; such deficits may be indicative of impending neurological dysfunction. A comparison of CT and SPECT results in these patients further suggested that SPECT may be more sensitive in detecting early tissue abnormalities. Of the 35 individuals having a normal CT, 28 showed pathological SPECT results. By comparison, 12 of the 13 individuals with abnormal CT results also showed abnormal SPECT findings (see Figure 8.3).

ELECTROPHYSIOLOGICAL TESTING

In general, the neurological and neuropsychological dysfunction seen in HIV-infected persons has an insidious onset, starting as subtle, subclinical changes in motor skills, memory, and mood. Detection of early neurological dysfunction may be important to successful treatment of individuals infected with HIV. A number of research groups have focused on the development of sensitive, early predictors of neurological disease. A major focus in this research has been neurophysiological tests, which may detect the first signs of CNS dysfunction.

Evoked Potentials. Many of these neurophysiological tests are based on "evoked potentials." The subject's brain activity is monitored by means of surface electrodes placed on the head. A visual, auditory, or somatosensory stimulus is presented to the subject, and the brain wave evoked by this stimulus is recorded. Normally, a particular stimulus results in a reproducible pattern of waves in the brain. Each time the stimulus is delivered, these waves are of the same size (amplitude) and occur at the same time interval from delivery of the stimulus (latency). In general, little variation is seen among individuals in the waves evoked in response to the same stimulus. However, individuals with cognitive deficits appear to have differences in the amplitude and/or latency of certain components of their evoked potentials.

Of critical importance (and with particular relevance for HIV-infected persons) is how early these differences in evoked potentials can be measured. Dr. John Messenheimer and coworkers (University of North Carolina, Chapel Hill) found that certain types of evoked potentials may be affected early in HIV disease: Somatosensory evoked potentials (SEPs) differed significantly between seronegative and seropositive asymptomatic individuals, and these differences were more evident with increasing disease status. In a study of HIV-infected patients in later stages of illness, Dr. Don Jewett and colleagues (University of California School of Medicine, San Francisco) found that approximately half of individuals with ARC or AIDS had an abnormal auditory brainstem response (another type of evoked potential). In a number of these individuals, the diagnosis of ARC or AIDS was made strictly on the basis of systemic findings, such as *Pneumocystis carinii* pneumonia; no sign of CNS involvement existed. In the authors' opinion, these results suggest that the auditory brainstem response is sufficiently sensitive to detect the earliest CNS abnormalities and may be useful in detecting early CNS dysfunction in asymptomatic individuals. Likewise, Dr. Harry Hollander (University of California, San Francisco) reported that the occurrence of electrophysiological abnormalities in the absence of neuropsychological dysfunction suggests that evoked responses may provide a sensitive way of screening for subclinical HIV-associated CNS disease.

Other studies indicate that some types of evoked potential may not be so useful in assessing early CNS abnormalities. One of the most commonly used electrophysiological tests measures a component wave of the evoked potential known as the P300 (so-named because it normally occurs 300 milliseconds after the stimulus is presented). In a study of HIV-infected homosexual men at various stages of illness, Dr. Ruth McAllister and colleagues (Middlesex Hospital, London) found no consistent differences in P300 amplitude or latency among these men or between this group and seronegative individuals tested as controls.

Otoneurological & Other Electrophysiological Tests. In addition to evoked potentials, other electrophysiological tests may signal subclinical neurological dysfunction in HIV-infected persons. A study reported by Dr. Igor Koralnik and coworkers (University Hospital, Geneva) compared asymptomatic HIV-positive homosexual men with HIV-negative homosexual men in a battery of tests. No differences were seen between the groups on various standard tests of neurological or neuropsychological function, and no differences were seen on radiological examination using MRI. However, significant differences were seen when otoneurological tests evaluating certain brain pathways were administered (see Figure 8.4). Abnormalities in these tests were seen in 34–44% of HIV-positive subjects, compared with similar abnormalities seen in only 6–7% of seronegative men. In addition, electroencephalographic (EEG) measurements found significant abnormalities in 30–40% of the HIV-positive group, while none of the seronegative individuals exhibited abnormal EEGs. The HIV-infected group showed a significant worsening in test performance on follow-up 6 to 9 months later.

Taken together, the results of these electrophysiological tests suggest that subclinical neurological dysfunction is a fairly common occurrence in HIV-infected individuals. However, the significance of these subtle changes in testing patterns remains open to speculation. Studies have yet to show that individuals with electrophysiological abnormalities are more prone to progress to clinical neurological illness than individuals without such early abnormalities. Until such a link is established, the diagnostic and therapeutic usefulness of neurophysiological tests will remain unresolved.

OCULOMOTOR TESTING

Oculomotor (eye movement) performance may serve as another means by which subclinical neurological involvement can be detected in HIV-infected persons. Dr. Pauline Merrill and collaborators (Washington University School of Medicine, St. Louis) examined various

aspects of oculomotor function in both HIV-infected and HIV-seronegative populations. Abnormalities were noted in the oculomotor performance of nearly all HIV-infected individuals, and eye motor performance generally worsened with disease progression. Deficits were often seen in the absence of overt neurological difficulties, suggesting that eye movement disorders may be an early sign of subclinical dysfunction.

PSYCHIATRY

Common psychiatric disturbances in HIV-infected individuals include emotional reactions, such as anxiety or denial, that occur in almost 90% of patients following notification of seropositivity. These adjustment reactions rarely lead to chronic functional impairment. Indeed, one of the more frequently asked questions relating to the psychiatry of HIV disease is the impact that seropositivity has on the mental state of the infected individual. Addressing this issue, Dr. Danitza Jadresic and coworkers (St. Stephen's Clinic, London) studied the effects of HIV diagnosis on mood and on patterns of drug and alcohol use 6 months after the individual first learned of their seropositivity. Somewhat surprisingly, it was found that knowledge of HIV seropositivity had little or no effect on mood or patterns of drug use in these persons. One likely explanation for this finding was revealed in the results of tests conducted prior to notification. In these tests, individuals subsequently identified as seropositive and individuals found to be uninfected exhibited similar borderline pathological levels of anxiety.

Adjustment disorders—as opposed to the adjustment reactions described above—are generally associated with chronic functional impairment and can be ameliorated by medication or psychotherapy. Included in the category of adjustment disorders are depression and mania, examples of affective disorders. In a study of psychiatric disorders in a group of 225 HIV-positive individuals, Dr. Dieter Naber and colleagues (University of Munich) found that 27% of these individuals suffered from major depression. Additionally, 6% had symptoms of clinical anxiety, and 1% reported suicide attempts after learning of their seropositivity. No significant differences among the various disease stages were seen in terms of these disorders. In a supplementary study of more than 700 HIV-infected persons, only 9 were found to suffer from some form of psychosis (for example, manic-depression or schizophrenia). All of these individuals had ARC or AIDS. Thus, although the incidence of psychiatric disturbances seems to be rather high in people with HIV infection, no elevation appears in the incidence of psychoses.

Dr. Henry Chuang and coworkers (University of Calgary, Alberta) obtained similar results in a study of psychiatric morbidity in patients infected with HIV. The only psychiatric disorder found with increased frequency in HIV-positive individuals was adjustment disorder, seen in nearly half of all HIV-infected persons. In comparison, only 21% of control individuals suffered from this disorder. All other psychiatric disturbances were found in approximately equal prevalence in the two populations.

Dr. Jaime Smith and collaborators (University of British Columbia, Vancouver) presented evidence that psychosis in the context of HIV infection may be of organic origin. A study of 16 HIV-positive individuals whose first clinical evaluation revealed acute psychosis found that all of them subsequently developed HIV encephalopathy. This result suggests that certain psychoses may be attributable to the direct effects of HIV on the brain, rather than the result of the stress associated with a serious illness.

A study conducted by Dr. Michael Tuite and coworkers (Rochester Medical Center, New York) examined radiological findings in persons suffering from acute mood disorders, such as mania and depression. A common finding in these individuals was an enhancement of the meninges, seen using MRI. Such enhancement is characteristic of meningeal inflammation, and the authors speculate that this may represent activation of HIV in the CNS.

Dr. David Ostrow (University of Michigan, Ann Arbor) discussed some of the more important psychiatric issues confronting HIV-infected persons, their friends and family. Three fundamental questions served as the focus for his talk:

- What is the incidence of major psychiatric illness in persons infected with HIV?
- What roles do alcohol and recreational drug use play in the development of such illness?
- Are there safe and effective psychopharmacologic treatments for these individuals?

(This last topic is discussed in detail below, in "Psychopharmacologic Issues.") Unfortunately, the answers to these questions may be partially masked by confounding factors associated with dealing with a life-threatening disease such as HIV infection.

A tremendous overlap is bound to exist between symptoms that are directly related to HIV infection and symptoms resulting from psychological difficulties in adjusting to the disease. Dr. Ostrow cited the example of weight loss in an HIV-infected person: how does one distinguish between weight loss due to the direct effects of viral infection and weight loss resulting from the depression associated with confronting HIV infection? With respect to recreational drug and alcohol use, since 1984, a precipitous decrease in recreational drug use among HIV-positive individuals has been noted, while alcohol use has shown virtually no change during this time. (The decline in drug use likely also resulted in a decline in high risk sexual behaviors, since there is a strong association between drug use and measures of sexual risk.) As suggested by Dr. Ostrow, the continued use of drugs and alcohol among infected individuals may represent a method of dealing with the anxiety associated with HIV seropositivity.

Several studies have suggested that drug use depresses immune function, and acute alcohol or cocaine abuse hinders the ability of lymphocytes to eliminate or control HIV. However, Dr. Ostrow pointed out that such data contradicts the results of some studies showing that HIV-infected men using drugs fare about as well as their non-drug using counterparts.

PSYCHOPHARMACOLOGIC ISSUES

Many HIV-infected individuals require pharmacologic intervention in the treatment of psychiatric problems associated with infection. Depending on the psychiatric disorder, a number of drugs may be prescribed, including antidepressants (clomipramine, imipramine), benzodiazepines (Librium, Valium), neuroleptics (haloperidol, chlorpromazine), and lithium. Dr. Ostrow provided certain guidelines for the use of these agents in HIV-infected persons:

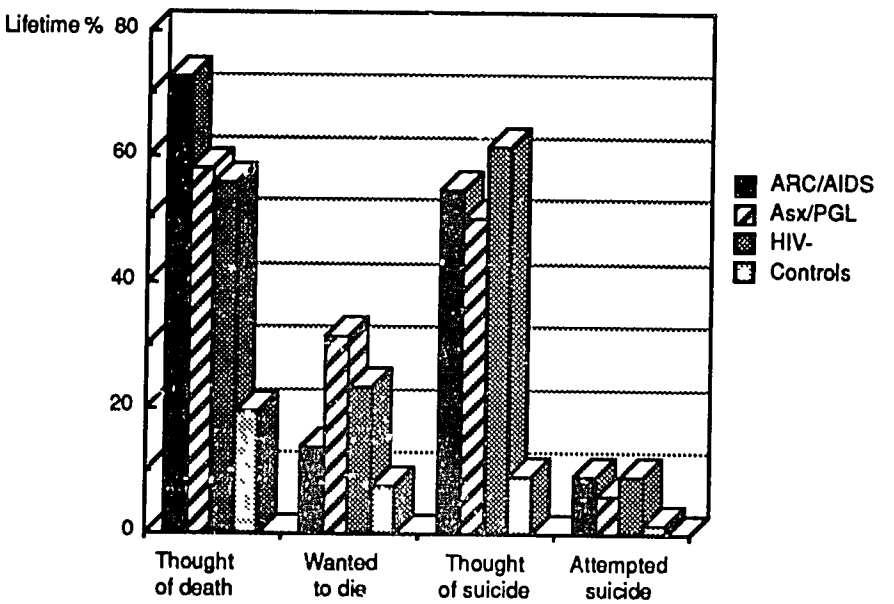
- The agent should be chosen based on the patient's symptomology and potential side effects of the drug.
- The patient should be monitored to ensure administration of an adequate dosage.
- Adequate maintenance therapy, usually consisting of half the initial therapeutic dose, must be provided.
- A proper period must be allowed for termination of the drug; generally, 6 months after recovery is adequate.

If used properly, psychopharmacologic therapy can be extremely effective in the treatment of psychiatric disorders in the HIV-infected population. An example was provided by Dr. Donna Manning and colleagues (New York Hospital-Cornell Medical Center), whose group found that imipramine was effective in countering depression in HIV-positive homosexual men.

An important concern when treating HIV-related psychiatric disorders with pharmacologic agents is that the agent used may interfere with normal immune function. Many of the drugs used, including the benzodiazepines, neuroleptics, and lithium have been shown to have immunosuppressive effects *in vitro*. Although the *in vivo* effects of these drugs have yet to be established, the possibility of immunosuppression should be considered when prescribing these and other agents.

Figure 8.5. Suicidal Thoughts and Attempts

In an study correlating clinical status with suicidal ideation and attempts, a group of 105 homosexual men (HIV seropositive and seronegative) were compared with 294 matched heterosexual men as controls. Shown are their lifetime percentages for suicidal thoughts and attempts. The interviewees were homosexual men with ARC or AIDS (ARC/AIDS); HIV-infected homosexual men who were either asymptomatic or had persistent generalized lymphadenopathy (Asx/PGL); HIV-uninfected gay men (HIV-); and the control group from the general population (Controls). The authors noted a higher lifetime incidence of a major psychiatric disorder (e.g., depression or substance abuse) in the homosexual men, regardless of HIV serostatus, than in the controls. (Based on data presented by Dr. Hampton Atkinson and colleagues, University of California, San Diego and VA Medical Center, San Diego.)



Psychostimulatory agents—although unnecessary and inadvisable for the majority of HIV-infected individuals—may occasionally be necessary in the treatment of certain HIV-associated psychiatric disorders. Dr. Ostrow provided a profile of situations in which the use of such agents may be warranted:

- hospitalized patients exhibiting a "failure to thrive" and in need of acute intervention
- patients in whom apathy outweighs depression and severe fatigue is also evident
- patients who are intolerant or nonresponsive to standard antidepressants or who become delirious

In addition to the beneficial effects that have been documented for neurological and neuropsychological performance, evidence suggests that the antiviral drug AZT alleviates some of the psychiatric problems seen in HIV-infected individuals. Drs. Diana Perkins and D. L. Evans (University of North Carolina, Chapel Hill) reported that patients with organic mood disorder (clinical depression) responded with a significant improvement in mood on institution of AZT treatment. However, attributing this improvement to an organic effect of AZT on the brain is at this point premature.

SUICIDE

As with any life-threatening illness in which little hope of a timely cure exists, contemplation of suicide occurs fairly often in the HIV-infected population. Dr. Hampton Atkinson and colleagues (University of California, San Diego and VA Medical Center, San Diego) studied the frequency of suicidal thoughts and suicidal attempts in HIV-infected homosexual men at various stages of illness. Whereas thoughts of suicide and suicide attempts

were elevated in homosexual men compared with heterosexual controls, little difference was found in the frequency of suicidal thoughts or attempts among HIV-infected versus uninfected homosexual men (see Figure 8.5).

However, these results should not be interpreted as suggesting that the risk of suicide is not increased within the HIV-infected population. Alcoholics are known to have a suicide rate that is 10-20 times higher than that in the general population. In a comparative study of HIV-infected subjects and alcoholics, Dr. Karen Drexler (Wilford Hall U.S. Air Force Medical Center, San Antonio) and collaborators found these two populations to have comparable levels of suicidal thoughts and suicidal behaviors. They suggest that clinicians treating HIV-infected persons should be acutely aware of the increased frequency of suicidal behaviors in this population of patients.

Although the incidence of suicidal thoughts and actions is elevated among the HIV-infected population as a group, it appears that certain individuals are more susceptible than others. In examining an HIV-infected population of homosexual men, Dr. Rosben Gutierrez and colleagues (University of California, San Diego and VA Medical Center, San Diego) found that men with suicidal tendencies had a smaller network of social support, and relied more heavily on avoidance as a strategy to cope with their disease than nonsuicidal HIV-infected men. Thus, having a strong network of supporting friends, family, and other individuals, as well as trying to confront HIV disease more directly, may result in a healthier psychological approach to living with HIV illness.

NEUROPSYCHOLOGY

MANIFESTATIONS OF NEUROPSYCHOLOGICAL DYSFUNCTION

In addition to the devastating neurological and psychiatric effects of HIV infection, many of these individuals suffer from neuropsychological deficits as well. Neuropsychological dysfunction typically is manifested as a decline in cognitive abilities, which can range from mild, quite subtle changes to extremely severe disorders, as in AIDS dementia complex. As summarized by Dr. Karl Syndulko (VA Medical Center, West Los Angeles), neuropsychological function is generally assessed with respect to a number of specific skills: verbal, visuospatial, visuomotor, visual attention (memory), verbal attention (memory), manual dexterity, motor speed, and ambulation.

Dr. Igor Grant (University of California School of Medicine, San Diego) offered that neuropsychological or neurobehavioral disorders associated with HIV infection can be divided into two major categories: neurogenic and psychogenic. Neurogenic disorders result from organic brain disease associated with HIV. This can be primary disease, due to the direct effects of HIV on the CNS, or secondary disease, due to other biological factors. In contrast, psychogenic disturbances represent adjustment disorders, such as changes in mood, chronic or acute anxiety, and difficulties coping with the illness. The most severe neuropsychological deficits seen in HIV-infected persons, including ADC, are likely neurogenic.

PREVALENCE OF NEUROPSYCHOLOGICAL DEFICITS

A survey of the literature reveals considerable disagreement over the prevalence of ADC in the HIV-infected population, a fact that was emphasized by Dr. Grant in his discussion. The best evidence to date suggests that approximately one-third of all HIV-infected persons will develop ADC (although values ranging from 16% to 70% have been cited). Generally seen in the most advanced stages of HIV disease, ADC is characterized by a global cognitive decline.

Milder cognitive disorders are seen at earlier stages of disease and in a greater percentage of the HIV-infected population. According to Dr. Grant, these milder disorders are characterized by a subjective sense of cognitive decline and an impairment in neuropsychological function in at least two of the above areas, but without substantial effects on social functioning. The incidence of these less profound cognitive disorders is even harder to pinpoint than that of severe ADC; estimates on the prevalence of non-ADC neuropsychological deficits in ARC and AIDS patients range from 33% to 75%.

The largest controversy, however, appears to revolve around the prevalence of neuropsychological deficits in *asymptomatic* HIV-infected individuals. The figures range between 0% and 50%, with a significant number of studies suggesting that no difference exists between HIV-positive asymptomatics and uninfected individuals in terms of cognitive function. A number of reasons can account for these large differences, some of which were discussed by Dr. Grant:

- *Sampling.* Differences in these values may be, in part, a function of sampling; studies that show differences in cognitive functioning among asymptomatic and control individuals may be drawing from a population of asymptomatics that is further along in disease progression than the asymptomatics used in studies that show no differences.
- *Testing.* Each study uses a different battery of tests to measure different aspects of cognitive performance, making comparisons between these studies virtually impossible.
- *Criteria.* No standard criteria has been established for deciding whether or not a person is neurologically impaired. Thus, a person classified as neurologically impaired using one set of criteria, may be considered unimpaired using a different set of criteria.
- *Confounding Factors.* A number of confounding factors—including drug use, head injury, and a history of previous psychiatric illness—may strongly influence the results of neuropsychological tests. If not properly controlled for, these factors may result in serious discrepancies from test group to test group.

Dr. Grant illustrated the legitimacy of these concerns by using one of the largest studies on the neuropsychological aspects of HIV infection, the Multicenter AIDS Cohort Study (MACS), as an example. To date, the MACS finds that asymptomatic HIV-infected individuals do not demonstrate neuropsychological deficits beyond control values. Sampling is of great concern with this study group, since the cohort of individuals tested is self-referred. With such a selection system, impaired individuals may not readily volunteer, biasing the sample towards less impaired persons. In a study as large as the MACS, spread nationally over a number of institutions, the logistics of gathering and analyzing the data are enormously complicated. One fear is that without a central facility to standardize the administration and scoring of the neuropsychological tests, the resolution of such tests in picking out subtle cognitive deficits will be rather poor.

In contrast to the MACS results, most studies today suggest that there is some neuropsychological dysfunction early in the course of HIV infection. In a survey of the presentations given at the satellite conference in Monterey, Dr. Grant found that 12 of the 18 studies comparing asymptomatic seropositives with uninfected controls reported a difference in neuropsychological function between these groups. According to his own data, Dr. Grant found that 36% of HIV-infected individuals in CDC Stage IV (diagnosed with some form of HIV disease) are neurologically impaired. By comparison, neurological impairment was found in 25% of CDC Stage II/III individuals (asymptomatic or diagnosed with progressive generalized lymphadenopathy) and in 15% of HIV-negative controls. The primary areas of impairment were in learning, attention, recall of information, and problem solving.

NEUROPSYCHOLOGICAL TESTING & EVALUATION

Dr. Agnes Kocsis (St. Mary's Hospital and Medical School, London) also discussed some of the difficulties associated with neuropsychological testing of HIV-infected persons. Dr. Kocsis stressed that in her opinion, many of the neuropsychological tests used for the detection of mild cognitive dysfunction in HIV-infected persons are inadequate. These tests, originally designed to evaluate cognitive function in trauma and head injury patients, may not be sensitive enough to detect the more subtle signs of neuropsychological dysfunction seen in the early stages of HIV illness.

Dr. Kocsis also cautioned that the methods used to evaluate the neuropsychological data obtained from HIV-infected persons are fraught with problems. Which system of scoring is most appropriate in presenting the results of these tests? Table 8.2 outlines the ability of three scoring methods in detecting varying degrees of focal or global neuropsychological deficits. Simply using the mean (average) can be misleading, since a single low or high score can wildly skew the results from the whole group. Percentage scores, used by many evaluators, are ultimately dependent on the thresholds used to define cognitive dysfunction. Summary scores, such as the Z score, can magnify subtle differences between individuals and groups. Dr. Kocsis proposed that pooling the results of all of these methods would give a better profile of neuropsychological function in the various groups.

A number of studies rely on self-reporting to evaluate neuropsychological function in HIV-infected persons. In such studies the individual is asked to quantify the degree of cognitive impairment, if any, that they feel they are experiencing as a result of HIV infection. As is any type of subjective evaluation, such analysis is fraught with interpretational problems. In a study of neuropsychological self-evaluation, Dr. Jonathan Canick (University of California School of Medicine, San Francisco) and Dr. Lydia Temoshok (Henry M. Jackson Foundation, Walter Reed Army Medical Center, Washington, D.C.) found that the degree of cognitive dysfunction described by an individual was more a function of the level of psychological distress experienced by the patient, than of objective neuropsychological dysfunction.

NEUROPSYCHOLOGICAL DYSFUNCTION & DISEASE STAGE

In an effort to explain the cognitive decline seen in many HIV-infected individuals, some researchers and clinicians have proposed a direct correlation between immune status and neuropsychological performance. This relationship, if it exists, has previously been difficult to document. The results of research conducted by Dr. Charles Hinkin and coworkers (University of California, Los Angeles, School of Medicine), however, support such an association. In their study, cognitive function was measured in a group of HIV-positive men with CD4 counts *above* 200/mm^3, and the results were compared with those from a similar group of men but with CD4 values *below* 200/mm^3. The immunocompromised group (CD4 counts below 200) scored significantly worse in measures of memory, sequencing, psychomotor speed, and depression, suggesting a strong association between immune function and neuropsychological performance.

Most studies today find some degree of cognitive impairment in asymptomatic seropositive individuals, although, as previously mentioned, the topic is still controversial. Dr. David Clifford and colleagues (Washington University School of Medicine, St. Louis) examined neuropsychological function in several groups of control and HIV-infected individuals at various stages of disease. The tests were designed to evaluate various aspects of cognitive function, including attention, memory, motor skills, and language. The most sensitive tests were those measuring tasks with relatively long reaction times, such as memory scanning. Whereas no difference was seen in the cognitive performances of control individuals from either the general population or from seronegative high-risk groups, HIV-infected persons showed poorer overall performance.

Table 8.2. Generating A Profile of Neuropsychological Impairment

The methods used to analyze data collected from neuropsychological tests of asymptomatic HIV-infected individuals can result in quite different conclusions. As indicated in the table, each of the three methods of statistical analysis—means (averages), percentage scores, and summary statistics—are of differing sensitivities in detecting varying degrees of global or focal neuropsychological dysfunction. Consideration of all three scoring methods together may yield the best overall profile. (Based on data presented by Dr. Agnes Kocsis, St. Mary's Hospital and Medical School, London.)

Scoring method	Global dysfunction		Focal dysfunction	
	Mild	Severe	Mild	Severe
Means	No	?	No	?
Percentage	No	Yes	?	Yes
Summary	Yes	Yes	?	Yes

Other studies have provided similar results. Data presented by Dr. B. E. Levin and colleagues (University of Miami School of Medicine, Florida) suggest that subtle cognitive deficits can be found in some individuals early in the course of HIV infection. Dr. Dieter Naber and coworkers (University of Munich) also found indications of early neurocognitive dysfunction in certain HIV-infected individuals. In a battery of neuropsychological tests administered to HIV-infected persons in the early stages of disease (asymptomatic or with lymphadenopathy), 75% scored normally, 20% exhibited mild impairment, and 5% were classified as extremely impaired. In subjects in later stages of disease, these scores were significantly worse. Only 60% of ARC/AIDS patients scored normally, with 25% showing slight impairment and 15% showing severe impairment. In a study of HIV-infected homosexual men, Dr. Robert Bornstein and collaborators (Ohio State University, Columbus) reported that 22.5% of the asymptomatic men had summary neuropsychological scores categorizing them as cognitively impaired. By comparison, 36.5% of ARC patients, and 45.5% of AIDS patients were impaired, while none of the control individuals exhibited cognitive deficits.

Although many studies point to a subtle cognitive decline in the early stages of HIV infection, the cause of this decline is unknown. Early cognitive impairment may represent the first functional effects of viral CNS infection; alternatively, these declines may be more a function of the psychological effects of coping with HIV disease. In a study reported by Dr. Pamela Krell (Veterans Administration Medical Center, San Francisco) and colleagues, mild levels of cognitive impairment were strongly associated with high levels of anxiety, depression and emotional distress-reactions commonly experienced by individuals after first learning of their seropositivity.

PROGRESSION OF NEUROPSYCHOLOGICAL DYSFUNCTION

Once cognitive deficits are first manifested in HIV-infected individuals, what is the progression of the decline in function? Dr. Kevin Robertson and colleagues (University of North Carolina School of Medicine, Chapel Hill) examined this question by looking at neuropsychological changes in HIV-infected persons over time. They found that early in the asymptomatic stage of disease, little or no decline was seen in tests of cognitive function. However, at follow-up evaluations, by the symptomatic period of infection, most notably during AIDS, neuropsychological dysfunction rapidly progressed. Similar results were reported by Dr. Christina Marra and colleagues (University of Washington, Seattle); their prospective study, restricted to individuals in the earliest stages of HIV disease, found no neurological or neuropsychological deterioration in these individuals over a 1-year period. Dr. Robert Velin and collaborators (University of California, San Diego and VA Medical Center, San

Diego) found that a larger percentage of homosexual men with ARC/AIDS showed mild neuropsychological decline over a 1-year period than did homosexual men in the asymptomatic stage, or men with progressive generalized lymphadenopathy followed for the same period. Assessment of change in neuropsychological function was based on evaluations at baseline, and seronegative controls were included in the study.

DRUG USE AND OTHER CONFOUNDING FACTORS

Long-term drug users constitute one of the most difficult of the HIV-infected subgroups to assess for cognitive dysfunction. A number of studies have suggested that these individuals are especially susceptible to the damaging effects of HIV on the nervous system. However, HIV-infected drug users, many of whom come from deprived backgrounds, commonly have little or no education, may have suffered brain damage due to extended drug use, and are likely to have scored poorly on tests of neurocognitive function even prior to their infection with HIV.

In a study of HIV-infected drug users, Dr. Vincent Egan and collaborators (City Hospital, Edinburgh) suggest that the cognitive impairment seen in these individuals is more likely the result of their extended drug use rather than their HIV infection. Further, Dr. Egan reported that in these individuals—despite an average 6-year duration of infection—no evidence was seen of declining neuropsychological performance with progression of HIV disease. In addition, this study indicated that the early onset of ADC was no more likely to occur in infected drug users than in populations of HIV-infected homosexual men.

Another potential confounding factor in evaluating the neuropsychological effects of HIV infection is past or current infection with other neurotropic agents. Such infections may contribute to cognitive deficits, rendering analysis of neuropsychological function difficult, if not impossible. To examine the possible confounding effects of other neurotropic infections on cognitive function in HIV-infected persons, Dr. Ola Selnes and colleagues (Johns Hopkins School of Medicine, Baltimore) examined the effects of prior or current syphilitic infections on the neuropsychological performance of asymptomatic HIV-infected persons. Their preliminary results suggest that syphilitic infection has little effect on cognitive function in this population.

AZT THERAPY AND NEUROPSYCHOLOGICAL PERFORMANCE

The antiviral drug azidothymidine (AZT, or zidovudine) has met with some success in prolonging survival and improving quality of life. In addition, a body of evidence now suggests that AZT improves neuropsychological function in certain HIV-infected persons. Dr. Frederick Schmitt (University of Kentucky School of Medicine, Lexington) presented data showing significant neurocognitive improvement in a group of ARC and AIDS patients following treatment with AZT. These individuals showed advances in attention, memory, motor skills, and general mental speed.

Data from the MACS show a similar trend for all HIV-infected groups. Dr. Justin McArthur (Johns Hopkins University, Baltimore) and colleagues reported that significantly fewer subjects showed a decline in neuropsychological performance when evaluated up to 6 months after AZT treatment (although the overall number of HIV-infected individuals exhibiting cognitive decline in this study was low). An interesting presentation by Dr. Valerio Tozzi (Spallanzani Hospital, Rome) gave evidence of reversal of neurological dysfunction in persons suffering from HIV encephalopathy after institution of AZT therapy.

How does AZT mediate these improvements? The most obvious possibility is that by improving general health, AZT improves neuropsychological performance; alternatively, AZT may exert these effects through direct action on the brain. Indeed, Dr. Giulio Costanzi

(University of Milan) and colleagues reported that, in their study of brain autopsies in AIDS-related deaths, patients treated with AZT had a lower frequency of lesions associated with multinucleated giant cells (syncytia) than did untreated individuals.

The mechanism of action of AZT on the brain, however, is open to speculation. The drug may modulate the level of endogenous cytokines; some evidence indicates that individuals with symptomatic HIV disease have elevated levels of the cytokine alpha-interferon. High levels of this substance may lead to neuropsychological impairment. Dr. Joseph Bigley (Burroughs Wellcome Company, Research Triangle Park, North Carolina) and members of the AZT Collaborative Working Group sampled sera from patients in the original double-blind AZT study. The administration of AZT was associated with a significant decrease in serum alpha-interferon titers. Although only correlative, these results suggest a link between cognitive function and alpha-interferon levels.

Some scientists believe that neuropsychological testing can be an important means of evaluating the efficacy of new drugs being developed for treatment of the HIV-infected population. Dr. E. C. Cooper and colleagues (Food and Drug Administration, Rockville, Maryland) pointed out that improvements in the management of opportunistic infections in HIV-infected persons has made it increasingly difficult to judge the effectiveness of new anti-HIV drugs. New strategies to evaluate prospective therapies must therefore be developed. Changes in neurocognitive function—which seem to occur early in the course of HIV disease and which seem capable of responding to pharmacologic intervention (as seen with AZT)—may provide new and more sensitive measures of drug efficacy.

REHABILITATION OF THE COGNITIVELY IMPAIRED

As has been shown for individuals who have suffered cognitive impairment due to stroke or traumatic head injury, HIV-infected individuals with neuropsychological dysfunction can be retaught many cognitive skills. Dr. Lynne Rich (Florida Psychological Association AIDS Network, Ft. Lauderdale) presented the results of some recent research suggesting that learning can improve brain function in HIV-infected individuals, and that new synapses are created in the process. Strategies for coping with cognitive deficits are a major part of any rehabilitation program. As an example, HIV-infected persons frequently complain of problems in performing "higher executive functions," such as remembering lists of objects, focusing on more than one thing at a time, and remembering where items are put away. In rehabilitative therapy, individuals are taught to slow down and focus their attention on precisely what they are doing while they are performing an activity. Dr. Rich reports that when persons with AIDS engage in behaviors they believe will bolster their health, the body appears to respond in a positive way. Thus, participating in rehabilitation may have both physical and psychological benefits for HIV-infected individuals.

NEUROPEDIATRICS

NEUROLOGICAL/NEUROPSYCHOLOGICAL MANIFESTATIONS

Children infected with HIV (whether by perinatal transmission or transfusion) exhibit neurological abnormalities and neuropsychological dysfunction similar to those observed in infected adults. As in adults, CNS manifestations of HIV disease in children can be static or rapidly progressive, with encephalopathy being one of the most common HIV-associated diseases. Dr. Rosemary Vazeux (Institut Pasteur, Paris), reported that HIV-associated encephalopathy in children is manifested by loss of developmental milestones, deterioration of motor functions, and microcephaly. Neuropathologies are similar to those seen in adults, and include calcification, white matter pallor, inflammation with numerous

macrophages and lymphocytes at the site(s) of inflammation, and multinucleated giant cells. HIV viral particles can be seen by electron microscopy, and HIV RNA and DNA can be detected by PCR and *in situ* hybridization.

In examining the brains of 7 HIV-infected children (born to seropositive mothers) who died with severe encephalopathy, Dr. Vazeux's group found productive HIV replication in the CNS of only 3 of the children. In tissues from these 3 cases, HIV antigens and HIV RNA (by *in situ* hybridization) were readily detectable, with macrophages and microglial cells being the only cells infected by HIV. In the other 4 children, little or no HIV replication was found associated with the encephalopathy. In addition, although HIV DNA was detectable by PCR, HIV antigens and HIV RNA were either undetectable or present in very small amounts. These observations demonstrate that encephalopathy in HIV-infected children is not always associated with HIV replication in the CNS at the time of death. Dr. Vazeux summarized the following possibilities to explain these results:

- The lesions seen in encephalopathic brains could be the result of previous, or latent, rather than productive HIV infection.
- Tissue damage seen in encephalopathy could be caused by soluble factors rather than as the direct result of HIV infection in the area of damage.
- Encephalopathy could result from other causes. In fact, brain lesions in 3 of the 4 children who did not have evidence of productive HIV replication were associated with ischemic necrosis, aspergillosis, and CMV encephalitis.

Several research groups are examining the neurological and neuropsychological manifestations of HIV infection in children with respect to the mode of viral transmission. Scientists want to know, for example, if HIV-infected children, who acquired the virus via transfusion, including hemophiliacs, exhibit the same types of neurological and neuropsychological disorders as children with perinatally acquired HIV infection. Do differences exist with respect to the *degree* of neurological involvement between the two populations? Furthermore, is there a temporal difference between the two populations—that is, do neurological symptoms appear earlier in one population than the other?

Among all of the subgroups of HIV-infected individuals, children exhibit the most severe cognitive difficulties as a result of their infection. It is likely that exposure to HIV during the formative years of brain development has profound neurological and neuropsychological consequences.

In a study of cognitive and motor development in infants (1-2 years of age) who acquired HIV from their infected mothers, Dr. Cynthia Chase and coworkers (Boston City Hospital) found significant differences when these children (with perinatally acquired HIV infection) were compared to children born to seronegative mothers, or compared to seronegative children born to seropositive mothers. Measures of mental development, psychomotor development, memory, problem solving, and fine and gross motor skills were all significantly lower in the HIV-infected infants. Indeed, cognitive deficits and gross and fine motor deficits appear to be prominent in HIV-associated encephalopathy in children aged 6 to 24 months. Dr. Chase pointed out that—although the entire sample population (HIV-positive and HIV-negative) were from an inner-city hospital and therefore at high risk for developmental delay (due to poverty, maternal drug use, poor nutrition, and other features of low socioeconomic status)—the static encephalopathy exhibited by the HIV-positive group could not be accounted for by environmental variables.

PREVALENCE OF PEDIATRIC NEUROPSYCHOLOGICAL DYSFUNCTION

Findings reported by several studies suggest that approximately 50% of HIV-infected children show neurodevelopmental delays. Dr. Uwa Kabagabo (Projet SIDA, Kinshasa, Zaire) and colleagues examined the neurodevelopment of HIV-infected children in Kinshasa.

Motor development was found to be clearly impaired in well over half (56%) of the perinatally infected children by the time they were 2 years old. Similarly, tests of mental development found 40% of the HIV-infected children to be performing at least one standard deviation below the norm. Two relatively small U.S. studies gave evidence of rather significant developmental delays among HIV-infected children, compared to their seronegative counterparts. Dr. Anita Belman (State University of New York, Stony Brook) reported that of 13 HIV-infected children (aged 13–39 months), 7 had HIV-related neurological disease; 6 of whom showed extremely low developmental scores. Similarly, Dr. Joan Hittleman (State University of New York, Brooklyn) and colleagues found 8 of 16 HIV-infected children to have some kind of developmental disability (when evaluated at 15 months). Dr. Hittleman's team pointed out that the 50% prevalence was in sharp contrast to the 11% found in HIV-negative children born to seropositive mothers and the 9% found in the control group of seronegative children.

HIV-INFECTED CHILDREN WITH HEMOPHILIA

Dr. Kenneth Whitt (University of North Carolina, Chapel Hill) reported his team's preliminary findings on the initial (baseline) neuropsychological assessments of HIV-positive hemophiliac children and adolescents compared with their seronegative counterparts. (Although significant differences were found in CD4 cell counts and CD4: CD8 ratios between the two groups, none of the seropositive children met the criteria for AIDS). While lower motor, attention, memory, and sensory perceptual functioning was observed for the HIV-infected group, no measure yielded a statistically significant difference between the two groups. Dr. Whitt indicated that continued assessment, at 6-month and 12-month intervals, will provide an ideal opportunity to monitor the subsequent neurological and neuropsychological manifestations of HIV infection in children with hemophilia.

EFFECT OF ANTIVIRAL THERAPY

Cognitively impaired HIV-infected children, as do their adult counterparts, appear to respond to antiviral therapy. A study conducted by Dr. Pamela Wolters and colleagues (National Cancer Institute, Bethesda, Maryland) examined the effects of the antiviral agent dideoxyinosine (ddI) on cognitive function in infants and children with symptomatic HIV infection. In this study, children treated with ddI showed minimal decline in cognitive function after 3 and 6 months. Since progressive neuropsychological decline is a characteristic of many HIV-infected children, this stabilization of cognitive function is likely to reflect a therapeutic effect of ddI.

DEFICITS IN UNINFECTED CHILDREN OF SEROPOSITIVE WOMEN

Work presented by Dr. Thomas Koch and colleagues (University of California, San Francisco) suggests that even uninfected infants born to HIV-infected mothers experience deficits in mental development. Although no differences in neurological findings or psychomotor development were seen between uninfected controls (born to seronegative mothers) and uninfected children born to HIV-positive mothers, a significant difference between these groups was noted in the mental development index during the first year of life. This suggests that uninfected infants born to infected mothers may be at risk for a nonprogressive neurodevelopmental problem. Dr. Koch offered that even in the absence of viral transmission, a mother's seropositivity may be detrimental to the cognitive development of her fetus.

CHAPTER 9

Page 265 **Background**
266 **Occupational Risks**
266 Specific Occupational Hazards
268 Universal Precautions
270 Epidemiological Studies
273 **Post-Exposure AZT Prophylaxis**
274 **Risk to Patients**
274 **Attitudes, Beliefs, & Practices**
275 Physicians
277 Medical Students
277 Dentists
278 Nurses
278 First-Response Personnel
279 **Education in the Workplace**
279 Educating & Training the Health Care Worker
280 Education in Business & Industry
282 **Ethical & Legal Considerations**
282 Health Care Setting
285 Business & Industry

WORKPLACE ISSUES

BACKGROUND

Depending on the workplace setting and the types of work activities involved, the risks of HIV transmission vary from real to illusory. Increasingly, as the epidemic lengthens, people are having to confront their own attitudes and fears about coworkers, clients, and patients with HIV infection or AIDS. Worldwide, as medical science becomes more facile in delaying the progression of advanced HIV disease, more and more HIV-infected persons and persons with AIDS will remain sufficiently healthy to continue work. Indeed, early diagnosis and prophylactic and therapeutic interventions have been shown to favorably affect patients' survival time as well as quality of life (see Chapter 7: CLINICAL MANAGEMENT for details.)

Although the relative risk of exposure may vary for each workplace setting, the routes of HIV transmission remain the same: direct (primarily invasive) contact with HIV-infected blood, body fluids, or other HIV-infected substances (such as those used in HIV laboratory research); see "Specific Occupational Hazards," below. Furthermore, not one case has been reported in which HIV infection or AIDS resulted from casual contact with an HIV-infected person or AIDS patient in the workplace—nor in any other setting.

Because the age group most affected by the HIV epidemic constitutes the bulk of the work force, the workplace, as the primary common denominator, is considered a potentially significant conduit for disseminating AIDS information. Workplace educational efforts are widely accepted as a means of dispelling fears and replacing them with accurate information and effecting attitudinal changes. Although the business community as a whole was

initially slow in its response to the AIDS epidemic, more businesses are now providing HIV/AIDS education for their employees: Dissemination of accurate information on HIV transmission—particularly addressing concerns regarding casual contact with HIV-infected persons—can be cost-effectively integrated into the workplace setting to minimize HIV-related fear and discrimination.

All employees, in all workplace settings, should be made aware of their organization's policies regarding AIDS, as well as the availability of specific educational materials or programs. Employer insurance benefits relating to occupational exposure to infectious diseases, including HIV infection, should be explicit. Workers should also be made aware of what to do in the event of accidental exposure.

OCCUPATIONAL RISKS

Occupational risk is not a new concern. Long before the AIDS era, occupational health and safety regulations were instituted—in some instances, legislated—in an effort to protect workers in various jobs from exposure to hazardous materials. In the health care and biomedical/biopharmaceutical research settings, infection control measures encompass precautions regarding infectious materials and persons with infectious disease. The emergence of the HIV epidemic in the early 1980s has engendered a heightened awareness of and concern for appropriate precautions.

Some occupations carry an inherent risk of exposure to potentially infectious materials—blood or body fluids from HIV-infected patients as well as research reagents containing HIV. Medicine, dentistry, biomedical research, allied health professions, emergency response jobs, and correctional facility officers bear varying degrees of occupational risk for HIV transmission. Those occupations at the higher end of the risk-of-exposure scale include:

- health care personnel (for whom precautions against exposure to HIV need to be integrated with existing infection control procedures)
- firefighters, police officers, ambulance and rescue workers, and other first-response personnel
- personnel whose work involves use of skin-piercing instruments (such as morticians, acupuncturists, cosmetologists, and tattooists)

Certain jobs are considered to have a relatively higher risk than others, based on the number and types of exposures. Additionally, risk assessment must also take into account the relative prevalence of HIV in a given geographic area. Among hospital staff positions, operating room, delivery room, and emergency room personnel are considered to bear the highest risk of transmission, due to the greater number of exposures and the greater volume of blood and/or other body fluids per exposure. Nursing and clinical laboratory research often entail *daily* exposure to potentially infectious blood and body fluids from patient specimen samples. Workers involved in pre-hospital emergency situations are not only exposed to large amounts of blood, but may be subject to injury themselves during this contact, further increasing their risk of exposure. Any industry or workplace setting in which workers come in contact with blood and/or other body fluids of HIV-infected persons carries some degree of risk.

SPECIFIC OCCUPATIONAL HAZARDS

Numerous ongoing studies are attempting to quantify the risks involved in specific occupational activities. Each exposure to HIV-contaminated blood or other body fluid or HIV-contaminated research reagents carries with it a small but not insignificant probability of infection. Although the risk of *exposure* to HIV-infected substances may be relatively high, the risk of *infection*, as demonstrated by seroepidemiological studies, is relatively low: Relatively few cases of HIV seroconversion have been documented as a direct consequence

of occupational exposure. (Seroconversion is the point at which a person's antibody status changes from seronegative—no detectable antibodies to HIV in the blood—to seropositive.) No cases of seroconversion attributable to occupational exposure have occurred in settings other than those involving health care. Potential routes of occupational HIV transmission under investigation include:

- needlestick or cut by other sharp instrument; these exposures are generally classified as parenteral or percutaneous
- splashes of fluid onto mucous membranes (for example eyes, mouth)
- skin contact (blood or body fluids coming in contact with non-intact skin)

Ongoing prospective studies of health care workers, in which persons reporting accidental exposure to potentially infectious substances are followed, seek to determine which types of exposures carry what level of risk. Although needlesticks and other percutaneous exposures are currently associated with the greatest number of documented cases of occupationally acquired HIV infection, exposures involving skin or mucous membrane contact are not negligible and have also resulted in HIV infection. The worldwide incidence of occupationally acquired HIV infection is discussed in "Epidemiological Studies," below; what follows here is a discussion of the occupational risks associated with different hospital settings and the need for health care workers to self report accidental exposures.

Operating Room. Dr. Douglas Owens (Stanford University Medical Center, California) and colleagues presented data relating the risk of exposure to HIV with the risk of exposure to hepatitis B virus, per operation on an infected patient. (Hepatitis B virus, or HBV, infection has traditionally been considered a serious occupational hazard for surgeons.) The risk of exposure to both infectious agents was quantified in terms of loss of life-expectancy based on data from a cohort of hemophiliacs and homosexual men (for HIV) and from survival data (for HBV). For unvaccinated (HBV-susceptible) surgeons, the performance of surgery on an HIV-positive patient is associated with a loss of life-expectancy of 0.70 days, compared to a loss of life-expectancy of 0.12 days for surgery on an HBV-positive patient. This calculation took into account the higher probability of seroconversion after an HBV-contaminated needlestick injury (1 in 10) than after an HIV-contaminated needlestick injury (1 in 250). Quality-adjusted loss of life-expectancy also differed: 0.90 days for surgery on an HIV-positive patient and 0.55 days for surgery on an HBV-positive patient. For a vaccinated (HBV-immune) surgeon, the risk related to surgery on an HIV-positive patient is at least 10-fold greater than surgery on an HBV-positive patient.

Emergency Room. Emergency care providers are frequently at the forefront of occupational exposure to HIV. Unlike surgical personnel, who are more likely to know the serostatus of the surgical candidate, emergency room workers typically have no knowledge of the patients they encounter. In addition, particularly in large inner-city hospitals, a significant percentage of emergency room patients are at a high risk of carrying the virus. In a study examining blood contact among emergency health care providers, Dr. Ruthanne Marcus and coworkers (Centers for Disease Control, Atlanta) reported that emergency room patient seroprevalence at inner-city hospitals ranged between 4.2% and 8.9%. In comparison, seroprevalence rates at suburban hospitals ranged between 0.4% and 6.4%. Blood contacts were observed in 388 (4%) of the 9,385 emergency procedures examined.

Delivery Room. Dr. Adelisa Panlilio (Centers for Disease Control, Atlanta) and colleagues examined the frequency of blood and body fluid contact during obstetrical procedures in an urban hospital. Although emergency room and surgical personnel are generally considered the groups most at risk of accidental exposure, other health care providers are frequently in high-risk situations, and many of them are less cognizant of the need to adhere to precautions associated with minimizing blood and other fluid contact at all times. Delivery

room personnel represent one such population. In their study of 202 vaginal and 28 cesarean deliveries, Dr. Panlilio's group reported observing 118 inadvertent contacts with blood or amniotic fluid (the fluid that surrounds the fetus). Of these, 112 were classified as skin contacts, 2 as mucous membrane contacts (eyes), and 4 as needlesticks. At least one blood contact was observed in 30% of the vaginal deliveries and in 43% of the cesarean deliveries. Surprisingly, blood or amniotic fluid contact was seen to occur in 9 (43%) of the 21 deliveries in which the mother was known to be either HIV seropositive, a carrier of the hepatitis B virus (HBV), or an intravenous drug user.

Self-Reporting of Accidental Exposures. In an attempt to improve the support and treatment of health care employees accidentally exposed to HIV, several institutions have established self-reporting systems. Dr. Julie Gerberding (San Francisco General Hospital) reported on the first of these programs, a "needlestick hotline" instituted in 1989 at San Francisco General Hospital. Since the hotline was begun, self-reporting of potentially infectious exposures to HIV or hepatitis B virus have increased 184%. In the 10-month period of study, 250 occupational exposures were self-reported; in the same period for the previous year, 136 had been reported. The hotline, which operates 24 hours a day, 7 days a week, offers immediate counselling and advice provided by a physician or nurse practitioner. The employee is immediately offered prophylactic AZT therapy (see "Post-Exposure AZT Prophylaxis," below), consultation with a licensed counsellor, and testing for HIV infection. All information is kept confidential. Data suggests that prior to the hotline, less than 30% of accidental exposures to HIV were reported. The reasons for such a low value include: lack of incentive, inconvenience, no expectation of benefit (such as access to AZT) and fear of lack of confidentiality. In dealing with some of these employee concerns, the hotline and institutional employee post-exposure care appears to have markedly improved the reporting rate.

UNIVERSAL PRECAUTIONS

Prevention of exposure to HIV through infection control practices, such as barrier clothing, has been outlined for health care workers in a set of guidelines referred to as "Universal Blood and Body Substance Precautions" by the U.S. Centers for Disease Control; see Appendix D: UNIVERSAL PRECAUTIONS. These recommendations emphasize that all patients should be treated as potentially infectious regardless of lack of knowledge of their HIV serostatus. Such a practice would greatly limit the possibility of HIV acquisition via exposure to the blood or body fluids of an individual with undetected or undocumented HIV infection. Under these guidelines, all blood, blood products, and body fluids are to be handled as potentially infectious materials.

The guidelines include specific precautionary measures for emergency personnel, dentists, morticians, dialysis personnel, and all persons in clinical or research laboratories. Sterilization and disinfection of equipment and areas, cleaning and decontaminating spills, laundry and housekeeping, and waste management are also addressed in the CDC guidelines.

Preventing Exposures. The most serious risk to the health care worker lies in percutaneous exposure—that is, skin penetrating, primarily needlestick, injuries. Although reasonably effective in limiting the risk of skin and mucous membrane exposures, universal precautions cannot prevent the inadvertent stick with a potentially infectious needle. One possible way to reduce the risk of accidental needlestick is to develop safer needles. Needleless alternatives also reduce injuries; eliminating the use of needles in medical devices is a practical and achievable goal. For example, stopcocks and luer locks are already in use to replace hypodermic needles as connectors for intravenous lines.

Dr. Janine Jagger (University of Virginia, Charlottesville) discussed some of the pros and cons of safer needle devices. The basic requirement of safer needle devices is that they

Table 9.1. Compliance with Universal Precautions

Nurses of all services completed questionnaires regarding procedures performed, the number of times gloves were worn, and, when not wearing gloves, the number of times blood contact occurred. Responses were obtained from 265 nurses before implementation of universal precautions (and in-service education, accompanied by presentation of the first study's results) and were compared with responses obtained from 541 nurses after implementation. Overall, blood contacts have decreased significantly despite only partial compliance with universal precautions. The frequency of needlestick injuries did not change: A total of 6 injuries were reported on the day of survey, before and after implementation, yielding a frequency of 1.06% per nurse per day and 1.10% per nurse per day, respectively. (Based on a presentation by Patrick Francioli and colleagues, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.)

	Before Implementation		After Implementation		
Procedure	Blood Contact per Procedure (%)		Blood Contact per Procedure (%)		% No Gloves
Venous puncture	57/824	(7)	46/797	(6)	82
Blood drawn by IV catheter	35/193	(18)	10/184	(5)	58
Capillary puncture	111/190	(58)	67/212	(32)	70
Hemoculture	8/33	(24)	2/68	(3)	43
IV line insertion	51/157	(33)	43/256	(17)	76
Arterial compression	7/44	(16)	1/92	(1)	51
Dressing change	29/186	(16)	11/208	(5)	83
Resuscitation	4/13	(31)	4/10	(40)	67

rigidly cover the needle after use. Some designs feature a separate accessory, rather than one integrated into the needle/syringe, in an attempt to improve safety, but these are not likely to reduce injuries, Dr. Jagger pointed out. Several designs are currently on the market or soon will be, including syringes with sliding sleeves, telescoping covers, and retractable needles. Cost-effectiveness, however, is not an unimportant consideration. Some new safety needles sell for 2 to 4 times the cost of their conventional, hazardous counterparts, which makes them prohibitive. Perhaps a full 85-90% of all needlestick injuries could be prevented with the use of safer needle designs. Dr. Jagger also pointed out that although several manufacturers have developed or are developing protective needle devices, the majority of manufacturers have not developed safer technology. Aggressive regulation, legislation, and litigation may be the only means by which to force manufacturers to develop safer devices.

Compliance. As a set of voluntary guidelines, universal precautions are only effective to the degree to which they are followed. The inconsistent adoption of universal precautions by health care personnel remains a significant problem: Health care staff have been noted to follow universal precautions consistently only when caring for a patient diagnosed as having AIDS. Rather surprisingly, however, when handling patients who belong to high-risk groups (of unknown serostatus) or those known to be seropositive (but asymptomatic or pre-AIDS), health care workers do not consistently adhere to universal precautions.

In a study examining the rate of compliance, Dr. Patrick Francioli and colleagues (Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland) looked at a group of nurses before and 1 year after the implementation of universal precautions. Although the noncompliance rate for gloving precautions was surprisingly high (for example, 76% of those starting an intravenous line did *not* wear gloves), the results pointed to a significant reduction in the frequency of blood contacts per procedure; see Table 9.1. These results suggest that partial compliance, along with a heightened awareness of the dangers associated with handling blood and blood products, resulted in a significant decline in the occupational contact with potentially infective blood; however, no reduction of needlestick exposures occurred.

In a similar study examining compliance among emergency department personnel, Dr. Scott Campbell (St. Paul-Ramsey Medical Center, St. Paul, Minnesota) and coworkers found significant, but less dramatic, noncompliance with universal precautions at a university-affiliated Level One trauma center. Based on observations of 270 hours (1,018 patient–health care worker interactions), it was found that when appropriate, gloves were worn in 80% of the interactions; goggles, in 19%; gowns, in 12%; and masks in only 2%. Gloves were worn for only 55% of all IV line placements, and in only 44% of all phlebotomy (blood taking) procedures. Needles were discarded as recommended by the precautions 43% of the time, and recapping was observed 53% of the time. Comparison of direct observation with self-reported compliance with universal precautions revealed that self-reporting considerably overestimates compliance with certain recommendations. For example, self-reported rates for masking, goggling, and handwashing were significantly higher than those observed. When questioned, the principal reasons given for noncompliance with the precautions were:

- "insufficient time" (by 68% of respondents)
- "reduced dexterity" (by 61%)
- "patients appear to be at lower risk for HIV/hepatitis B" (by 48%)

EPIDEMIOLOGICAL STUDIES

Worldwide epidemiological studies indicate that the risk of acquiring HIV infection in the workplace is limited to those activities involving exposures to

- blood and body fluids from HIV-infected persons
- blood and body fluids from HIV-infected laboratory animals
- HIV-infected laboratory materials (such as research reagents containing HIV)

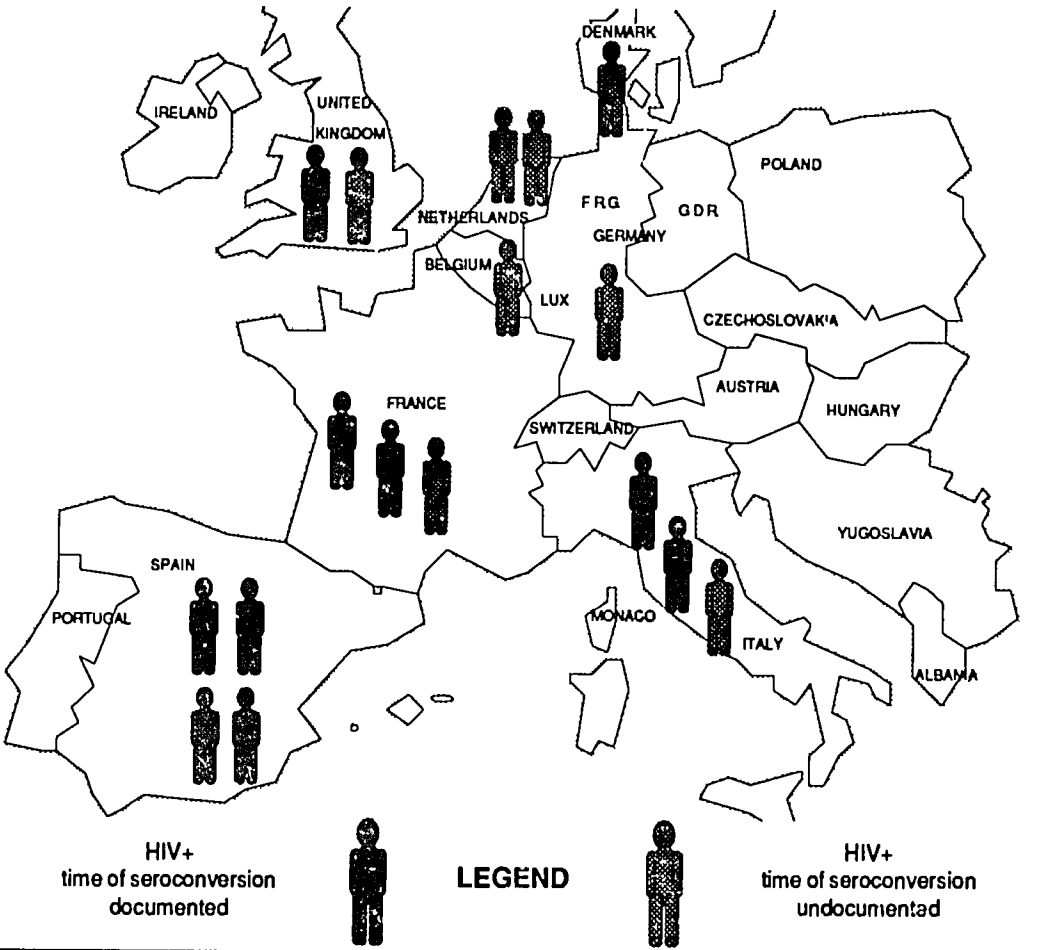
Information and statistics on occupationally acquired HIV infection among health care workers comes from surveillance data (on workers with AIDS or HIV infection reportedly resulting from occupational exposure) and risk assessment data (from prospective studies of exposed health care workers and HIV seroprevalence surveys). Crude risk estimates are made from patient seroprevalence studies, combined with frequency of blood contact by the health care worker and by risk of infection per exposure. The Centers for Disease Control (Atlanta) has estimated the risk of seroconversion (that is, of HIV transmission) following an occupational exposure to HIV-infected blood or other body fluid to be in the range of 0.1% (1 in 1,000 exposures) to 0.4% (1 in 250 exposures).

According to CDC (Atlanta), as of Conference time, 34 cases of occupationally acquired HIV infection were reported among health care workers in the United States and 54 worldwide. (Currently under investigation are an additional 23 cases in the United States and 4 in Europe.) No case of AIDS or HIV infection has been reported as resulting from casual contact with an HIV-infected patient or patient with AIDS, and no case of HIV infection or AIDS due to occupational exposure has been reported in settings other than the health care industry. Although the frequency of inadvertent exposure to blood or body fluids from HIV-infected persons is alarmingly high in the health care setting, the number of seroconversions resulting from such exposure is relatively low.

Seroconversion Data. A number of ongoing seroepidemiological studies have been following health care workers accidentally exposed to potentially infectious sources. (Exposure to *potentially* infectious blood or body fluids only means that the patient was known to be HIV-infected, not that HIV was known to be transmitted.) The major focus of these seroepidemiological studies is to determine the probability of seroconversion after occupational exposure to the virus. Most of the studies that examine occupationally exposed health care workers follow these individuals for periods up to a year after accidental exposure to HIV. Blood samples are usually taken daily for the first 15 days after exposure, then at 3, 6, 12,

Figure 9.1. European Cases of Occupational HIV Infection

Reports from 18 countries in Europe reveal a total of 18 cases of occupationally acquired HIV infection. (*Editors' note:* Only 17 cases were specified by country.) In 7 cases, seroconversion has been documented following the exposure. In the remaining cases, the health care workers were seropositive on inclusion in the study. (Based on a presentation by Dr. Giuseppe Ippolito, Lazzaro Spallanzani Hospital, Rome.)



and 18 months. Such a follow-up ("window") period is used based on other modes of HIV transmission (by blood transfusion or sexual contact, for example), in which the period between exposure and the development of detectable antibodies to HIV (seroconversion) is about 2 to 4 months—although longer periods have been reported. However, some debate whether occupational exposure to the virus results in a longer time between exposure and seroconversion. To examine this possibility, Dr. Cary Littell and colleagues (University of California San Francisco and San Francisco General Hospital) studied health care workers occupationally exposed to HIV for periods of up to 3 years, looking for delayed seroconversions (as identified by positive p24 tests, ELISA/Western blot, virus culture, or polymerase chain reaction, or PCR, tests). In their study population, 3 possible delayed seroconversions (out of 237 workers followed for 1 to 2 years) were identified; no delayed seroconversion was observed in 62 workers followed for 3 or more years. These 3 cases were PCR-positive and are awaiting repeat PCR; other tests for virus, however, have been negative: p24, ELISA/Western blot, and virus culture. David Bell (CDC, Atlanta) concluded that based on these findings and those from other studies involving several hundred health care workers (in which no delayed seroconversions have been documented after up to 2 years follow-up), it appears that seroconversion beyond 6 months in health care workers occupationally exposed is uncommon.

In a Needlestick Surveillance Group study reported by Dr. Jerome Tokars (CDC, Atlanta) and coworkers, which has been following 1,351 health care workers exposed from 1983 through April 1990, a total of 4 individuals have seroconverted. The 4 individuals were exposed percutaneously, via a needlestick or other puncture wound. The overall infection rate among all exposed health care workers (including skin and mucous membrane exposures) was 0.3 per 100 workers. In essence, this means that 1 in approximately every 300 health care providers exposed to HIV actually became infected.

A similar study of 1,300 health care workers followed since 1933, conducted by the National Institutes of Health (Bethesda, Maryland) and reported by Dr. Susan Beekmann and colleagues, found only a single seroconversion among a group of 525 health care workers inadvertently exposed to HIV. This individual was 1 of 179 parenterally exposed to the virus. As was noted in the previously cited study, no mucous membrane exposures (of 346 total) resulted in HIV infection. Dr. Beekmann's group is now using polymerase chain reaction (PCR, a highly sensitive means of detecting HIV DNA) to check for false negatives in this exposed population (see Chapter 4: DIAGNOSTIC TESTS FOR HIV).

The results of a large collaborative European study on HIV infection in health care workers was reported by Dr. Giuseppe Ippolito (Lazzaro Spallanzani Hospital, Rome). From 1984 to 1990 in Europe, 18 documented cases of occupational HIV infection have been reported. In 7 of these cases, seroconversion was seen soon after HIV exposure. In the remaining cases, the patients were seropositive at the time of inclusion in the study and had a history of occupational exposure. Of the 18 cases, 10 (55%) were nurses; 4 (22%), surgeons and; 2 (11%), physicians. To estimate the risk of seroconversion among occupationally exposed health care employees, Dr. Ippolito pooled the data from 13 longitudinal studies conducted in 9 European countries; see Figure 9.1. Of 2,781 health care workers who had been followed for a mean time longer than 9 months, 4 (0.14%) have seroconverted. In Italy, where the most data has been collected on European health care providers, 2 seroconversions were seen among 1,154 individuals, for a seroconversion rate (0.17%) very close to that observed for all of Europe.

Esperanca Santos Abreu (Emilio Ribas Hospital, São Paulo, Brazil) and colleagues reported on 84 health care workers (from a single hospital in Brazil) who self-reported parenteral or mucous membrane exposure to infected blood or body fluids between August 1985 and January 1990. Of those followed for at least 3 months, none were found to be infected. Although a total of 217 health care workers were identified as having had inadvertent exposures, 108 had less than 3 months of follow-up (most subjects were lost to follow-up), and 25 refused to participate in the study. There were 298 total reported incidents of exposure, some health care workers had multiple exposures:

- 134 needlestick or other puncture wound
- 73 mucous membrane or non-intact skin exposed to blood
- 91 mucous membrane or non-intact skin exposed to other body fluid

Together, these data suggest that the chances of seroconversion after occupational exposure to HIV is relatively low, ranging from 1 in 300 to 1 in 600. Nonetheless, the risk of transmission is far from negligible, and all health care employees must be trained in risk reduction and appropriate precautions must be taken in order to minimize their contact with potentially infective blood and blood products.

Behavioral or Occupational Exposure? One of the difficulties in determining the actual risks of occupational exposure is that any occupationally exposed individual may also have engaged in high risk behaviors, which must be taken into account. As David Bell (CDC, Atlanta) pointed out, of all health care workers with AIDS in the U.S., 94% of them have non-occupational risk factors; in only 3 cases has development of AIDS following exposure to HIV-infected blood been documented. Dr. Mary Chamberland (CDC, Atlanta) and colleagues looked at a group of blood

donors who had proved seropositive to determine the rate of occupational HIV acquisition. Of the 513 (out of 849) thus far interviewed, 22 were identified as health care workers. Ten of the 22 acknowledged HIV risk behaviors, leaving 12 individuals with possible occupational transmission. Follow-up studies are underway to establish whether infection was behaviorally or occupationally acquired in these 12 health care workers.

POST-EXPOSURE AZT PROPHYLAXIS

Recent work using animal models suggests that treatment with the antiviral agent AZT soon after HIV exposure (24-48 hours) can prevent HIV infection at least some of the time (as demonstrated by lack of subsequent seroconversion). On the basis of these results, a number of health care facilities have instituted AZT prophylaxis for employees accidentally exposed to HIV.

Dr. Stanley Bauer (Beth Israel Medical Center, New York) and colleagues reported on the development of such a protocol. A 72-hour supply of AZT is kept on hand at high-risk locations in the hospital, such as the emergency room, operating room, and nursing stations. All health care workers are counselled on the use of AZT and instructed to take 400 mg immediately after parenteral exposure to blood or body fluids and 200 mg every 4 hours for the next 72 hours. Within this 72-hour time frame, assessment of the magnitude of exposure and risk is based on laboratory tests and patient diagnosis. Based on the findings, the employee is counselled as to whether to continue prophylaxis for the next 6 weeks. While on AZT the health care worker is monitored for possible toxic effects and followed closely for the next year.

Although it is too soon to determine whether AZT is effective when given prophylactically, ongoing studies are accumulating valuable data on the frequency of use of AZT after occupational exposure, and the toxicity associated with such use. Dr. Jerome Tokars and colleagues (Centers for Disease Control, Atlanta) reported on their study examining post-exposure AZT use in health care workers enrolled in a CDC surveillance project. Between October 1988 and April 1990, 348 exposed health care workers were enrolled and, of these, 41 (12%) received AZT soon after HIV exposure. If broken down quarterly, the data show that by the latest quarter examined (Jan-Mar 1990), more than 29% of all exposed health care workers were receiving AZT. Of the individuals receiving AZT who were followed up for at least 6 weeks, over half (58%) exhibited reversible, short-term toxic effects. No seroconversions have been identified among 14 health care workers taking AZT and followed for at least 6 months.

Several studies confirm the increasing prophylactic use of AZT among occupationally exposed health care workers. As reported by Dr. Julie Gerberding (University of California San Francisco and San Francisco General Hospital), 45 individuals reported parenteral exposure to HIV at San Francisco General Hospital in a 10-month period between mid-1989 and early 1990. More than half (23) of these persons elected to take AZT prophylaxis (1,000 mg/day for 4 weeks); no severe toxicities were reported. Similarly, Dr. Susan Beekmann and coworkers (National Institutes of Health, Bethesda) reported that in their study of 23 health care workers inadvertently exposed to HIV since April 1988, 17 opted for the prophylactic use of AZT. Of these 17 individuals, 4 failed to complete the recommended 42-day treatment regime due to AZT intolerance.

Similar studies are currently in progress in Europe. Dr. Vincenzo Puro (Lazzaro Spallanzani Hospital, Rome) and colleagues reported results of a study conducted by the Italian Study Group on Occupational Risk of HIV Exposure. To date, 48 of 1,154 occupationally exposed health care workers have been enrolled in a study on the prophylactic use of AZT; 2 have stopped therapy due to adverse effects. After a mean follow-up period of 7 months, none of these individuals have seroconverted. Examination of toxicity data, however, revealed a

dose-related toxicity associated with AZT use: 15 of 36 of health care workers on a daily dose of 1000–1250 mg AZT showed adverse effects. Overall, 33% of those receiving post-exposure AZT at dosages ranging from 500–1250 mg per day showed adverse effects.

Dr. Giuseppe Ippolito (Lazzaro Spallanzani Hospital, Rome) related that two cases of failure of AZT prophylaxis in health care workers had been recently reported, one occurring in the Netherlands after an inadvertent inoculation with a high concentration of virus. Dr. Ippolito offered that AZT failure may be due to an inadequate dose or to reduced virus sensitivity to AZT. Continued evaluation of post-exposure use of AZT is needed to demonstrate the efficacy of prescribed prophylactic regimens and assess the relative toxicities in cases of inadvertent exposures.

RISK TO PATIENTS

Evidence for the transmission of HIV from infected health care workers to patients has, until this year, been lacking. In August 1990, the Centers for Disease Control (Atlanta) announced that HIV transmission from a dentist with AIDS (who has since died) to his patient during a tooth extraction may have occurred. The CDC's announcement was based on DNA analysis of the HIV strains isolated from serum samples of the dentist and his patient: the similarity between the two strains was close enough to suggest transmission. (Sexual transmission was ruled out.) Neither the dentist nor the patient recalled visible blood contact during the operation. Some researchers, however, opine that at this point in the investigation, transmission cannot be unequivocally demonstrated. Two other patients of this dentist have been found to be HIV-infected; the CDC reported that neither of these patients belongs to a high-risk category and, according to preliminary analysis, the HIV strains isolated from the dentist and from these patients are relatively close. An investigation is being conducted regarding the route of transmission; evidence of insufficient sterilization of the dentist's instruments is being examined. The American Dental Association has recently issued a policy recommending that HIV-infected dentists disclose their HIV status to patients or stop performing oral surgery or other invasive procedures.

Previous studies following patients of known HIV-infected physicians (surgeons) in the United States and United Kingdom have failed to demonstrate health care worker-to-patient transmission. At the Conference, Dr. Ban Mishu (CDC, Atlanta) and colleagues reported that no patients who were operated on by a surgeon diagnosed with AIDS in 1988 were found to be HIV-positive (except one patient who was a member of a high-risk group). Dr. Mishu's team identified 2,160 patients on whom the surgeon had operated in the 7 years prior to his diagnosis; 264 had already died of causes unrelated to HIV. The names of the remaining patients were compared with the AIDS registry and no matches were found. Of the 1,896 living patients, 1,652 were traced, contacted, and offered free HIV antibody testing; 616 (37%) have accepted testing and none have been found to be infected.

Although a singular finding, the aforementioned case of HIV transmission from the infected dentist to his patient has raised the public's concern for the safety of patients of HIV-infected health care workers—notably, doctors and dentists. The case has brought to the public's consciousness the ethical question of whether doctors and dentists have a duty to inform their patients of their seropositivity. (The dentist, who was diagnosed with AIDS 3 months before the tooth extraction procedure, did not inform his patient that he was infected.) See "Ethical & Legal Considerations," below.

ATTITUDES, BELIEFS, & PRACTICES

Surveys among health care personnel reveal that health care practices and clinical decision making are strongly influenced by attitudes and beliefs or perceptions. Nancy Kass (George Washington University, Washington, D.C.) reported that denial of health care to

homosexual men because of seropositivity or diagnosis of AIDS is not an insignificant problem. Furthermore, although to a lesser extent, seronegative homosexual men are also denied medical care. Based on data collected from the Baltimore and Los Angeles MACS (a prospective, multicenter study of homosexual men), more homosexual men with AIDS were refused treatment than seropositive homosexuals.

Basis for Refusal to Treat	Profession	HIV-negative (Number=852)	HIV-positive (Number=585)	AIDS (Number=96)
Homosexuality	Doctor	2%	4%	3%
Homosexuality	Dentist	1%	3%	4%
HIV seropositivity	Doctor	1%	3%	5%
HIV seropositivity	Dentist	1%	4%	16%
HIV seropositivity	Doctor or dentist	1%	5%	18%

Survey results from other Conference presentations agree with those of Ms. Kass's findings: a disturbing number of physicians and health care professionals were found to hold negative attitudes toward members of the major HIV-risk groups. Other surveys, including those of medical and dental students and nurses, demonstrate wide variances in attitudes and beliefs—and consequently, care practices. Some are very willing to treat HIV-positive persons; some report a desire for more knowledge to be more effective, confident care givers; and some acknowledge fear of HIV transmission from patient contact.

PHYSICIANS

As the HIV epidemic lengthens, more and more doctors will be seeing HIV-infected patients. This means that more and more doctors are now facing the question of whether they want to or intend to treat patients infected with HIV or patients with AIDS. Not surprisingly, as a group, physicians hold a wide range of attitudes and beliefs concerning HIV and AIDS, which directly influence how they deal with (or intend to deal with) HIV-infected patients. (See "Ethical & Legal Issues," below, for a discussion of physicians' obligation to treat.)

Several Conference reports noted that the *perceived* risk of occupational transmission is much higher than is currently accepted as accurate—based on worldwide data, the CDC estimation of risk of HIV transmission following an occupational exposure is 0.1 (1 in 1,000) to 0.4% (1 in 250). Denise Nadeau and colleagues at Centre Hospitalier de l'Universite Laval (Quebec), for example found that among physicians surveyed in the Province of Quebec, 12.3% of respondents (100 of 815) perceived the risk of HIV infection to be very high in their medical practices; in fact, the risk was overestimated by 10-fold over the CDC's 0.4% estimate. Comparable survey results from other reports indicating poorly informed physicians (on the issues of risk of transmission due to occupational exposure) and/or distrust of AIDS information underscore the need to explore strategies to better educate and change attitudes—and practices—among doctors.

Another survey of 200 house staff and staff physicians in the departments of medicine, pediatrics, and surgery revealed similar findings—that many physicians have misconceptions concerning the occupational risk associated with HIV. In a study of physician beliefs, Dr. R. F. Nease, Jr. (Decision Focus Inc., Los Altos, California) and colleagues found that 24% of those surveyed believed the risk of seroconversion following needlestick to be 10 times greater than the actual value. In addition, although certainly a factor, it was found that the physicians' perception of risk was not based solely on the chance of seroconversion. A significant factor in their assessment of risk was the morbidity and mortality associated with HIV infection.

In an effort to identify differences between physicians who treat AIDS patients and those who do not, Dr. Molly Cooke and colleagues (University of California, San Francisco) surveyed 1,045 medical residents throughout the United States. By pinpointing these differences, Dr. Cooke's team hoped to uncover ways of increasing the willingness of doctors to deliver care to HIV-infected patients. The study found that given a specified (hypothetical) seroconversion risk of 1 in 100 for physicians, 64% of the survey group would not provide life-saving care to a person with AIDS. Even if the risk were 1 in 100,000, 5% of those surveyed would withhold treatment.

In addition, 64% of the residents indicated that they would fashion their future medical practices to accommodate the care of HIV-infected patients, but only 17% had summary scores indicative of a strong intent to provide care to HIV-infected patients in the future. One of the primary factors contributing to a physician's unwillingness to treat HIV-infected individuals was found to be prejudicial attitudes toward homosexuals and/or intravenous drug users. Conversely, those physicians expressing a willingness to treat individuals with AIDS were found to possess a strong sense of professional responsibility toward the HIV-infected community. Pointing out that fear of contagion was not the factor that distinguished residents who intend to treat and those who do not, Dr. Cooke presented three factors that, in this study, accounted for more than half of the variance between the two groups of residents;

Residents who intended to care for HIV-infected patients

- are less negative in their attitudes toward members of the predominant risk groups
- are more comfortable with the demands of AIDS medicine
- have a stronger sense of professional responsibility

These factors, Dr. Cooke said, are modifiable and therefore should be addressed at this point so that problems of access to care for HIV-infected people will not be compounded in the future.

Intimately tied into a physician's attitudes and beliefs is the manner in which they deal with their patients. Since HIV can be classified as a sexually transmitted disease (STD), one of the important aspects of health care promotion is the complete and accurate documentation of a patient's sexual history. However, many physicians, as a result of their attitudes and beliefs, find it difficult to obtain a sexual history from a patient, particularly if that patient is homosexual. Such a practice can serve as a significant impediment in the prevention and diagnosis of HIV-related disease. In examining the sexual history-taking practices among a group of primary care physicians, Drs. Kathleen Montgomery and Charles Lewis (University of California, Los Angeles) found that in 1989, only 42% of those physicians interviewed asked about a patient's sexual orientation. Only 30% inquired as to the number of sexual partners. Nonetheless, such numbers are a marked improvement over 5 years ago, when only 23% of the physicians asked about sexual orientation and 10% about number of partners. Even with such improvements, more than two-thirds of those physicians interviewed believed their colleagues suffer significant distress in discussing sex with homosexual patients.

Leonard Calabrese and colleague (Cleveland Clinic Foundation, Ohio) reported similar findings. In their questionnaire-based study of 301 physicians in northeast Ohio (administered 1987–89), only 41% of the physicians routinely obtained risk assessment histories or histories of sexual function and orientation in new patients. The authors pointed out that this is in contrast to the nearly 90% rate in obtaining histories of smoking or alcohol use in the same patients. Additionally, it was found that the physicians most likely to obtain sexual histories were those with continuing education or post-graduate courses concerned with AIDS. Such a result highlights the importance of education, even within the physician community, in bettering AIDS prevention and care.

A singular report on knowledge, attitudes, and beliefs of minority physicians in the United States, conducted by the National Medical Association (Washington, D.C.), was given by Virginia Caine and coworkers. They reported that, based on a survey of 118 black physicians, knowledge of HIV transmission was generally good, but only 65% felt knowledgeable about counselling a patient with a positive antibody test. In addition, 23% had moderate-to-considerable discomfort discussing oral and anal sexual activity with their patients.

MEDICAL STUDENTS

Without a cure on the horizon, HIV infection and AIDS represents one of the major problems for the new generation of health professionals. The attitudes of these new care providers will have an enormous impact on the way in which the epidemic is handled. With the steady increase in the number of new AIDS and other HIV-related cases, today's medical students (especially those who will practice in an urban setting) are more likely than not to be called on to treat an HIV-infected patient.

What are the attitudes of medical students toward individuals with AIDS, and more importantly, do these students want to treat the HIV-infected? To answer these questions, Drs. Arthur Culbert and L. Strunin (Boston University Schools of Medicine and Public Health) administered an anonymous questionnaire to a number of first- and second-year medical students at the four Massachusetts medical schools. More than 25% of these students wanted to be allowed to refuse to treat persons with HIV infection or AIDS; their primary concern was fear of becoming infected. These individuals also stated that the epidemic will influence their choice of speciality and final choice of a hospital for residency. In contrast, knowing someone with AIDS was a significant predictor of a student's willingness to treat an HIV-infected person. On the basis of these findings, it was suggested that early association with HIV-infected individuals by medical students may help ameliorate the problem of physicians' unwillingness to treat the HIV-infected.

Other studies have found unwillingness to treat an HIV-infected person among medical students to be a relatively rare occurrence. In a study of medical students at Johns Hopkins University, Drs. Keith Loring and Gabor Kelen (Johns Hopkins University, Baltimore) reported that 94% of the students responding to their survey would not hesitate to care for an AIDS patient. Additionally, HIV infection and AIDS appears to have had little impact on speciality choice among these students, with only 14% saying that they would avoid a speciality dealing with a large number of HIV-infected persons. As in past years, internal medicine (42%) and surgery (31%) were the most popular specialities.

DENTISTS

Another group of health professionals who routinely deal with HIV-infected individuals are dentists. As with physicians, dentists as a group hold a wide range of attitudes, beliefs, and practices concerning the care of HIV-infected persons. To study some of these issues, Drs. Diana Shin and A.Y. Wong (University of California, Los Angeles) questioned nearly 300 dental faculty and students in an area of high HIV prevalence as to their infection control practices, risk assessment, and willingness to treat HIV-infected patients. The survey revealed an inconsistent use of suggested infection-control practices (such as use of gloves and masks) among faculty and students. The perceived risk of contracting HIV from an infected patient was often overestimated: the highest perceived risk was by pre-clinical students, while faculty showed the lowest perceived risk. A substantial unwillingness to treat HIV-infected persons was uncovered; rather surprisingly knowledge levels did not have a statistical correlation with willingness to treat. Higher willingness-to-treat scores and lower perceived risk were associated with those who reported having treated HIV-infected patients.

James Cottone and colleague (University of Texas Health Science Center, San Antonio) reported findings from a survey of 296 dental students, 49 faculty, 63 dental staff members, and 125 participants at a dental conference. (The anonymous survey was administered following an AIDS education session at a dental conference.) The majority of dental students and conference participants felt that 1–5% of their patients were at risk for HIV (the authors calculated the risk at an estimated 11–20%). Roughly 33% of dental students, 18% of faculty and conference participants, and 4% of staff felt that no patients were at risk. These results suggest that less than 10% of those surveyed had a true perception of the patient population at risk for HIV infection. About 30% of respondents reported no concern about HIV infection, while 25% reported that they were very concerned about the risk.

When surveyed about being tested for HIV, 18% of the dental students reported being tested, as did 13% of the staff, 23% of the faculty (of whom 4% reported being HIV-positive), and 33% of the conference participants (of whom 7% reported being HIV-positive). Not all tested had received their test results at time of survey. Based on the survey answers obtained, the authors concluded that continued education efforts are warranted.

NURSES

To examine whether AIDS care practices and knowledge and attitudes about AIDS differ between nurses at high AIDS experience hospitals or facilities and those at low AIDS experience facilities, Donna Anderson (University of Colorado Health Sciences Center, Denver) and associates surveyed 136 rural and 311 urban nurses registered in Colorado. Differences on knowledge, attitudes, and reported practices were found to exist between the two groups. Not unexpectedly, it was noted that these differences diminished when looking only at survey answers from nurses with equal number of times caring for an AIDS patient. The authors suggested that clinical mini-sabbaticals be instituted to provide an opportunity for nurses in rural areas to spend time with AIDS patients.

Vuyelwa Ndiki Ngcongco (World Health Organization, Regional Office for Africa, Congo) reported that the situation for AIDS nursing care in Africa is, to a large extent, dictated by material/monetary inadequacies. Based on a survey of nursing and midwifery personnel in 39 countries in Africa (conducted between October 1989 and January 1990), all 39 countries cited supplies as inhibiting factors in the provision of effective nursing care to AIDS patients and families. Although self-reported as willing and committed to caring for AIDS patients, 98% of respondents stated that they were not professionally and technically equipped with knowledge or skill to provide effective nursing and health care to HIV/AIDS patients and families. (Based on focus-group discussions, interviews, and on-site observations, WHO-coordinated efforts are being made to develop nursing standards and patient care protocols to guide the provision of nursing care appropriate to HIV/AIDS patients.)

FIRST-RESPONSE PERSONNEL

Janine Patrone-Reese and colleagues (University of Miami, Florida) reported on results from a voluntary, anonymous, self-administered questionnaire given before and after a peer educational program on AIDS: Of 203 emergency medical technicians, paramedics, and firefighters who completed the survey, 122 (60%) perceived themselves to be at high risk for acquiring HIV infection through job-related activities. Regarding practices, 90% reported that they changed job-related behavior according to CDC infection control recommendations—as compared with 80% prior to the education program. Negative attitudes toward AIDS patients and worry about contracting HIV from patients did not change following the program.

EDUCATION IN THE WORKPLACE

Education in the workplace varies in content depending on the type of workplace: Health care workers, for example, receive very different instruction than most other categories of workers. The goals of the education programs are also quite different: In the health care industry (and in occupations where there is a potential risk of HIV transmission), education is aimed at preventing or minimizing inadvertent exposures to potentially infectious blood and body fluids (or other substances); in most other workplace settings, general information on HIV infection is disseminated with the objective of dispelling myths and fears.

EDUCATING & TRAINING THE HEALTH CARE WORKER

Education programs for health care personnel focus on instructing workers how to avoid inadvertent exposure in the workplace as well as emphasizing the need for rigorous adherence to universal precautions. Ongoing education programs geared toward increasing the health care worker's AIDS knowledge are necessary for maintaining high levels of knowledge. Evelyn McBride and associates (Kingston General Hospital, Ontario) used data from their AIDS knowledge surveys to 540 non-nursing health care workers (one administered immediately following an AIDS education program and another, 2 months later) to demonstrate that AIDS knowledge scores drop significantly as time from the program increases. Continuous programs rather than one-time efforts are therefore recommended.

Training community health center staff to collect behavioral data from clients at risk for HIV infection is also being pursued. Isabel Fernandez (Centers for Disease Control, Atlanta) and coworkers presented a training program model that would eliminate the costly utilization of outside researchers to collect reliable and valid behavioral data from clients enrolled in HIV-risk reduction programs. Two-day training sessions targeted persuasion, skill acquisition, testing, and quality assurance. Preliminary evidence, based on observing data collectors and assessing their reliability suggest that on-site program staff can reliably collect behavioral data.

Professional organizations for health care workers are also active in providing education and support to their members. The Association of Nurses in AIDS Care and the Physicians Association for AIDS Care, for example, seek to promote professional development in AIDS care practice and HIV/AIDS education.

Teaching Methods and Strategies. Innovative strategies for educating health care personnel are continually being explored in efforts to achieve or maintain high-level understanding of all aspects of HIV disease. Ann Ogden and colleagues (Southern Illinois University School of Medicine, Springfield) have incorporated AIDS patients or HIV-infected patients into education programs for health care professionals who, because of the low AIDS incidence in the area in which they reside, may not yet have cared for HIV-infected patients. Based on survey answers from program participants, which strongly supported inclusion of patients in education programs, the authors recommended this educational strategy for other audiences with little prior direct experience with AIDS.

In response to a demand from nurse educators, clinical specialists, and staff nurses for a comprehensive nursing care manual, Joan O'Brien and associates (Professional Standards Review Council of America, Inc., New York) reported that they have developed a usable, easily updatable manual of nursing care standards that reflects the minimal acceptable level of care for patients with HIV infection. The nursing care component of this criteria manual details standards, describes revising care plans, and the anticipated patient outcomes for each of the involved body systems.

Elaine Gross (University of Medicine & Dentistry, New Jersey and Children's Hospital of New Jersey, Newark) and colleagues reported that interactive teaching methods (adult edu-

cation with goal setting, games, exercises, and group work) was more successful than didactic methods (lectures/case studies) in increasing HIV/AIDS knowledge of perinatal and pediatric nurses. Either teaching method was randomly assigned to 275 nurses from 6 tertiary care hospitals in New Jersey attending a one-day workshop on pediatric HIV infection. The two groups were compared using pre- and post-test scores: overall higher scores were found among the "interactive group."

HIV-prevention training programs for health care providers seek to increase the knowledge and skills necessary for educating and counselling clients about HIV-risk reduction practices. Interactive programs with physicians, nurses, social workers, psychologists, and substance abuse counsellors have been used to train U.S. Department of Veterans Affairs staff to promote HIV prevention among their patients and also to educate and provide counselling for them. Pamela Herbert and coworkers (Department of Veterans Affairs, Washington, D.C.) reported that more than 600 hospital staff members—from inpatient and outpatient settings—across the country have undergone training since 1988. (The Department of Veterans Affairs oversees a national health care system of 172 hospitals and associated outpatient clinics.)

Train-the-Trainer Programs. Numerous programs exist for the training of health care personnel who will act as HIV/AIDS educators to train different levels of health care workers at their own facilities—including physicians, nurses, clinical laboratory personnel, and housekeeping staff. These trainers may also serve as on-site AIDS information officers. Richard Durnan and colleagues (Department of Veterans Affairs, Washington, D.C.) reported that evaluations of train-the-trainer programs should be conducted to identify program weaknesses and strengths since training needs will likely increase as the HIV epidemic lengthens. Appropriate modifications can be made based on assessment of training frequency, effectiveness, trainer support, staff participation, adaptability and usefulness of educational strategies and materials, and obstacles to training.

Stress Reduction Programs. Numerous HIV/AIDS-dedicated organizations, as well as traditional care centers, which provide services to HIV-infected persons, have cited "burnout," or stress as an impediment to effective care giving among health care workers. The growing number of reports of burnout indicate that most health care workers including social workers find their work stressful. Mari Rogers and coworkers (Denver Disease Control, Colorado) studied 134 health care workers serving HIV-infected clients; their findings suggest that specific stressors can be identified and interventions can be implemented to decrease workplace stress. Survey responses regarding individual stressors, such as management style or difficulties in HIV-care delivery, were examined in terms of perceived level of distress and interference with job tasks. Ms. Rogers' team found that after identifying specific high-level stressors, targeted intervention was able to significantly reduce stress.

Jane Healy-Chidekel and colleagues (University of Miami School of Medicine, Florida) reported that their stress reduction program for the nursing staff of an AIDS unit has favorably influenced staff retention and workplace burnout. Although burnout has been associated with high employee turnover rates, Ms. Healy-Chidekel's team found that of 24 nurses in an AIDS unit participating since January 1988 in a stress reduction program, 20 (83%) have continued employment; 2 nurses resigned because of relocation for personal reasons, 1 transferred for a promotion, and 1 transferred for personal reasons. The authors also reported that an overall improvement in the working environment was noted.

EDUCATION IN BUSINESS & INDUSTRY

Workplace educational strategies need to identify the best way to reach employees. Strategies may vary according to geographic location of the workplace and the nature of the business or industry. In Africa (where the primary route of HIV transmission is hetero-

sexual contact and approximately 80% of all AIDS cases occurs among sexually active members of the general population, aged 15–55), the size of the infected work-force population is not insignificant. Ntambanzondo Mposo from Projet SIDA, Kinshasa, Zaire, reported that HIV prevalence among workers at a large service corporation is already high: 6.3% of employees are infected. The information content in educational programs, then, must reflect regional differences in prevalence of HIV transmission modes and specific prevention measures.

The nature of the business may also require that educational strategies be tailored to the audience. "Generic" workplace educational programs may be all that is necessary—and quite useful—for most businesses, but inappropriate for law enforcement personnel. Educational programs for law enforcement, other first-response occupations, and corrections systems personnel need to address issues not usually encountered in other types of businesses. (Attitudes and behaviors toward HIV-infected individuals, for example, may directly affect the handling of incidents involving blood and/or body secretions.)

In attempting to evaluate existing strategies for effecting the greatest change in knowledge, attitudes, and behavior among employees, a number of groups found that AIDS workplace education is sorely lacking in most companies. Steven Humes and coworkers (Gay Men's Health Crisis, New York), in collaboration with New York Business Group on Health, Inc., reported that surveys sent to 2,637 human resources administrators of New York metropolitan firms and nonprofit agencies reveal that, on the whole, employers there have made little effort to implement employee AIDS education programs; AIDS information is generally supplied only in response to crises. In trying to assess the AIDS issue among employees needing to be addressed the most, many company executives pointed to the continued fear of HIV transmission in the workplace.

Train-the-Trainer Programs. A presentation by Moreira Lima (Ministry of Health, Brazil) and colleagues gave evidence of the widespread lack of AIDS information being provided to employees. Among Brazilian companies with 100 or more employees, 10,000 employees were randomly selected and sent questionnaires. Almost 7,000 responses revealed that AIDS educational materials are completely lacking in the majority of companies. Based on these findings, the STD/AIDS Division of Ministry of Health has formulated educational materials, specifically tailored to companies, to be used in a train-the-trainer program. From such a program, regional tutors and "multipliers" will in turn train company tutors and multipliers so that a trickle-down effect will guarantee that all workers receive AIDS information. Francis Rwakagiri (Federation of Uganda Employers) outlined his organization's AIDS education and prevention program for the workplace. A train-the-trainer program, in which staff from selected companies are designated to instruct company AIDS education trainers, constitute the first stage in the Federation of Uganda Employers' initiative. Information and materials (such as posters and condoms) are distributed by peer educators (multipliers) within a given company.

Targeting Knowledge and Attitudes. Workplace education programs may influence attitudes as well as increasing HIV/AIDS knowledge. Edward Cowen (Mid-Atlantic AIDS Education & Training Center, Washington, D.C.) reported on survey results from more than 700 federal and privately-employed workers in the Washington D.C. area who attended a 2-hour "AIDS in the Workplace" workshop: significant changes in attitudes toward persons with AIDS and male homosexuals were noted. Mr. Cowen and his associates compared answers to pre- and post-intervention questionnaires: While scores showed increases in knowledge and more positive and compassionate attitudes toward persons with AIDS and male homosexuals, male respondents had the most dramatic change; attitudes of female respondents remained at about the same level of compassion, pre- and post-intervention. The following questionnaire statements revealed the greatest degree of change:

- "Persons with AIDS should be quarantined for the protection of society" (29% of participants agreed with this statement before attending the workshop; 7%, afterward)
- "I feel more negatively about homosexuals since AIDS" (31% of participants agreed with this statement pre-intervention; 16%, post-intervention)

Similar findings were reported by John Leonard and colleagues (Seattle-King County Department of Public Health): Knowledge scores increased from 76% correct to 90% correct following attendance at a 2-hour "AIDS 101" intervention program. Attitude scores among the 162 municipal employees tested also showed improvements but to a lesser extent. The finding of relatively high knowledge scores and persistent negative attitudes suggests that educational strategies should place greater emphasis on effecting attitudinal changes.

ETHICAL AND LEGAL CONSIDERATIONS

HEALTH CARE SETTING

Occupational exposure to potentially infectious materials, including blood and body fluids of persons with HIV infection or AIDS, has raised serious ethical and legal concerns. Two particularly controversial issues are the confidentiality of patients' HIV test results and the health care professional's duty to care for HIV-infected patients and patients with AIDS. Recently, there has been much discussion and debate regarding the *patient's* right to know the HIV status of their health care providers.

Patients' Right to Confidentiality. Within the context of patients' rights in general, is the specific issue of whether the patient's right to privacy supersedes the desire of the health care team to known the patient's serostatus. Some health care workers argue that specific procedures and maneuvers can be modified to minimize potential exposures to body fluids if knowledge of HIV positivity is available. Others counter that, especially in high-risk areas, it is wisest to proceed as though each patient is HIV infected. Clearly, any approach that factors in the safety of the health care team is rational—as long as it does not increase the risk to the patient. The possibility that knowledge of a positive HIV test result could be prejudicial to the patient is often cited as sufficient reason for keeping patient serostatus confidential. While mandatory testing of all hospital patients has been deemed inappropriate, health care workers at elevated risk of occupational exposure call for selective testing.

Duty to Treat. Dr. Laurence McCullough (Baylor College of Medicine, Houston) outlined the arguments relating to the ethical obligation of health professionals to treat HIV-infected patients. Those arguing that health care professionals have the absolute obligation to risk their own health and lives in treating HIV-infected patients cite various reasons, including the altruistic and virtuous nature of the professions. However, as Dr. McCullough pointed out, such views are untenable; the more realistic view is that one has a duty to assume personal risk when the level of risk is reasonable, but not when it is unreasonable.

Defining "reasonable risk" then becomes the crux of the matter. From the currently available data, the consensus is that most health care workers are exposed to only minimal (reasonable) levels of risk and more-than-minimal (unreasonable) levels are rare. Dr. McCullough offered that if a procedure or setting is deemed to involve unreasonable risk, then volunteers should be sought and fully indemnified against health care costs, disability, and loss of lifetime earnings should they become occupationally infected by HIV.

While Dr. McCullough's lecture was based on philosophical analysis and research of the medical ethics literature, the American Medical Association has taken the stance that physicians may not ethically refuse to treat HIV-infected patients. At the Conference, Carol Stocking and colleagues (University of Chicago) reported on a survey of orthopedic surgeons who practice in U.S. cities with high AIDS incidences. Of 388 survey respondents,

181 (47%) reported having operated on an HIV-positive person in the last year. Another 89 (23%) said they had examined an HIV-positive person in the last year; 29 surgeons reported that they declined to operate on one or more HIV-positive persons, but of these, 16 had operated on an HIV-positive person in the past (5 of the 29 reported that they declined for nonmedical reasons). Surgeons who had operated on an HIV-positive persons were more likely to agree that surgeons are ethically obligated to operate on HIV-positive persons in emergencies or whenever it might affect quality of life. Dr. Molly Cooke (University of California, San Francisco) reported that in a national cross-sectional survey of 1,045 medical residents in the United States, the majority agreed that private practitioners have a duty to treat HIV-infected patients, but they were divided on the issue of whether surgeons have a duty to operate on an HIV-infected person.

Duty to Accept New Patients. Dr. McCullough (cited above) pointed out that more ethically complex than the "duty to treat" issue in HIV care is the question of whether physicians have an obligation to accept HIV-infected individuals as *new* patients. Some of the more poignant complexities outlined include the following:

- In Western democracies at least, health care professionals have the right to choose whom they will serve.
- Some patients with HIV infection may not be able to pay for the care. In the U.S., private health care professionals are not required to accept as patients individuals who are unable to pay for their medical care.
- A patient's need for an elective procedure may not have the same ethical claim on a professional as the need for an emergency or life-prolonging procedure.
- Professionals who become HIV-infected can place their sexual partners, and potentially their unborn children, at risk of infection, none of whom can be presumed to have consented to such risk.

Dr. McCullough concluded that there is at present no ethical obligation on the part of health care professionals to accept HIV-infected individuals as new patients.

Health Care Providers: HIV Testing and Disclosure. Following the U.S. Centers for Disease Control's announcement of the first probable case of HIV transmission from an infected health care professional (a Florida dentist with AIDS) to one of his patients during a medical procedure (an extraction), much public concern has mounted with respect to the ethical responsibility of health care professionals to inform their patients of their seropositivity. At the Conference, Harvey Skinner (University of Toronto) and colleagues reported results from a survey of 2,922 Canadian and U.S. medical students, interns, residents, and practicing physicians regarding this issue: When asked "If you knew you were seropositive, would you tell any patients?" 77% of responding physicians, 91% of interns and residents, and 76% of students said "No."

Dr. Hacib Aoun, a U.S. physician, told Conference participants in a plenary lecture that after informing hospital officials of his HIV-positive status he was virtually abandoned by that institution with respect to benefits and continued employment. Dr. Aoun felt that the medical profession had discriminated against him—after asking him to work the front lines. Dr. Aoun's points—likely to be fully addressed in the near future—included the need for the following:

- employer insurance coverage for all front-line health care workers (life, disability, health, and workmen's compensation)
- active, ongoing education on prevention of occupational exposure to infectious diseases
- restriction of inexperienced medical students from performing invasive procedures on high-risk patients

Failing to take care of health care workers, Dr. Aoun concluded, may lead to these workers becoming less willing to take care of AIDS patients.

Guidelines for Corporate AIDS Policies

The following principles, drafted by The Citizens' Commission on AIDS (New York City and Northern New Jersey), have been endorsed by almost 400 corporate and nonprofit organizations in the United States. These corporate AIDS policies reflect our current understanding of the epidemiology of HIV infection, prudent business practice, and a human and compassionate attitude to all individuals.

(1) People with AIDS or HIV infection are entitled to the same rights and opportunities as people with other serious or life-threatening illnesses.

(2) Employment policies must, at a minimum, comply with federal, state and local laws.

(3) Employment policies should be based on scientific and epidemiological evidence that people with AIDS or HIV infection do not pose a risk of transmission of the virus to coworkers through ordinary workplace contact.

(4) The highest levels of management and union leadership should unequivocally endorse nondiscriminatory employment policies and educational programs about AIDS.

(5) Employers and unions should communicate their support of these policies to workers in simple, clear, and unambiguous terms.

(6) Employers should provide to employees with sensitive, accurate and up-to-date education about risk reduction in their personal lives.

(7) Employers have a duty to protect the confidentiality of employees' medical information.

(8) To prevent work disruption and rejection by coworkers of an employee with AIDS or HIV infection, employers and unions should undertake education for all employees before such incident occurs and as needed thereafter.

(9) Employers should not require HIV screening as part of general pre-employment or workplace physical examinations.

(10) In those special occupational settings where there may be a potential risk of exposure to HIV, employers should provide specific, ongoing education and training, as well as the necessary equipment to reinforce appropriate infection control procedures and ensure that they are implemented.

In the United States, the American Medical Association and American Dental Association have recommended that HIV-infected doctors and dentists disclose their HIV status to patients or give up surgery and all other invasive procedures. The AMA also added that doctors who perform surgery or other procedures that carry a risk of transmitting HIV should be tested; those who test positive have an ethical obligation not to engage in any professional activity that has any identifiable risk to their patients.

The existing CDC guidelines for HIV-infected doctors recommend that decisions regarding what medical procedures can and cannot be performed by them be made on a case-by-case basis by local community/hospital expert panels. New guidelines that specifically identify procedures by their associated degree of risk (based on experience with other infectious diseases, such as hepatitis B) should be issued in early 1991.

It is anticipated that the forthcoming CDC guidelines will recommend HIV testing of all health care workers who perform invasive procedures. But already advance news of this subject's inclusion in the guidelines has raised opposition from professional organizations and human rights' advocates. Questions such as "how often should health care workers be tested?" and "Who would learn the test results?" are of primary concern. Many say that it is easier to suggest that doctors and health care workers be screened (or counselled to avoid invasive procedures) than to execute such a policy.

Duty to Warn. Another ethical aspect of testing and confidentiality is the "duty to warn," or to breach patient confidentiality—or that of an HIV-infected health care provider—in an effort to protect unknowing persons at risk of HIV infection. Does the health care professional have a moral obligation to warn unsuspecting third parties, such as other health care providers or a patient's sexual partner, if a patient or client knowingly places that third party at risk for HIV infection? Will health care professionals be legally liable if they do breach confidentiality? Although no current law resolves this dilemma, law experts say that the professional must determine if a warning (or other action) to the at-risk third party is the highest "good."

BUSINESS & INDUSTRY

As more companies and businesses are incorporating specific HIV infection and AIDS clauses in their employee policies, attention focuses on the issue of job discrimination. In major international corporations, at least, workplace policies thus far developed—clearly outlining the company's position on issues relating to HIV-infected employees and their coworkers--appear to be consistent with nondiscriminatory practices. (See "Guidelines for Corporate AIDS Policies.")

In the United States, federal and state antidiscrimination legislation protects HIV-infected individuals and persons with AIDS from job discrimination. As pointed out by Charles Nau (Syntex Corporation, Palo Alto, California), state and local handicap statutes, privacy statutes, defamation and other potentially relevant tort laws, and wrongful termination laws—as well as the principles of "reasonable accommodation"—all become relevant when examining the rights of HIV-infected employees. To prevent expensive and time-consuming work stoppages and litigation, Mr. Nau recommended that managers and supervisory personnel of American corporations become familiar with the "law of AIDS" in the United States. Crisis control, as well as crisis prevention, can benefit from implementation of HIV-related policies and training of managerial personnel on how to respond to HIV in the workplace.

CHAPTER 10

Page 287 **Background**
288 **Social Marketing Approach**
288 Message Variables: The Nature of the Message
290 Source Variables: Characteristics of the Message Producers
295 Channel Variables: How the Message is Communicated
296 Receiver Variables: Nature of the Target Population
296 **Knowledge, Attitudes, & Behavior Surveys**
297 General Population Surveys
299 Surveys in Developing Countries
301 Surveys among Homosexual/Bisexual Men
303 Surveys among Intravenous Drug Users
306 Surveys among Ethnic Minority Groups
307 Surveys among Adolescents
309 Surveys among College Students
310 Surveys among Women
312 **Education**
312 Changing High-Risk Behaviors
313 Educational Efforts in Target Populations
316 **Counselling HIV-Infected Persons**
316 Counselling Approaches
317 Partner Notification
317 Consequences of HIV Diagnosis
318 Psychosocial Consequences
319 Family and Care Giver Needs

EDUCATION & COUNSELLING

BACKGROUND

From the earliest days of the AIDS epidemic, public health officials have referred to education as the only currently available "solution" to the threat of the disease. Despite major progress on the biological and medical frontiers, education remains the most powerful intervention to stem the threat of AIDS. This challenge has resulted in a wide variety of efforts on the international, regional, national, and community levels:

- increased understanding of the correlates of high-risk behavior
- dissemination of accurate information on transmission routes of HIV, geographic transmission patterns, demographic patterns, as well as specific preventive measures
- research on relationships among knowledge, attitudes, and high-risk behavior
- increased understanding of the cultural and behavioral characteristics of specific populations that make them particularly susceptible to HIV infection
- establishment of programs to effect specific behavioral changes—notably educational programs and intervention programs such as needle-exchange and condom distribution
- implementation of pre- and post-screening test counselling

While education and counselling are widely agreed to be central to any strategy for curbing the epidemic, for a number of reasons this seemingly simple "solution" is neither simple nor well understood. Because the primary transmission modes for this virus involve activities that bear social and moral import (sexual activity and IV needle sharing), the need to discuss them further complicates AIDS education efforts. Not surprisingly, teaching people how to take protective measures during activities that are perceived as undesirable, devalued, illegal and/or immoral raises objections by some on religious or ethical grounds. (See Chapter 3: EPIDEMIOLOGY for a discussion on HIV transmission routes.)

Furthermore, the reinforcements for sexual activity and drug abuse are immediate, powerful and unmistakably connected to the antecedent behavior. AIDS messages and their presentation must perforce deal with some of the most powerful biologically reinforced behaviors in the species. The short-term consequences of sexual and drug-taking behavior are pleasurable; educational attempts to alter such behavior can be perceived as punitive because the benefits are long-range and not immediately apparent. As such, they do not have a forceful presence and seem remote to individuals in high-risk groups. Additionally, AIDS prevention messages that are, for reasons of accuracy, couched in probabilistic rather than absolute terms, are meaningless to these individuals.

Some social scientists are not optimistic that long-lasting behavioral change is possible given these facts, particularly when the physical nature of drug addiction is also considered. Yet, the evidence of success in modification of high-risk behavior is building.

This chapter will first consider a heuristic framework borrowed from business management and communications theory—social marketing theory—as a way of organizing the concerns of

- "what" (the nature of the message)
- "from whom" (the nature of the source)
- "how" (the nature of the communication channel)
- "to whom" (the nature of the target population)

An in-depth consideration of each of the high-risk and emerging at-risk target populations will follow, leading to an examination of various educational approaches.

SOCIAL MARKETING APPROACH

Social marketing concepts have been used by a number of researchers interested in the question of how to sell the idea of adopting risk-reducing behaviors to members of a high-risk group. Just as commercial marketing refers to planning, implementing, and evaluating programs to affect consumer behavior, so social marketing concerns itself with designing, implementing, and evaluating programs that seek to increase the acceptability of a social idea, cause, or practice in a target group(s). Among the strategies used to maximize target group response are those of market segmentation (identifying and describing consumer subgroups); consumer research (identifying barriers to acceptance of the program); message analysis (consideration of alternative ways of making the point); communications (consideration of alternative media); and facilitation (utilization of multiple message sources and social supports). Thus, social marketing entails a careful analysis of

- message variables—the nature of the messages, products, or packages to be disseminated
- source variables—the characteristics of the message producers
- channel variables—alternative modes through which the messages can be sent
- receiver variables—relevant characteristics of the target populations

MESSAGE VARIABLES :THE NATURE OF THE MESSAGE

What should constitute AIDS education messages? Should the emphasis be on "facts" about the disease? Should the information be simple or detailed, subtle or direct? Is an appeal to fear likely to result in behavioral change? Consideration of these and other questions are essential to the framing of messages that seek to effect behavioral change. This consideration should entail not only analyzing content but also identifying the idiom, metaphor, or image in which messages must be cast for effectiveness.

Health Belief Model. AIDS education interventions have relied heavily on theories and findings from previous behavior modification research (directed toward smokers and the obese, for example). This research made it evident that mere awareness of an unhealthy

outcome is insufficient to bring about desired behavioral change. The theoretical model most widely used to guide research on health-related behavioral change is the "Health Belief Model," which has grown out of the work on Social Learning Theory by Albert Bandura and his associates.

Social Learning Theory emphasizes the social and environmental events that trigger and reinforce behavior change, and it provides a rich source of behavior change methods (for example, modeling and shaping). Bandura's notion of self-efficacy is also particularly relevant to health enhancement, because it has been shown to be a modifiable mediator of health behavior change. Using this theoretical approach, many researchers have concluded that before making behavioral change a person must:

- consider the problem serious
- feel personally susceptible
- perceive the benefits of action
- believe that the recommended action is efficacious
- overcome the barriers to performance and respond to a cue to action

For these reasons, the more successful AIDS education efforts augment simple messages about the "facts" of the disease with specific instruction that is designed to

- strengthen the learner's perception of the disease as representing a real, personal threat
- lead the learner to see that the disease can be controlled by changes in behavior
- strengthen the learner's belief in personal self-efficacy by making the desired behavioral changes.

AIDS prevention messages that also provide some opportunity to put these these beliefs into operation will likely be most effective.

Analyzing the AIDS Message. Michael Ramah (AIDSCOM/Porter Novelli, Washington, D.C.) and associates reported on the nature of condom promotion messages directed at gay men in Mexico. A series of focus groups exploring attitudes and perceptions about existing condom education/promotion campaigns led to the conclusion that condom messages targeting lifestyle might have the opposite of the desired effect. Many messages were found to be "translated" into the following equations:

UNDESIRABLE MESSAGE

GAY = AIDS

AIDS = DEATH

GAY = CONDOMS

GAY = AIDS = CONDOMS = DEATH

Thus condoms become associated with death and AIDS in gay men rather than with prevention of HIV transmission and AIDS. Messages targeted to the general community may be more successful in creating more accurate formulations, such as:

CONDOMS ≠ HIV INFECTION

The authors used the analogy of cigarette advertising as a desirable model for condom messages. "Slice of life" ads depicting happy people enjoying life, male and female, are culminated with the message, "use condoms."

Lorraine Sherr (St. Mary's Hospital, London) reported on a comparison of fear and humor tactics in the United Kingdom national AIDS campaign, using 200 subjects and a set of visual and audiovisual materials. It was found that the two approaches evoke markedly different responses. Subjects who were intravenous drug users had initially higher levels of anxiety and thus were more vulnerable to anxiety-producing materials. Risky sexual behavior was not greatly affected by either approach. Fear arousal as a model fared less well than humor.

SOURCE VARIABLES: CHARACTERISTICS OF THE MESSAGE PRODUCERS

Messages to change or maintain behavior are deeply embedded in and legitimated by a number of elements in individuals' social ecology. The messages of family, church, school, peers, and the media, for example, are often inconsistent. Individuals continually evaluate the source of such messages for their relevance and credibility, and for the consistency of their messages with already held beliefs. If individuals cannot relate to the source, their messages are usually disregarded. AIDS educators, therefore, need to take into account the existing sources of explicit or implicit messages about AIDS-related issues when attempting to formulate a program for reducing high-risk behaviors.

This year's Conference was striking in the number of reports that explored a variety of different sources for targeting populations at risk for AIDS, and to a lesser degree, persons that work with these populations.

Mass Media. In recent years, increasing interest has been paid to the role of mass media in informing people about the disease and how to reduce the risk of becoming infected. A 1988 French national survey of 2,295 persons was reported by Mitchell Cohen (INSERM, Villejuif, France) and colleagues. They found that approximately 90% mentioned television as their source of information about AIDS, and more than half mentioned newspapers, magazines, and radio. Doctors, friends, brochures, and posters are mentioned by about a third. Persons under age 24 are more likely to mention interpersonal sources and more-targeted media. Knowing someone with AIDS and mentioning friends as a source of information is related positively to unprotected sex. The authors offered that those seeking information from people they know may already be engaging in risky behavior. Changing to less risky activity is related to interpersonal and targeted media sources such as brochures and pamphlets. Obtaining information from television is not related to behavioral change, indicating that more personal sources of information may be required for respondents to change to less risky behavior.

M. K. Jinadu (Obafemi Awolowo University, Nigeria) and O. Ogunyankin and associates (Department of Disease Control, Akure, Nigeria) independently reported their studies of AIDS messages by mass media in Nigeria. Both groups surveyed AIDS messages in newspapers, radio, television and other print materials between 1986 and 1989. Most mass media messages were characterized as noninformative and fear-laden. A survey of rural and urban dwellers indicated that radioed AIDS messages were most frequently heard, with all urban residents and 40% of rural persons having been reached at least once. About 85% of the rural and 46% of the urban dwellers rated the credibility of these messages as poor. In another African study, M. Kyungu and associates (Population Services International, Washington, D.C.), in collaboration with Bureau Central de Coordination de la Lutte Contre le SIDA, found that between 57% and 69% of residents in Kinshasa, Zaire, had seen television spots or dramas about AIDS and could recall the key AIDS messages 2 months later. A study by Noemi Fisman and colleagues (Grupo Argentino de Tratamiento de la Leucemia Aguda, Buenos Aires) looked at a stratified sample aged 15 to 75 and compared their interests in and sources of information about AIDS and cancer. Only 42% reported looking for additional information about cancer, as compared with 74% for AIDS. Newspapers (59%) and television (56%) were the major sources of information about AIDS.

In two reports investigating the use of mass media for reaching intravenous drug users (IVDUs), Janine Jason and Liza Solomon and their colleagues at CDC, Atlanta, and the Johns Hopkins School of Hygiene & Public Health (Baltimore, Maryland), found that 55% of a group of 218 IVDUs responding to a survey had heard of AIDS by 1985; 78% were male; 94%, black; 48%, seropositive; and their mean age was 34 years. Television was most often cited as the source of information (53%), with other mass media sources cited much less frequently. Over half of the respondents watched up to 4 hours of television each day, most

often between 8 and 11 P.M. Because of the overlap in viewing hours with those of young children, the detail and explicitness of AIDS messages on television is limited.

Two presentations reported content analyses of newspapers' coverage of AIDS. Rosa Feijoo and coworkers (University of Zaragiza, Spain) analyzed 1,166 articles in national and local Spanish newspapers. It was found that impact aspects (such as stories about actors or AIDS-related legal problems) were more frequently reported than epidemiological and scientific advances. The most common educational orientation was described as negative and defeatist in nature. A study of the four largest Brazilian newspapers by Sergio Adeodato Filho and Paulo Longo (Jornal Do Brasil and the Instituto Brasileiro de Inovacoes em Saude Social, Rio de Janeiro) found that coverage of AIDS actually had decreased 58.9% from 1987 to 1989 despite the greatly increasing number of AIDS cases in Brazil during that period. On average, every month, each newspaper published 25 reports about AIDS. Of these, 71% came from international agencies. Little local coverage was provided, and much of the information was deemed inaccessible to the general public.

Kim Miller and William Darrow (CDC, Atlanta) reported an analysis of the U.S. media's portrayal of crack cocaine and the risk for HIV infection. Only 13% of a total of 31 articles were found to be based on scientific evidence, as compared with 75% of articles on this topic in the scientific literature.

Two studies that attempted to compare respondents knowledge and attitudes regarding AIDS before and after a major media intervention were reported. One study, reported by Jennifer Bryce (CDC, Atlanta) and colleagues at Michigan Department of Public Health (Lansing), found that a major statewide HIV-prevention mass media campaign resulted in increases in the number of people seeking information (through an AIDS hotline) and services (HIV testing and counselling centers). Surveys of IVDUs and the general public, retrospective analyses of data from previous counselling and testing records, as well as television Arbitron estimates were also used as data sources, confirming the results, and establishing the usefulness of routine service statistics to evaluate the effectiveness of mass media campaigns. Dr. Rukarangira (Harvard School of Public Health, Boston) and collaborators (Projet CONNAISSIDA, Lubumbashi-Zaire) reported that a 1-year mass media campaign resulted in a change from 25% to 85% of respondents (Lubumbashi residents) knowing that AIDS can be fatal.

Governmental and International Initiatives. A presentation on the role of the federal government in prevention of HIV infection among IVDUs in Canada, by Betsey MacKenzie and K. Elmslie (Federal Centre for AIDS, Ottawa), concluded that federal initiatives focusing on research and collaboration receive strong support from public health and community groups. Joycelyn Sue Woods (Narcotic and Drug Research Inc., New York) and associates reported that the goal of the International Working Group on AIDS and Drug Use is to share among researchers and policy makers information about HIV infection related to drug use. Epidemiological and behavioral data on IV drug use accumulated in Western Europe and North America may prove invaluable to countries now experiencing increases in HIV infection among IVDUs (such as Brazil and Thailand) as well as those regions possibly on the verge of an epidemic (such as Eastern Europe). The development of five different data bases, an international surveillance report, and a quarterly newsletter will, it is hoped, contribute to the active exchange of information for use in developing countries and Eastern Europe.

The development and implementation of a Central American and Mexican information exchange, sponsored by World Health Organization, was reported by Blanca Rico and coworkers (Pan American Health Organization, Mexico City). So far, 16 regional and 8 international centers of the network have been established. The goal is to exchange information on positive strategies on AIDS prevention and education, taking into account the common cultural and historical features shared by the region.

Local health departments also play an active role in sending out AIDS messages. As David Werdegar and coworkers (San Francisco Department of Public Health) reported, the local health department may serve as the focal point in the community response to AIDS. Among the AIDS-related functions of the San Francisco Department of Public Health were the following:

- gathering epidemiological information
- developing education/prevention programs
- organizing treatment and support services
- coordinating funding
- providing linkages to other local, state, and national agencies

It was concluded that a health department cannot provide all required services, but rather can coordinate and maximize the community response.

Higher Education Agencies. Institutions of higher education are widely recognized as credible sources of many kinds of information. Given the teaching nature of much the activity at these centers, it is fitting that AIDS education be undertaken by these institutions. A model interdisciplinary university-wide course on AIDS was described by Ronald Strauss and colleagues (University of North Carolina, Chapel Hill). The course dealt with content from immunology, epidemiology, and the impact of AIDS on society. Student evaluations were especially positive on panel discussions with patients or on sexuality and lectures on prevention behaviors and treatment. Michael Closen and Scott Isaacman (John Marshall Law School, Chicago) described the development of an HIV/AIDS coursebook *AIDS: Cases and Materials*, which has been adopted by more than 20 law schools. A continuing need to address course materials is cited.

A novel approach to increasing AIDS awareness among college students was reported by Dr. Mark Goldstein and colleagues (Massachusetts Institute of Technology, Cambridge). They reported the successful use of a visual arts program around AIDS themes, including a student art contest and exhibition, a quilting project, displays of AIDS quilts and photographs of individuals in the terminal phases of AIDS.

Dr. Mary Redick of San Francisco Community College District described AIDS-related activities of a 60,000-student community college over the past 6 years. Activities engaged in included the following:

- policy: nondiscrimination and confidentiality
- education: materials production for employees and students
- service: worksite and classroom integration of HIV-infected employees or students, outreach and liaison with community, regional, state, and national groups

Religious Organizations. The potential importance of religious organizations as sources of helpful AIDS messages is implicit in the new religious initiative being undertaken by the Centers for Disease Control's National Partnerships Program reported by Richard Jimenez (CDC, Atlanta). The CDC will encourage, assist, and support religious communities in the fight against HIV infections and in providing AIDS services to those in need. Models of national, regional, and local interfaith partnerships among government, churches, and community-based organizations were presented.

Two reports dealt with religious groups' responses to AIDS issues. Mary T. Wilson of the Christ Church Cambridge AIDS Concern Committee (Cambridge, Massachusetts) described that parish's responses to AIDS-related needs: collection of AIDS-related information in the church library; provision of educational and discussion opportunities; and collection, storage, and distribution of food for persons with AIDS, among other activities. A more national effort was described by Michael Rankin and Ian Gilson (Union of American Hebrew Congregations Committee on AIDS, New York). Formed in 1985, this group has

provided resource material on AIDS to all 840 affiliated congregations, proposing social action resolutions, planning and staffing workshops and addresses, arranging special liturgies and services, and providing other human and material resources for regional congregational and regional forums.

Some scanty evidence is emerging that religious affiliation together with social class is related to commitment to the civil rights of persons with AIDS. Homer D.C. Garcia and others (Pitzer College, Claremont, California) and associates reported results of a telephone survey of 906 African-American and Latino adults in Los Angeles County indicating that religious tolerance promotes beliefs that the civil rights of people living with AIDS ought to be protected. Higher socioeconomic subjects were found to be more religiously tolerant.

The credibility and resources of the Christian church in Africa as an integrative influence in AIDS care and prevention was the topic of a report by Ian Campbell and others (The Salvation Army Chikankata Hospital, Zambia). They made the case that the church in Africa represents a vast, strongly organized, but underutilized resource to help make balanced decisions about lifestyle in the context of AIDS control. A report from Kampala, Uganda, by E. Maxine Ankrah and others (Makerere University), however, was less optimistic about the potential contribution of the African church. These researchers assessed the perceptions on a number of AIDS issues of a random sample of Protestant clergy. As a group, they lacked factual knowledge about the disease, most having never heard of the virus, HIV. While few believed AIDS to be God's curse, all advocated sympathetic care. It was concluded that this group needs much additional health education in order to be an instrument of service or behavioral change.

Community and Nongovernmental Organizations. Increasing numbers of persons concerned with the continuing spread of HIV are recognizing that HIV disease is most effectively combated at the local, grass-root level. Community-based organizations (CBOs) and nongovernmental organizations (NGOs) can provide unique commitment and enthusiasm, as well as authentic understanding and access to the target groups to whom AIDS outreach is being directed. For this reason, the Center for Disease Control (CDC) in Atlanta has launched several major initiatives involving these types of organizations in attempts to stem the spread of AIDS. Several Conference reports described these initiatives.

As early as 1985, the CDC, in collaboration with the U.S. Conference of Mayors, initiated a program that provided "seed money" and intensive technical assistance for CBOs. A follow-up questionnaire to the 85 organizations funded by this program was undertaken and reported by Stephen Schindler and others of CDC and U.S. Conference of Mayors. At the time of the survey, 96% of the CBOs were engaged in HIV prevention activities; 83% had successfully obtained funding from other sources, and 17% had applied for such funding. It was concluded that CBOs play a vital role in reducing the spread of AIDS. A description of the programmatic activities and interventions of 64 minority and other CBOs providing street outreach programs to reduce the spread of HIV was presented by P. Imania Thompson and collaborators (CDC, Atlanta). The largest number of these programs are targeted toward IVDUs and their sex partners; others serve high-risk teens and prostitutes; still others focus on men who have sex with men. Detailed case studies were presented by four of the CBOs.

To assure implementation of a comprehensive national HIV-prevention strategy, the CDC launched four initiatives to provide financial and technical assistance to assist NGOs in planning, implementing, and evaluating HIV prevention programs (reported by M. I. Fernandez and others, CDC, Atlanta). Mass media, theater, peer education materials development, and street outreach programs were among the types of interventions funded under the aegis of more than 600 NGOs in 1989. Hard to reach, high-risk populations that were targeted included runaway youth, homeless persons, men who have sex with men,

drug users, prostitutes, sexual partners of infected persons, and youth. Evidence supports the need for technical as well as financial assistance, especially on organizational matters.

Assisting local and national organizations to develop and promote community education about drug abuse and HIV infection is the purpose of a program effort by the National Institute on Drug Abuse (Rockville, Maryland), reported by Leona Ferguson and W. Link. Since October, 1987, 20 citywide networks or coalitions have been formed as a result of the training workshops conducted by this program. Regional conferences for various ethnic minorities have been arranged. Technical assistance to individuals and groups have been provided in the areas of community education, communications strategies, message/product development and evaluation.

The function and effectiveness of NGOs at the national and international level was analyzed by Robert Grose and others of the World Health Organization and Canadian AIDS Society. A key to success was reported to be the development of mutual networks for support, information exchange, skills development, fund-raising, and advocacy. The need for clarity of objectives was stressed. Kenneth Williams (CDC, Atlanta) discussed the role of NGOs in the United States, and governmental attempts to assist and coordinate such efforts.

Community organizations have focused on a number of missions. In the U.S., as reported at the Conference, these have included legal services (David Hansell and Teresa Calabrese, Gay Men's Health Crisis, New York); lobbying for appropriate state laws (David Jones, North Carolina AIDS Service Coalition, Chapel Hill); and establishing of a legal needle exchange (Jeffrey Schouten and colleagues, Life Foundation, University of Hawaii, Hawaii Medical Association, and Governor's Committee on AIDS, Honolulu). Jacalyn Lee and coworkers (Hunter College Center for Community Action to Prevent AIDS) reported that they conducted a survey of community and religious leaders to identify AIDS-related activities and obstacles to future actives. Technical assistance on networking and overcoming obstacles was provided.

Internationally, community organizations have had even wider missions: Hans Hengelein (Deutsche AIDS-Hilfe, Berlin) reported a novel workshop approach to further education, exchange of experience, leisure activities, and political networking. Roy Mwilu and others (Chikankata Hospital, Zambia) described the role of community based volunteers in home care and prevention programs, community counselling, and Village Health Advisory Committees. An umbrella organization—CONASIDA—was described by Gloria Ornelas and others (Ministry of Health, Mexico) as effective in the promotion of the interaction of public, private, and social sectors to achieve congruency in adopting measures to confront AIDS. Assessment, training, and provision of educational materials were among the activities described. In Ecuador Germania Brito and others reported that the Ministry of Health, using USAID funds, has organized a series of 70 community-based seminars for high-risk groups such as homosexuals and prostitutes (Ministry of Public Health and Centro Medico Entre Amigos, Quito).

Alison Rader and Ian Campbell (Salvation Army Chikandata Hospital, Zambia) raised the question of how community based programs can become well implemented, so that goals can be met and evaluated. They described the following principles as underlying successful task achievement:

- team approach
- ability to sustain people, programs, and finance
- hope (based on the capacity of the community to realistically incorporate AIDS into normal life, to share information respectfully and confidentially, and to reach consensus about significant change in lifestyle)

Given the many demands on community organizations, it is not surprising that a number of groups, particularly those engaged in direct services to people with AIDS, have experienced "burnout." This was the topic of a report by Levinia Crooks (University of

Wollongong, Australia). The volume of work done by community volunteers was found to be so overwhelming as to require an innovative approach to the care of these volunteers, if burnout is to be avoided.

Persons with AIDS. Credibility of the sources of any message, especially AIDS messages, is always an issue. Persons to whom such messages are directed may question the motives of the source or the relevance of the message to the source. For both of these reasons, persons with AIDS (PWAs) have excellent credibility. In turn, sharing their story may imbue PWAs with respect, compassion, and an understanding of self-worth. Wendy Arnold and M. Bard of "Being Alive," Los Angeles, describe a speaker's bureau of PWAs that provides messages of how HIV is and is not transmitted. Indeed as demonstrated in a study reported by Nancy Evans and J. Fetro (Department of Public Health, San Francisco, and Unified School District, San Francisco), incorporating PWAs into HIV prevention programs positively influences the relative success of the program: This team evaluated the effectiveness of an adolescent HIV education program in promoting changes in (self-reported) sexual activity. It was found that of all the components of the program (addressing facts, attitudes, feelings), a session that had a PWA had the most impact.

CHANNEL VARIABLES: HOW THE MESSAGE IS COMMUNICATED

The seriousness of the threat of AIDS has spurred much creative activity in the design of AIDS education materials in a variety of modes. A number of traditional and nontraditional modalities for the dissemination of AIDS messages were described. These included a report on the use of audiocassettes for Haitians with low levels of literacy (Nicole Prudent and coworkers, Haitian American Public Health Initiatives, Boston University); the use of rap and rock videos (Iris Page and others, North York Public Health Department, North York, Ontario); information campaigns during music and culture festivals (Merete Lindholm and Bente Hansen, Municipal School System of Copenhagen); role plays (Tukumbe Eke, Central Bureau of AIDS Coordination Programme, Kinshasa, Zaire); culturally specific videos (Susan Muska and others, Projet San Francisco, Kigali, Rwanda); national pamphlets specific to a particular population, such as women (C. Hankins and others, Federal Centre for AIDS, Ottawa); and use of traditional herbalists (Theresa Odero, Ministry of Health, Kenya).

Computer and Other Data Bases. With information regarding the HIV epidemic changing rapidly, it is not surprising that computers have been turned to as a way of keeping information up-to-date and accessible. A computer-aided information system on diagnosis and treatment of AIDS-related conditions capable of accelerating traditional ways of knowledge transfer to a broad public was the focus of a report by Bernd Sebastian Kamps and W. Stille (Johann Wolfgang Goethe Universitat, Frankfurt). Regularly updated public domain software is the core of the system. A more focused and differentiated database is described by Leonard Indyk and colleagues (Neptune Computer Group, Stamford, Connecticut and Mount Sinai School of Medicine, New York). They described a detailed encoding of AIDS information into standard database format (dBASE-III). The purpose is to capture, translate, and store the information for multiple uses; in this way users can be more efficient and facile in accessing information. Ben Gardiner (AIDS Info BBS, San Francisco) described the planning, construction, and operation of a computer bulletin board with a database of published material and anecdotal information from callers. It is used by people in HIV prevention, containment and management, by press, health officials, and persons with AIDS. The use of the French telephone network to tap into a knowledge base system for risk evaluation and prevention management was described by Claude Beuscart and associates (Universite de Lille, France). This system enables anyone with a telephone to obtain information, an anonymous consultation, and follow-up connection to a mailbox number for a specialist's response to a personal problem.

Christopher Hall and Lisa Flam (National Hemophilia Foundation, New York) described the design and implementation of an information center for hemophilia community: Hemophilia and AIDS/HIV Network for the Dissemination of Information, or HANDI. Information dissemination focuses on HIV risk reduction, treatment for HIV-infected persons, living with hemophilia and HIV, and discrimination against hemophiliacs with HIV. Services are available directly to people with hemophilia as well as health-care providers and related others.

RECEIVER VARIABLES: NATURE OF THE TARGET POPULATION

The risk of infection is not equal for all groups: risk is related to a group's geographic location, age, lifestyle, gender, and cultural variables. It therefore follows that not all groups can be accessed with AIDS prevention messages in the same way. Relevant issues include not only the obvious ones, such as presenting messages in that group's language, but a consideration of how can they be motivated to believe that the issue is one they should attend to, care about, and act on—in short, that they should "own."

Substantial research has been conducted with groups identified as at high risk (such as IVDUs and men who have sex with men), as well as emerging at-risk groups including sexual partners of infected individuals or those at high risk, members of racial minorities (especially blacks and Latinos), sexually active adolescents, and more recently, women.

For most of the high-risk groups, a substantial body of literature describing group-based attitudes, beliefs, values, and behaviors is available. Surveys of current perceptions and attitudes among various populations assist in the design and/or modification of education and intervention programs. Readable and meaningful literature regarding specific aspects of HIV infection, including transmission routes and risk-reducing behaviors, can be tailored to meet the needs and deficiencies of well-defined audiences.

KNOWLEDGE, ATTITUDES, & BEHAVIOR SURVEYS

Assessing specific behaviors or practices within well-characterized populations—unprotected sex among college students, for example—and within specific cultures is essential since it has been repeatedly demonstrated that high knowledge levels do not necessarily translate into behavioral changes. The relationship between the perceived need for behavioral change and actual behavior modification can vary considerably, as is illustrated by numerous presentations at this year's Conference.

Knowledge, attitudes, and behavior surveys typically assess the following:

- knowledge about transmission of HIV and specific behaviors that place persons at risk of exposure
- self-perception of risk
- attitudes toward adopting risk-reducing behaviors
- fears about contracting the virus
- changes or preventive measures taken since becoming aware of AIDS
- attitudes toward HIV-infected persons and persons with AIDS (PWAs)

Surveys collect both qualitative and quantitative information: For example, in cases where heterosexual transmission is the primary risk factor, it is important to identify the types of sexual activity as well as the number of incidents or the number of sexual partners. Inherent to surveys that ask such personal questions, however, is the possibility of nonresponse and/or underreporting. Acknowledging these problems, several independent investigators at the Conference pointed out that survey methods may influence compliance and accuracy. A singular report dealt with the hazards of street-based research.

William Darrow (CDC, Atlanta) assessed the reliability and validity of self-reporting of sex practices, sexually transmitted diseases, and IV drug use. Confidential interview data from 1,410 prostitutes who consented to be interviewed, tested, and examined from eight U.S. regions were compared with laboratory findings and clinical observations. Self-reported information about HIV antibody status, treatment for syphilis, and intravenous drug use were substantially confirmed by objective laboratory and clinical data, suggesting that valid comparative AIDS-related data can be collected in large-scale studies.

David Gibson and colleagues (University of California, San Francisco) reported on a study to validate self-reporting of needle sharing by IVDUs by correlating them with the results of forensic tests designed to detect multiple users of the syringes. Comparing residue in 31 syringes donated by multiple users with reference fingerstick blood specimens for discrete biochemical traits, it was found that the results were remarkably consistent with self-reports of the users. The researchers concluded that IV drug users' self-reports of needle sharing appear to be surprisingly valid.

Robert Broadhead and associate (University of Connecticut, Storrs) reported on occupational risks of indigenous workers who conduct street-based outreach research interviews with IVDUs. The three major risks presented were addiction relapse, burnout and staff turnover, and physical and psychological assault.

GENERAL POPULATION SURVEYS

Several studies have looked at large samples of the general population in attempts to gauge knowledge about AIDS. Arlene Davis (Peoria City/County Health Department, Illinois and Illinois State University, Normal) investigated factors which inhibit safe sexual behavior in an unmarried heterosexual population aged 35 and older. The 139 respondents were generally knowledgeable about HIV transmission (correctly answering greater than 80% of the questions). However, knowledge level did not significantly affect the decision to be sexually active or inactive. Only 2.6% of the subjects always asked prospective sexual partners about previous high risk behavior (IV drug use, homosexual activity, sexual contact with prostitutes, multiple sexual partners, lack of regular condom use). Almost 50% of the men and 29% of the women had not asked any potential partner about any of these behaviors during the previous year. Questions regarding condom use revealed that 16.9% of the men and 21.8% of the women had used condoms at least once during the previous year. Reasons for not using condoms included accurate perception of low risk; inaccurate perception of low risk; interference with pleasure; spontaneous sex; embarrassment; and the belief that condoms are ineffective in preventing transmission of HIV. Similar findings were reported from a national study of AIDS knowledge and sexual behavior in a representative sample of 1,000 Dutch citizens (Theo Sandfort and colleagues, University of Utrecht, The Netherlands). Overall, the level of AIDS information on transmission and prevention was high. About 12% of the sample reported engaging in risky sexual behavior during the previous year; interestingly, these persons scored slightly higher on AIDS knowledge. The authors conclude that prevention strategies should be more intensively directed at the subgroups who are relatively more at risk.

A comparison of the relationships among knowledge, beliefs, and condom use in the U.S., France, and U.K. was reported by Dr. James Wells (Project HOPE Center for Health Affairs, Chevy Chase, Maryland). Interviews were conducted with national probability samples with approximately 1,950 respondents in each country; factor loadings of the 14-item knowledge survey was similar in the 3 countries. Knowledge levels were not found to correlate well with condom use. However, certain items correlated with condom use in several specific populations: Fear of AIDS related to condom use during vaginal intercourse and to initiating use of condoms, especially by U.S. and French subjects. Knowing

someone with AIDS is related to condom use for anal intercourse with men in the United States only. Belief that AIDS is fatal did not equate with high condom use in any country.

Self-Assessment. The ability of heterosexuals to estimate their risk for HIV infection was the focus of a study presented by Lisa Feingold and associates (New England Behavioral Health Study, Providence, Rhode Island). Using data from the first 631 enrollees in a population-based study of heterosexual transmission of HIV infection, the following findings were obtained:

Perceived Risk	Actual Risk of HIV Infection		
	Low (n-128)	Moderate (n=132)	High (n=371)
None/slight	67%	66%	36%
50/50 chance	9	14	28
High	2	3	29
Do not know	21	17	7

Overall, the findings show a significant tendency to underestimate risk of HIV infection. Nonwhite women were less likely to underestimate their risk, compared to white women. Age and sexual orientation were not predictive of underestimation. Males were slightly more likely to underestimate their risk. Persons using condoms were more accurate than nonusers in assessing their risk of infection. The researchers concluded that high levels of misperception and inability to accurately classify one's risk of HIV infection put heterosexually active adults at substantial risk.

Sexual Behavior. A representative sample of 1,511 adults aged 18–59, living in the southeastern part of France was interviewed regarding their sexual behavior. Brigitte Lhomond (CNRS, France) and colleagues found that 15.8% of heterosexual respondents had more than one partner in the previous year; 3% had homosexual relations, and of these, approximately 75% had more than one partner. People with multiple partners were found to have a lower frequency of sexual activity than persons self-reported as monogamous, but they reported more varied practices. Condom usage among persons with multiple partners was higher than that of the general population: 3.3% used condoms with a regular partner, 9.8% with a known occasional partner, 13.1% with a new partner.

Joseph Catania and colleagues (University of California, San Francisco) tested a three-stage process model of changes in sexual risk behavior using data collected in a random household probability study of 1,781 white (41%), black (26%), Hispanic (25%), and other (8%) unmarried persons. Subjects were asked to make an assessment and label their risk for HIV based on sexual practices. The dependent variable was frequency of condom use averaged across sexual partners for the previous year. Hispanics labeled their behavior more accurately compared to whites, whereas gay men were more accurate than heterosexual men. Greater condom use during vaginal or anal intercourse was found to be significantly related to better sexual communication skills, higher perceived benefits and lower costs of condom use, but unrelated to religiosity, self-efficacy, and ethnicity. These latter findings held for both women and homosexual and heterosexual men. The finding of unrelatedness to self-efficacy is contrary to predictions of the Health Belief Model.

Condom Use. Two studies reported condom use by patients at clinics for sexually transmitted diseases (STDs). In a study reported by Hillard Weinstock (CDC, Atlanta) and colleagues, perceived risk for HIV was not associated with condom use in a random sample of 300 heterosexual patients aged 18-65 (mean age, 29); 50% were male, 43% were white, and 36%, black. While 99% knew that condoms could prevent transmission of STDs including HIV, only 18% regularly used condoms in the previous 2 months. Condoms were used 50% of the time with casual partners, only 28% of the time with their most important partners.

Patients were more likely to use condoms if casual partners endorsed their use and if they believed condoms do not detract from sexual spontaneity. The authors concluded that an understanding of partner relationships is necessary for increasing condom use. Soldiers with newly diagnosed STDs at an STD Clinic at Fort Bragg were the subjects of a study reported by Charles Magruder and associates (Womack Hospital, Ft. Bragg, North Carolina). Consistent with the findings of the study by Mr. Weinstock and colleagues (above), 96% of these men knew that condoms reduced transmission of HIV, but only 17% had used one the last time they had sex. Lack of availability was the major reason given for the lack of use. Black men more often than white men felt that condoms break or come off during intercourse, reducing their effectiveness.

Dominique Hausser (University of Lausanne, Switzerland) and colleagues presented findings of their study of condom use in Switzerland using two perspectives: interviews of representative samples of 27-30 year olds during the period January 1987 to October 1989 (approximately 1,200 subjects in 4 waves, the last wave including persons aged 31-45) and analyses of numbers of condoms sold per year in Switzerland. The reported increases in condom use (8% in January 1987 to 17% in October 1987 to 29% in October 1988 to 48% in October 1989) were confirmed by increases in condom sales. The authors noted, however, that although condom usage has steadily increased perhaps reflecting the effectiveness of prevention campaigns, persons under 31 were more likely than older persons to use condoms.

SURVEYS IN DEVELOPING COUNTRIES

As in developed countries, knowledge surveys can yield important information for the design of effective prevention campaigns and/or interventions. The majority of knowledge surveys were combined knowledge, attitudes, and behavior surveys.

HIV/AIDS Knowledge. Muhammad Bustan (Hasanuddin University, Indonesia) and colleagues reported an assessment of AIDS knowledge and risk perception among Indonesian public health students (residing in an area of low AIDS incidence). Wide variation in accuracy of responses to different questions was found: 99.2% correctly identified the main modes of HIV transmission, but 68.6% thought AIDS can be cured. Furthermore, 52% believed that HIV could be transmitted through casual contact. The researchers stressed the need for more education to erase misconceptions regarding HIV and AIDS.

Aurora Del-Rio and coworkers (Ministry of Health, Mexico) reported the results of a study of low-risk sexual transmission groups (general population, students, and health personnel) and high-risk sexual transmission groups (prostitutes and gay men) in six Mexican cities. Indices were calculated for each group for knowledge, attitudes, and risk of infection (modified cumulative infection probability). It was found that knowledge levels were highest among gay men (87.4%) and lowest among female prostitutes (79%). Attitude index was highest for those in the high-risk group and lowest for health personnel. Female prostitutes were significantly higher than the other groups on the infection-risk index. However, this latter index was sensitive to adjustments in seroprevalence rates, and gay men were at significantly higher risk when the Mexico City seroprevalence rates of potential sexual partners were used.

Sexual Behavior. The World Health Organization and other national and international groups have worked hard to disseminate relevant information about HIV and AIDS to every part of the world. Despite these efforts, and despite the capability of today's mass media to reach the most remote areas of the globe, there are still indications that not everyone has the basic information. Oswaldo Reyes (Ecuadorian Red Cross, Quito) and associates described an assessment of knowledge about AIDS and high-risk activity among donors at the Red Cross Blood Bank in Quito, Ecuador. Half of the 678 respon-

dents did not know that AIDS can affect people in Ecuador; 20% thought that only homosexuals could have the disease; and 17% were unaware of the protection that condoms offer against HIV. Almost 25% of the men had visited prostitutes; 29% of the men had more than 5 lifetime sexual partners; and 58% had never used condoms. Among women, 5% had more than 5 lifetime sexual partners and 85%had never used condoms. Only 1% of the sample had used IV drugs.

A number of researchers reported on aspects of knowledge, attitudes, and behavior related to AIDS in African groups. In a Ugandan study reported by Maako Musagara (AIDS Control Programme/Rakai Project, Uganda), 86% of 1,304 interviewed adults knew of sexual transmission of HIV and 83% believed that changes in behavior can reduce risk of AIDS. While a large majority of interview respondents self-reported changing to lower risk behaviors, this was not borne out by discussions in 35 focus groups from the same areas. Much risky behavior still persists. These findings were consistent with those of Ntsomo Payanzo and colleagues (IPN and Universite de Kinshasa, Zaire) who found that more than 90% of a sample of permanent residents, transients, and prostitutes had heard about condoms, but only a small percentage regularly used them.

Bombelenga Bomboko (Ecole de Sante Publique, Zaire) reported his team's findings on the determinants of risk behaviors among college students in Zaire. In their sample of 1,480 randomly selected, mostly Catholic, students (80% male), much high-risk sexual behavior was reported: 84% had already had sex, 37% with 2 to 3 partners in the last year; 19% had had sex for money; 29% had paid to have sex with someone other than a prostitute; 31% had visited a prostitute at least once a month. Among those who did not visit prostitutes, 48% gave as a reason the desire to avoid sexually transmitted diseases, 30% for religious reasons. Sexual practices reported were as follows: 64% vaginal intercourse only; 33% sometimes include oral-genital contact; 19% sometimes practice anal intercourse; 20% of the students reported not feeling any pleasure while having sex. In a separate report by the same group (using the same sample), it was found that 7% reported sexual contact with persons of the same sex at least once (36% before the age of 10; 25% between ages 10 and 15; 14% between ages 16 and 20; and 14% after age 20), with less than 1% expressing an intention to repeat the experience. Religion was the major reason given for this intention. It was concluded that homosexuality seems to be a sexual practice of early life, and cannot be considered as a risk factor for HIV, since none of the seropositive subjects had had sex with persons of the same sex.

Condom Use. Six surveys examining the heterosexual behaviors of 1,478 Central African farmers, truck drivers, teenagers, and health personnel were reported by Jean Paul Louis (Organisation de Coordination pour la Lutte contre les Endemies en Afrique Centrale, Cameroon) and collaborators. Among the findings were the wide variability of condom use: farmers, 2%; truck drivers, 12%; students, 36-54%; health personnel, 68%. The major motivations for condom use cited were contraception and prevention of STDs (more than prevention of AIDS). The researchers pointed out that the use of condoms is encouraged by publicity, wide accessibility, and low cost.

Mwana Jingu (Projet SIDA, Kinshasa, Zaire) and associates reported high condom use over a 2-year period in 175 (Zairian) married couples with discordant HIV serology. More than 70% of discordant couples were found to continue condom use during this period for all sexual acts. The desire to have children was the most important reason for noncompliance (10 seroconversions occurred during the study period). Similar high rates of condom use among HIV-discordant couples in Rwanda were reported by Jeffrey Tice and colleagues (University of California, San Francisco). In this study of 46 discordant couples, follow-up after about a year showed that the mean proportion of unprotected sexual episodes was higher in couples with an HIV-positive male (11 of 99 versus 4 of 93). None of the 21 HIV-negative men and 3 of 25 HIV-negative women had seroconverted.

SURVEYS AMONG HOMOSEXUAL/BISEXUAL MEN

The frequent practice of bisexual activities among men having sex with men was pointed out by several Conference presenters. Peter Davies (South Bank Polytechnic, London) and colleagues, for example, reported that in their sample of 930 homosexually active men, 61.6% reported women sexual partners in their lifetime, 52.5% reporting vaginal penetration and 10.7% reporting anal intercourse. In the year immediately preceding the interview, 11.7% reported having had female partners and most of these also had penetrative sex with male partners. Of men who had both male and female partners, condom use was less common in vaginal intercourse than in anal intercourse with men. No condom use was reported by 80% of those with women partners in the previous month, compared with nonuse of 50% with anal intercourse with men. These findings show significant cross over in terms of risk.

Fewer general surveys of sexual behavior in homosexual/bisexual men were presented at this year's Conference. More differentiated studies (for example, aged subjects and types of recruitment) were reported, leading to a more precise understanding of exactly which groups of homosexual/bisexual men are engaging in high-risk behavior. Lance Pollack and others (University of California, San Francisco) explored reasons why about 40% of the cohort from the 6-year prospective study of 540 gay men in San Francisco continued to engage in unprotected anal intercourse. In men who were in mutually monogamous relationships, the most common reasons were: having the same serostatus as one's partner (71%), being in love (55%), and responding to the partners request (32%). Men in non-monogamous or casual relationships reported: being sexually turned on (60%), responding to partner's request (60%), that unprotected sex felt better (51%), and assuming that partner didn't want to use condom (45%). The authors concluded that condom use needs to be eroticized, communication and resistance skills need to be taught, and community norms in support of condom use should be further developed.

Janet Harrison and others (CDC, Atlanta), in collaboration with San Francisco Department of Public Health, compared condom use in homosexual men in a cohort study (156 subjects) and a cross-sectional study (133 subjects). All participants were asked to give the single most important reason for not using a condom during their last unprotected anal sex experience. Cohort men were more likely to be white (90% versus 54%) and to have had their last unprotected anal contact with a steady partner (76% versus 57%). The following reasons were given for not using condom in the two studies:

	Cross-Sectional	Cohort
Usage problems/interrupts sex	22.3%	15.7%
Not available/sex unplanned	26.5%	20.9%
Partner's responsibility/relationship	14.1%	20.3%
Partner with same HIV status	5.8%	20.9%
Alcohol/drug use	6.6%	2.6%

The authors cautioned that these findings may be specific for homosexual men and therefore may not be generalizable or representative of other populations.

A study designed to answer the question "Why are young gay men engaging in high rates of unsafe sex?" was reported by Robert Hays and others (University of California, San Francisco). Self-report questionnaires, to be returned by mail, were distributed in settings frequented by young gay men (aged 18–25) in 3 mid-size California communities: Eugene, Santa Cruz, and Santa Barbara. From the surveys returned (99), 43% of respondents reported unprotected anal intercourse in the preceding 6 months, with reasons given as: greater enjoyment of unprotected anal intercourse, perceived lower risk of unprotected anal intercourse, poorer

communication skills with sexual partners, and greater likelihood of having a boyfriend/lover. Reasons for unsafe sex included being "in love," using drugs/alcohol, and being "too sexually turned on" to stop to put on a condom. Respondents believed that HIV infection risk was significantly lower with younger, as opposed to older, gay men. Thomas McManus (King's College Hospital, London) and colleagues reported similar findings in a study of 111 gay men under age 21 in London. They found that the subjects were engaging in sex which is likely to put them at a higher risk than older groups, especially receptive anal intercourse (average 14.4 in month prior to the survey, compared to 3.3 times for men older than 21).

Relapse. A rather telling number of studies at this year's Conference looked at the issue of relapse—that is, homosexual/bisexual men who had adopted safer sex practices, but then went back to previous risky behavior. Ron Stall (University of California, San Francisco) reported that in his team's 5-year prospective study of 575 gay men, 19% reported relapse after having only had safe sex. Reasons given by men in monogamous relationships were being "in love" or having the same HIV status as their partner, while men not in relationships reported being "turned on" or inebriated during sex or not having condoms available when sex occurred.

In an effort to to predict relapse in homosexual and bisexual men, a discriminate function analysis (DFA) was used in a study reported by Brad Bartholow (Denver Disease Control Service) and others. The DFA correctly classified 86% of 200 subjects in a longitudinal design into high risk, stable high risk, stable low risk, and protective behavior change groups. Discriminating factors included

- sexual behavior self-perceptions
- perceptions of risk regarding sexual behaviors with monogamous, nonmonogamous, and occasional partners
- use of drugs and alcohol
- reasons for sex
- practice of health seeking behaviors
- Hispanic ethnicity
- age
- number of partners prior to learning about AIDS
- number of years of AIDS awareness

In a longitudinal study of 2,306 gay men, reported by Kevin O'Reilly and others (CDC, Atlanta), only 4% of seronegative men were found to increase their risk. Factors associated with relapse included low self-efficacy, poor perception of community support, excessive alcohol consumption, and alcohol or drug use together with sexual activity. Janet St. Lawrence and associates (University of Mississippi Medical Center, Jackson) found that relapse was strongly predicted by strength, frequency, and reinforcement value of past unsafe behavior in a 16-month follow-up of 68 gay men. Younger age, lower educational level, and sex when intoxicated also predicted relapse. In a study of relapse of 212 gay men, Maurice Adib and others (University of Michigan, Ann Arbor) found that only serostatus reliably predicted relapse, with HIV-positive men more likely to revert to unsafe sex than HIV-negative men. Overall, 82% to 87% of the subjects continued to use condoms consistently for both anal receptive and insertive sex 1 year later.

Serostatus. Dr. Leon McKusick and colleagues (University of California, San Francisco) reported that in a survey of 540 homosexual and bisexual men, 83% of those who were HIV-negative preferred HIV-negative partners for romance and sex. Of those never tested, 74% preferred HIV-negative persons for romance and sex. Fewer HIV-positive persons expressed preferences based on HIV serostatus, but some HIV-positive preferred persons who were also HIV-positive, citing their concerns for infecting someone who was healthy, and that someone who was HIV-negative could not empathize emotionally with an infected person.

A major study of behavioral and sociological risk factors for HIV seropositivity and condom use in homosexual and bisexual men in Mexico was reported by Jose Izazola (Ministry of Health, Mexico and Harvard School of Public Health, Boston). Interviews to determine knowledge, attitudes, and practices about AIDS were conducted and blood samples were collected to determine serostatus for 715 men in gay gathering places in six cities. Seropositivity rates varied from 3% to 26% from city to city, with seropositivity being associated with: city, anal insertive/receptive behavior, history of syphilis, sex with a person with AIDS and sex in bath houses. Condom use rate was 30%, the higher usage rates in cities where prevention programs were initiated earlier.

SURVEYS AMONG INTRAVENOUS DRUG USERS

Intravenous drug users (IVDUs) have traditionally been hard to reach with ordinary educational and communication efforts. In addition, it has been difficult to introduce reinforcements that are powerful enough to offset the biological reinforcement obtained from the drug "high." For this reason, most intervention attempts have been directed at trying to get people to avoid needle sharing, or to clean needles with bleach before reusing them (see "Education," below).

Much initiation into IV drug use is through needle sharing. A shared needle is a sign of shared friendship, intimate relationship, trust, and belonging to a common culture or group. It can also meet concerns regarding safety of the product when drug dealer and buyer share a needle. Sharing of HIV-contaminated needles is currently a major and growing vector in the spread of HIV. Consequently, the study of needle sharing by IVDUs is a major research focus. Questions concern the extent and correlates of needle sharing in different populations and conditions under which needle sharing can be reduced or eliminated.

Extent of Risk Behavior. In his plenary lecture, Dr. Gerald Stimson (Westminster Medical School, London) presented an overview of the epidemiological and behavioral issues in HIV infection among IVDUs. Based on a review of the literature and Dr. Stimson's own experience and studies, several behavioral issues were summarized as follows:

- Variations in HIV seroprevalence among IVDUs appear to be linked with the time of introduction of HIV into a particular community, to the local mix of individuals in a community, and to drug-injecting practice and high-risk sexual behaviors.
- Cocaine use is associated with both high-risk sexual behaviors and unsafe injection practices.
- The high fertility of female drug injectors exacerbates the growing problem of perinatal transmission.
- Some evidence suggests that injectors are changing, albeit slowly, their behavior—adopting protective injection strategies and changing social etiquette.
- Long-term strategies must include treatment for drug use and measures to help HIV-risk reduction for those who continue to inject.

A number of the Conference presentations found that basic knowledge about HIV transmission has indeed penetrated into most IVDU communities. These same studies, however, also show that much risk-taking behavior continues to take place.

Peter Kernidt (Los Angeles County Department of Health Services) and others reported their findings from interviews with, and HIV testing data of, 1,072 IVDUs in a methadone treatment program in Los Angeles County. Overall, the seroprevalence rate was 2.4%, but it was 4% among IVDUs who reported having used IV drugs outside Los Angeles County. Needle sharing was reported by 77%, with 40% stating they had used bleach to clean their needles. Interestingly, but perhaps not surprisingly, IVDUs who had used drugs in New York state had a higher seroprevalence rate (29.2%), and were more likely

to report needle sharing with a homosexual or bisexual drug user. These findings support the summary statement by Dr. Stimson regarding differences in HIV-infection rates and risk behaviors in individual communities.

Similarly, results of a large German epidemiological study of IVDUs, reported by Dieter Kleiber (Sozialpaedogogisches Institut Berlin) and colleague, revealed that seroprevalence is related to geographic and sociological aspects of the particular IVDU community. A representative sample of 1,267 IVDUs was interviewed and tested. The overall HIV prevalence rate was 19.9%, with major differences by location, drug scene, subpopulations with different sociological backgrounds. Needle-sharing habits were reported changed by nearly 60% of the addicts, but only 48% reported changing sexual behaviors.

Sexual Behavior. Diane Lewis (University of California, Santa Cruz) and Dr. John Watters (University of California, San Francisco) reported on the high-risk sexual behaviors of two groups of 336 male IVDUs: those with both male (70%) and female (63%) partners, and those with exclusively female partners. Of the bisexual men, 12% reported heterosexual anal intercourse (compared to 12% of the exclusively heterosexual men) and 33% reported homosexual anal intercourse; 56% of the bisexual men never used condoms for anal intercourse. Bisexual men were significantly more likely to exchange sex for drugs or money (51%) than were the heterosexual men (16%). Of the men having sex with both males and females, 49% self-identified as heterosexual, 37% as bisexual, and 14% as homosexual. This population requires special risk-reduction outreach since so many self-identify as heterosexual but may be inadequately served in heterosexual AIDS prevention campaigns.

A study describing sexual risk behavior in female IVDUs was presented by Carolyn McKay (University of Miami School of Medicine) and associates. Of 150 Miami women interviewed and tested for HIV, 70% reported sex with multiple partners; they were more likely to use condoms than women having a single partner. Both groups tend to have partners who are also IVDUs. Rates of seropositivity were above 30% in this population.

Les Pappas (San Francisco AIDS Foundation) and others presented data indicating that a large majority of female sexual partners of male IVDUs never use condoms during vaginal intercourse (71 of 100 women in this study). Kyung-Hee Choi and others (University of California, San Francisco) reported that the following variables were significantly associated with condom use in 77 female sexual partners of IVDUs: recent discussion of condom use with partner, partner's neutral or positive reaction to suggestion of condom use, and perception of AIDS risk. The authors argued that individual counselling and health education appeared to be insufficient in effecting adoption of safe sex practices; couples counselling was indicated.

Hilary Klee (Manchester Polytechnic, U.K.) described her findings from interviews of 303 IVDUs contacted through drug agencies or outreach work. Related to needle sharing were the following variables: low level of knowledge about drug-related health problems, homelessness, peer relationships with a history of sharing, and lack of close friends. Condoms were used by only 25%, mostly restricted to casual sexual encounters.

Rena Arshinoff and others (University of Toronto) presented data from 353 IVDUs in Toronto and Montreal treatment centers indicating that needle-sharing behaviors are associated with difficulty in obtaining personal injection equipment, injection of cocaine, and needles obtained from shooting galleries. (The seroprevalence rate was 3.1%.)

Several studies show that the form and context of needle sharing may be changing. Margaret Connors (Spectrum House, Amherst, Massachusetts) reported recent changes in needle-sharing practices. Needle sharing is giving way to needle lending, where users carry one needle for personal use, one for lending. The infection risk thus passes to the user who is forced to borrow. Additionally, many users are now making attempts to discern who has tested positive for HIV. Those who are HIV-positive or have avoided testing

are forced to go last during needle sharing, or are avoided altogether. A study reported by Jean-Paul Grund and others (European Addiction Research Institute, Rotterdam) suggests that the actual sharing of needles is only one of the dangers of infection with HIV in the daily injecting routine. Drugs were shared in approximately half of the observed drug-taking events, and in more than 80% of these events, it was done by "frontloading," a special technique using two syringes.

The Emerging Role of Cocaine. The upsurge in the use of cocaine, especially crack (smokable cocaine), in many countries, has given rise to a number of studies that have analyzed the relationship of the use of this drug to a variety of high-risk behaviors, including transmission of sexually transmitted diseases (STDs), including AIDS. Al Abramowitz (San Francisco Department of Public Health) and colleagues presented an analysis of trends in crack use, which is thought to be associated with higher risk of HIV infection. Data were collected from all clients (3,673) entering drug treatment during a 6-month period in 1986 and again in 1989 (4 446). Cocaine use was reported by 31% of the admissions in 1986, and by 44% in 1989. Cocaine was listed as the primary drug by 11% in 1986, and by 20% in 1989. Cocaine users who preferred smoking the cocaine increased from 53% to 78%. Latinos and youths under age 26 accounted for increasing proportions of crack users and blacks constituted the majority of users. In a separate presentation, Wayne Clark (San Francisco Department of Public Health) and others found that in IVDUs entering treatment, cocaine-using needle sharers reported more frequent drug use, and more multiple needle-sharing partners than non-cocaine users. Black and Latino sharers were more likely to use cocaine than other ethnic groups.

A series of focus groups with black, white, and Latino IVDUs was conducted by La Mar Hasbrouck and associates (University of California, San Francisco and Bayview-Hunter's Point Foundation). These researchers reported that drugs, specifically crack, have become the prominent currency used to buy sex. Black male IVDUs were less likely to use condoms when having sex while using crack—as compared to heroin users—because of perceived lower risk for HIV. In all racial groups, sexual partners also used drugs. The authors concluded that the fear of AIDS does appear to be modifying some practices, such as increased sterilizing of needles, avoidance of needle sharing, and condom use for most sexual acts except oral sex.

Relationships among gender, crack use, and high risk behaviors in 340 black sexually active 13–19-year-olds were reported by Eve Golden (University of California, San Francisco and Bayview-Hunter's Point Foundation) and colleagues. Likelihood of STDs was predicted by the total number of drugs used by both male and female crack users and among male nonusers. Exchange of drugs for sex and having sex in association with crack or alcohol use predicted pregnancies for female crack users. (Among female nonusers, pregnancy was predicted by age of first intercourse.)

A survey of HIV-related risk behaviors among male cocaine users was reported by Mark Kowalewski and coworkers (University of California, Los Angeles). Of 167 admissions for cocaine dependence to a VA hospital drug dependence unit, 20% used IV cocaine in the previous year, and 95% shared needles. Bleach use when sharing needles with strangers was reported by 25%, whereas 20% reported always using bleach. Sex with men during the previous year was reported by 7% and ,of these, 71% reported anal sex; 86% reported sex with females (of these 20% reported anal sex). Half the respondents reported being high on drugs or alcohol most of the time during sex; 21% paid for sex with drugs, 13% with money. Because of AIDS, 73% stated they reduced the numbers of sexual partners and 42% said they were more selective about partners.

Frederick Snyder (NOVA Research Company, Bethesda, Maryland) and colleagues reported that more-recent cocaine injectors exhibit less risky behaviors than those who have

injected for at least 2 years. Data presented showed that the following behaviors increase as number of years of cocaine use increases: use of shooting galleries, sharing needles with two or more others, and borrowing used "works."

SURVEYS AMONG ETHNIC MINORITY GROUPS

A number of U.S. researchers have found differences in various racial and ethnic groups regarding basic knowledge about HIV and its transmission as well as in HIV-related risk taking behavior. Robbie Hayes and others (R.O.W. Sciences, Inc.) reported on the findings of 36 focus group discussions that were conducted in six regions of the U.S. The focus groups were designed to explore knowledge, beliefs, and attitudes among African-Americans Haitians, Cuban-Americans, Mexican-Americans, and Puerto Ricans, and to identify strategies perceived to be potentially effective in providing AIDS information and education to these communities. The authors found that traditional communication methods are not effective and that culturally-specific information and strategies would probably enhance the impact of HIV prevention messages. Analysis of the focus group discussions also revealed that strategies that may be effective with one group will not be effective with others.

Daniel Minns (National Task Force on AIDS Risk Reduction, San Francisco) reported on a study measuring AIDS knowledge, attitudes, and behaviors among black homosexual men in the United States. The study also sought to assess the effectiveness of AIDS education efforts for this group and to determine possible ways to improve prevention and treatment policy and planning for them. Black men, 950 total, were interviewed on the basis of memberships in gay organizations (40%) or in locations where men meet men for sex in 24 areas by local interviewers. Generally accurate information about AIDS was reported; however, large numbers continue to practice unsafe sex. Correlates of high-risk behavior included beliefs about discrimination, or a Russian roulette approach to AIDS. The author believes that peers and word of mouth are especially important in motivating this group to change behavior. Felicity LaBoy (Midwest AIDS Biobehavioral Research Center, Chicago) and colleagues also found some evidence of a "pro-risk" social norm among black gay men. Indeed, the "safer sex" cultural shift appears not to have taken place. The authors reported that less change has occurred in minority communities in settings for high-risk or anonymous sex, such as bar "back rooms," bathhouses, or public parks, compared with the white gay community.

Self-Identification. Michele Russell (New York Hospital-Cornell Medical Center) pointed out that resistance to self-identifying as gay or homosexual should be taken into account in designing messages for black gay and bisexual men. Men who readily acknowledged "man to man sex" resisted terms such as "gay" or "bisexual" and denied behavior when these terms were used, apparently maintaining the idea that the experience is sexual and not an emotional relationship. Antonio Jimenez and others (University of Illinois, Chicago) reported on 45 interviews conducted in two black oriented gay bars in Chicago. Their findings confirmed those of others that self-identification as gay is lower among blacks than among homosexually active whites. In this study, not identifying as gay was the strongest predictor of more risk behavior, indicating the importance of this variable.

Acculturation. Two presentations dealt with the issue of acculturation among Mexican Americans. Barbara Marin and associates (University of San Francisco) reported findings on acculturation differences in condom use by Hispanics. Using random-digit-dialing, 523 Hispanic adults in San Francisco were interviewed (42% male; 24% U.S. born; mean age, 36). A five-item scale was used to measure acculturation (familiarity with U.S. culture and language): 28% of the men, 7% of the women reported risky sexual behaviors in the preceding years (multiple sexual partners, sex with a prostitute or with another man). Of men reporting high-risk sexual behavior, 46% reported always using a condom.

Acculturation, therefore, did not relate to condom use by men. Among women, however, 44% of the highly acculturated kept or carried condoms, compared to only 17% of the less acculturated. More than half of all women in the study were embarrassed to buy condoms, and more than one-third were embarrassed to ask partners to use condoms. The authors concluded that methods to reduce sexual embarrassment and enhance sexual communication are needed. Maria Fernandez and colleague (University of Arizona, Tucson) studied the influence of acculturation on the perceived risk for AIDS among 600 Mexican Americans. Educational level was found to be a more important predictor of the perception of risk for AIDS than acculturation (although the authors contend that the two are interrelated). Subjects who lacked accurate, specific knowledge about HIV and its transmission reported negative attitudes against those assumed to transmit it. The authors concluded that the role of fear and stigma need to be considered in designing AIDS prevention programs for this population.

SURVEYS AMONG ADOLESCENTS

Based on recent regional seroprevalence studies and AIDS incidence data, adolescents are fast becoming an at-risk group for HIV infection. This appears to be directly related to the fact that adolescents actively engage in the very behaviors known to facilitate HIV transmission—namely, sexual intercourse (often with multiple partners) and IV drug abuse. Perhaps most disturbing is the fact that while adolescents engage in risky behaviors, they are often remarkably poorly informed about their risk.

Sexual Activity and AIDS Knowledge in Adolescents. In the United States, it is estimated that 11.6 million teens have had heterosexual intercourse: 17% of males and 5% of females, by age 15; 64% of males, and 44% of females, by age 18. By the time Americans leave their teenage years, more than 80% of the males and more than 70% of the females have had sexual intercourse. These findings are consistent with those reported by Lloyd Kolbe and associates (CDC, Atlanta) in their summary of the CDC initiatives targeted toward preventing HIV infection in youth: A major survey of HIV knowledge, attitudes, and behavior was conducted in 34 states among 9th to 12th graders. It was reported that 1.3% to 5.4% had injected drugs; 0.2% to 2.8% had shared needles; 26.5% to 80.3% had engaged in sexual intercourse; and 6.5% to 40% had four or more sexual partners.

Other findings from this same study were reported by Deborah Holtzman and others (CDC, Atlanta). Of the 8,098 respondents, the mean number of correct knowledge items was 14.5 (range 0-17); 53.7% of the students had AIDS education in school. Overall, students with higher knowledge scores were less likely to have ever used drugs (paradoxically, for males, the relationship was in the opposite direction). HIV instruction was positively associated with HIV knowledge, and HIV knowledge was associated with other outcome measures of drug behavior. The results were interpreted to suggest that HIV knowledge may have a positive effect in reducing HIV-related drug behavior among high school students.

Ralph DiClemente and associates (University of California, San Francisco) reported findings indicating that risk factors in high-risk junior high students may be multiplicative—those with more sexual partners are progressively less likely to use condoms. Lee Strunin and associates (Boston University School of Health) reported research that addressed the question "Do beliefs about HIV, condom use, pregnancy, and STDs predict adolescent condom use?" Telephone interviews with 900 16–19 year-olds found that those who thought condoms are effective in reducing HIV transmission and carry them were 3.2 and 1.8 times more likely to always use them. Also predictive of condom use was the perceived likelihood of pregnancy without condoms, being asked by partners to use condoms, the perceived likelihood that women ask partners to use condoms, and beliefs about the social acceptability of condoms.

Steven Keller and colleagues (University of Medicine & Dentistry of New Jersey, Newark) reported a survey of 407 healthy inner-city heterosexual adolescents in northern New Jersey which found high levels of AIDS-related knowledge. However, high levels of sexual-risk behaviors were also found, with moderate use of alcohol or marijuana being the strongest predictors of high-risk sex. (1.24 % of the adolescents were HIV-positive.) In several studies of Massachusetts adolescents reported by Ralph Hingson and associates (Boston University), it was found that respondents were significantly less likely to use condoms after drinking compared to when they were sober.

Juan Ordonana (Direccion General de Salud, Murcia, Spain) reported his team's findings from an anonymous written questionnaire administered to youths and young adults whose mean age was 16.17 years. While most of the 1,750 respondents had a high level of knowledge, a significant number did not know you cannot get AIDS from donating blood (56%) or from insects (31%). Nearly 18% of the subjects had engaged in sexual intercourse; of these 65% had used condoms during the previous year. High knowledge of AIDS and its transmission was also reported by Saidi Kapiga and others (University of Dar es Salaam, Tanzania) among a group of youths (mean age 17.5) of whom 61% were sexually active.

Pal Kraft and associates (Institute of Public Health, Oslo) reported a Norwegian survey of 3,000 adolescents aged 17-19, in which those who had sexual intercourse at early ages were less likely to have used a condom than those who became sexually active at later ages. Condom use was lower if alcohol was involved in first intercourse. These findings are consistent with those reported by Bjarne Rasmussen (AIDS Secretariat, Frederiksberg, Denmark) and colleagues who also reported that students' main source of information about AIDS was television/radio and school, with 49% not talking about sexual problems at home. Only 11% go to their fathers regarding sexual questions.

In one of the earliest AIDS research reports from the Soviet Union, Galina Mozharova and V. V. Pokrovsky (Central Research Institute of Epidemiology, Moscow) discussed AIDS knowledge levels among Moscow youths aged 14-18. From 84% to 98% of the subjects correctly answered questions dealing with the modes of HIV transmission, and 73% to 90% were aware of preventive measures. These young people expressed a preference to get AIDS information from television and from the press.

Runaway and Homosexual Adolescents. Two related studies of gay and runaway male adolescents and female runaways were presented by Cheryl Koopman and colleagues (New York State Psychiatric Institute and Columbia University). Interviews with 121 gay and runaway (mostly black and Hispanic) male adolescents in New York City revealed that most had begun to engage in high-risk sexual activities at an early age (mean age of first receptive anal sex was 14.2; of penile-vaginal intercourse was 10.7). Condom use was infrequent (38% or less). Close to half had had more than 10 male or female partners. Receptive anal sex was engaged in by 47% of the gay males; insertive anal sex by 19% of the gay runaways. Interviews with 75 female runaways revealed a relationship between pregnancy and the following factors: trouble with the law; drug use; frequent, unprotected sexual intercourse; and high risk sex with multiple partners.

G. Cajedtan Luna and others (Mount Zion Hospital, San Francisco) compared homeless and runaway youth aged 16-19 from the streets of Rio de Janeiro and San Francisco. The results indicated that both groups engage in unprotected receptive anal sex, do not perceive the threat and consequences of AIDS, and minimize the need for condoms. Much prejudice, guilt, and shame associated with diseases in general, and AIDS and condoms in particular, complicates the view held by street youth. Appropriate intervention would include addressing these issues in educational programs for this population, and in providing condoms.

SURVEYS AMONG COLLEGE STUDENTS

At the college level, knowledge about HIV and AIDS is purportedly very high. However, knowledge alone does not insure that measures to reduce risk will be adopted. The large majority of college students do not perceive themselves to be at any risk of infection. Because of this mistaken perception they feel behavioral change is unnecessary for them. By not modifying their sexual behaviors, a large number of college students may be unwittingly placing themselves at elevated risk of becoming infected with HIV. (A recently published seroepidemiological study conducted on 19 U.S. college campuses—16,863 students—found an HIV prevalence rate of about 1 in 500, or 0.2%.)

A study reported by Karen Kotloff (University of Maryland School of Medicine, Baltimore) and associates followed up on 297 students who had been previously identified as "high risk" from a total pool of 3,394 students engaged in a voluntary serosurvey. Risk categories included male homosexuality/bisexuality (26%), IV drug use (15%), and high-risk sexual partners (89%). A history of STD was reported by 25%, which was related to numbers of sexual partners in both high-risk women and men; 30% had never used condoms, and only 55% reported increasing condom use as a result of HIV/AIDS knowledge.

A New Jersey study of 923 students from four campuses reported by Daria Boccher-Lattimore and others (Rutgers University, Newark and New Brunswick, New Jersey) found that pooling data from different race and gender subgroups masks effects that are obtained when the data are considered separately. Thus, in women, predictors of condom use included higher self-efficacy, but not in men. For black men, more homophobia was associated with less condom use; for Hispanic men, perceiving fewer barriers to condom use was associated with greater condom use. No significant predictors were found for white men. Social Cognitive Theory, the Health Belief Model, and the Theory of Reasoned Action had limited utility in explaining the findings of this study.

SURVEYS AMONG WOMEN

Until recently, in certain parts of the world, AIDS was viewed as a disease affecting mostly homosexual men, persons with hemophilia (male) who had received contaminated blood clotting factor, transfusion recipients, and IVDUs (mostly male). Reports from Africa and certain Caribbean countries, however, showed that females were just as likely as males to be infected. More recent evidence in North America and Western Europe shows a trend toward increasing numbers of HIV-infected females. Several epidemiological Conference studies provided AIDS incidence data and HIV seroprevalence rates among women (see Chapter 3: EPIDEMIOLOGY).

Risk Behavior in Women. A number of researchers have studied sexual risk-taking in women, as a way of exploring susceptibility to HIV infection. The availability of women in family planning clinics, prenatal clinics, or clinics for STDs has made such women the focus of several studies. The largest of such studies was carried out in the Philadelphia area and involved a description of HIV risk factors among 17,619 women. Among the findings of this study, reported by Susan Robbins and associates (Hahnemann University, Philadelphia), were the following: Women with a history of STDs were more likely to be African-American, aged 20-24, and from urban sites. This population was also found to have had a disproportionately higher number of sexual partners. Condoms alone or in combination with another method of contraception were used by only 20% of the total group. Heterosexual risk (56%) was found to be the most significant risk factor in women, particularly in those having two or more sex partners per year (48%), followed by those with a history of STDs (20%). While only 125 women in the total group were tested for HIV status, 8.8% of these were found to be HIV seropositive. HIV-positive women were disproportionately Latino, age 25 or older, having a history of STDs, and having two or more sexual partners per year. Only 30% of the HIV-positive women ever used condoms.

In a study of the ethnic and risk-perception associates of unprotected sex among young sexually active women, Denise Beirnes (Planned Parenthood, Alameda/San Francisco) and colleagues found that 88.3% of 210 women had unprotected vaginal sex with primary partners, 76.8% with casual partners. Anal sex with primary partners was reported by 5.3% of the women, 9.6% with casual partners. Only one of these women had ever used a condom. No statistically significant relationships were found among ethnicity, risk beliefs, and condom use.

Women attending a prenatal clinic in West Palm Beach, Florida, were studied by Tedd Ellerbrock (CDC, Atlanta) and coworkers. In the 219 women studied, 95% thought they should take precautions to avoid HIV infection; however, only 7% had used condoms during 80% of sexual contacts. The 5.5% of women who were HIV-positive were younger at the time of first sexual intercourse (48% of the total had had sex before age 16), and had more sex partners.

Prostitution. Street-based prostitution, by its very nature, increases the risk of HIV infection to the women involved, as well as to their sexual partners. Although surveys from all over the world reported that most women are familiar with AIDS and its risks, ignorance about risk reduction was still prevalent. Most of the studies of female sexual behavior revealed alarmingly low percentages of women using condoms. However a study reported by Rosemarie Santana (Department of Health Research Institute for Tropical Medicine, Philippines) found no relation between the frequency of condom use and prevalence of STDs in a sample of 680 Philippine commercial sex workers. Accuracy of reports of condom use and correct use of condoms, however, may be an issue.

A Philippine study reported by M. Lourdes L. Quintos (Kabalikat ng Pamilyang Philipino Foundation, Manila) and collaborators found that Philippine prostitutes believed that self-medication, careful inspection of customers, and personal hygiene (especially douching) were believed to be effective measures against STDs and AIDS.

In their attempts to control street-based sex work, law enforcement activities may have in fact increased the risk of HIV transmission to and by prostitutes, according to a study reported by Charlton Clay and associates (University of Washington, Seattle). Results of 25 ethnographic interviews led these researchers to conclude that carrying the tools of AIDS prevention (condoms, bleach, and clean syringes) flags illegal behaviors and escalates women's risk for police harassment and arrest. Enforcement strategies thus work at cross-purposes with community health outreach. An alternative to such law enforcement strategies was presented by Russell Reade (Nevada Brothel Association) and associates. They reported that despite high sexual activity rates among 350 female sex workers affiliated with Nevada's brothels, no HIV seroconversions have occurred in this group and the gonorrhea rate remains at about 1%. They concluded that a combination of licensing requirements, mandatory condom use, on-site AIDS prevention education for workers, and promotion of low-risk nonpenetrative sexual practices is critical to the AIDS prevention success of the Nevada brothel system.

Reproductive Decisions. A study reported by Jane Cowan and associates (University of Maryland School of Medicine, Baltimore) followed a group of 48 HIV-positive and 98 HIV-negative (but at-risk) pregnant women to assess their reproductive choices. Among those at or under 24 weeks gestation on initial prenatal evaluation, HIV-positive women were more likely to obtain a therapeutic abortion. However no significant differences were seen between the groups in numbers of subsequent tubal ligations or subsequent pregnancies. Similarly, Angelita Balanon and coworkers (New York City Department of Health) surveyed women (aged 15-44) pregnant or planning pregnancy, who had real or perceived risks of HIV infection about their intentions regarding the pregnancy. Of the 609 women interviewed, 56% had self-perceived risks for HIV infection, 26% were in fact at risk, and 19% had both perceived and real risks. All were asked if they would terminate pregnancy if tested HIV-positive. Responding "yes" were the following: 83% with perceived risks; 35%

with actual risk; 60% with perceived and real risks. Non-Hispanic (67% versus 33%) and less religious (60% versus 40%) women said they would terminate the pregnancy. These data indicate that perceived risk, ethnicity, and religious beliefs all enter into decisions to terminate pregnancy if HIV-positive.

A study of asymptomatic HIV-positive women was reported by Maeve Foreman and colleagues (St. James's Hospital, Dublin). These women were asked of their plans for their own and their children's future needs should they get an HIV-related illness. Women without children were much less likely to have made any plans than those with children. The authors concluded that despite significant handicaps—drug use, single parenthood, poverty, and stigmatizing illness—HIV-positive women with children plan ahead in making provisions for their children should they become ill and die.

In another study of HIV-positive women, Bryan Leibman and associates (Brown University, Providence, Rhode Island) prospectively followed three groups of infected women for 6 to 42 months: 11 women infected via heterosexual transmission; 21 women infected via IV drug use; and 26 women who had both IV drug use and prostitution as risk factors. Members of the heterosexual transmission group had fewer sexual partners and were more likely to have a stable heterosexual relationship.

Correlates of Risky Behaviors in Females. Adeline Nyamathi and colleague (University of California, Los Angeles) described the results of their study of 327 relatively high-risk black and Hispanic women from drug recovery programs and homeless shelters in Los Angeles. These women were IVDUs, sexual partners of IVDUs, women with a past history of STDs, or homeless women. Women who had high scores in any single resource—coherence, self-esteem, or support—reported less emotional and physical distress, and those with high levels of all three resources were less risky in their sexual behavior. Women with high self-esteem or coherence were less likely to engage in high risk activities. The implications of this study clearly point to the need for helping women to become empowered, thus reducing their vulnerability to high-risk sexual activities.

In an evocative study reported by Jacqueline Coberly (Johns Hopkins University, Baltimore) and associates, 83 recently pregnant Haitian smokers were compared to 34 former smokers and 303 nonsmokers. Smokers were found to be less likely to be married, have had first intercourse earlier, have had more lifetime sexual partners, be of a different religion, and to be infected with HIV, the latter association was still significant when adjusted for the effects of multiple variables. Still to be examined is the question of whether smoking has a biologic effect. The role of smoking in relation to STDs was also explored by Ernesto Guerrero and associates (Centro de Orientacion e Investigacion Integral, Santo Domingo, Dominican Republic) who found that female sex workers who smoked reported higher rates of STDs.

Interventions. A study of condom use by 77 female sexual partners of IVDUs reported by Kyung-Hee Choi and associates (University of California, San Francisco) found the following predictors of condom use: woman's perception of risk, discussion of condom use with partner, and man's anticipated attitude toward condom use. The authors concluded that future intervention should include work with men's groups to promote condom use, with women's groups to support self-protection and enhanced negotiation skills, and work with couples aimed at reaching mutually agreed upon strategies for avoiding HIV risk. These findings were consistent with the results of a series of focus groups comprised of 63 women of childbearing age and at high risk for HIV infection, reported by Chrystal Tunstall (University of California, San Francisco) and colleagues. These researchers found that many of the barriers to safer sex practices encountered by high risk women were not only institutional and socioeconomic, but also psychological. Among the psychological barriers that surfaced were feelings of inertia, isolation, stigmatization, powerlessness, and lack of

trust and self-efficacy. The conclusion of the researchers was a call to a multifocal intervention including counselling for behavioral strategies and support, drug treatment and recovery, and attention to normative health beliefs of the general community.

Using the theory of reasoned action as a framework with a sample of college women, Susan Middlestadt and Martin Fishbein (University of Illinois, Champaign-Urbana and CDC, Atlanta) concluded that sexually experienced and inexperienced women need different kinds of interventions to affect their intentions to tell their partners to use condoms. Interventions aimed at sexually inexperienced women should stress the potential health benefits of condom use, their intention being under attitudinal control; however, such an approach is not likely to be effective with experienced women. With this group, the normative pressure perceived by the women, especially from potential sex partners, must be addressed. This latter finding is consistent with the two University of California (San Francisco) studies cited above.

A study comparing interventions with 64 female sex workers comprised of counselling and testing only, condom skills training, or counselling, testing and condom skills training was reported by Peter Barchi (California State University, Long Beach) and colleagues. These researchers found that adding condom skills training to counselling and testing significantly increased condom use for vaginal sex.

EDUCATION

Because HIV transmission patterns vary geographically, education and intervention programs in different countries—and even in different regions of the same country—may require appropriately weighted differences with regard to emphasis. (In North America, Western Europe, South America, and Australia, for example, more than 50% of all AIDS cases occur among homosexual/bisexual males or IVDUs. In contrast, in sub-Saharan Africa and the Caribbean, heterosexual transmission is the primary mode of transmission. See Chapter 3: EPIDEMIOLOGY for a detailed discussion.) Education programs in major metropolitan areas in the United States, therefore, may focus on the risks of homosexual/bisexual behavior and IV drug use, whereas programs in Africa may target prostitutes and STD clinic patients.

CHANGING HIGH-RISK BEHAVIORS

Educational attempts at behavioral change can be implemented on three levels: societal, group, or individual. Societal efforts to change HIV-risk behaviors include those which attempt to inform the public at large, modify preferences for risky behavior, and prevent societal overreaction or discrimination.The majority of national programs have been attempts at the societal level. Examples include, the mailing of the U.S. Surgeon General's pamphlet "Understanding AIDS" to every household in the country and the television advertising campaign "America Responds to AIDS."

Group-based educational programs are those which are specifically targeted to groups who are deemed to be at high risk for HIV infection because of the likelihood of group members engaging in specific high-risk behaviors. Such groups include gay and bisexual men, IVDUs, sexual partners of IVDUs, persons with multiple partners such as commercial sex workers, adolescents, hemophiliacs, and others. Peers have been shown to be most credible for group-based educational programs. Most of the educational interventions reported at this year's Conference were group-based.

Individually based programs are frequently undertaken in a counselling format, and may be paired with HIV testing. Because this form of AIDS education is labor intensive and expensive, it is ordinarily used only with individuals at particular risk for being infected, or for infecting others (for example, HIV-positive persons).

Common to all risk-reduction efforts is the need to properly evaluate the program being implemented. Peter Aggleton and others (Bristol Polytechnic, Bristol, U.K.) reviewed a number of critically different strategies in relation to the evaluation of HIV education and health promotion. Alternative approaches to process and outcome evaluation were considered, differences among short-term, mid-term, and long-term evaluations were delineated, and consideration was given to evaluation techniques that can identify unintended consequences of particular interventions.

In the next section, group-based efforts to reduce high-risk behaviors reported at the Conference are summarized.

EDUCATIONAL EFFORTS IN TARGET POPULATIONS

Homosexual/Bisexual Men. A major project to evaluate the impact of four AIDS prevention programs on psychosocial factors thought to facilitate risk behavior change was reported by Christine Galavotti and others (CDC, Atlanta). In four U.S. cities (Denver, Seattle, Dallas, and Long Beach, California), 1,947 gay and bisexual men were recruited to participate in an intervention. A questionnaire was completed at study entry and at 6 months post-intervention (1,032 men returned for the 6-month assessment questionnaire). In all four cities subjects' confidence in their ability to protect themselves from AIDS increased; in three cities, risk-reduction skills increased; in two cities, perceived normative support for risk reduction increased; and in two cities a substantial increase in the belief that safe sex is effective was found post-intervention.

The effect of personal counselling on the use of condoms during oral/anal intercourse by gay men was reported by Sarna Seppo and S. L. Valle (University of Helsinki, Finland). It was concluded that repeated, closely argued personal counselling increased the use of condoms during anal intercourse from 78% never using to 68.7% always using or not practicing such sex. The percentage of men engaging in unprotected oral intercourse dropped from 97.2% to 66.1%, but most of the drop was accounted for in the "not practicing such sex" category.

Ted Myers and others (AIDS Committee of Toronto) evaluated the effects of two models of small discussion-group education on change in knowledge and behavior in 612 gay and bisexual men. It was concluded that a single, volunteer peer-led discussion group could have a positive change from unprotected to protected sex or abstinence.

Male Prostitutes. Paulo Longo and colleagues (Insituto Brasileiro de Inovacoes em Saude Social, Rio de Janeiro) reported on the effect of a street-based intervention on sexual behavior of 100 male prostitutes aged 15 to 23 in Rio de Janeiro. In 1988, 43% of the group was HIV-positive; this had increased to 69% by 1989. Before the street-based intervention program, 15% of the group used condoms with their clients; this increased to 65% after a 6-month intervention. These men are now beginning to develop their own prevention material and are organizing regular meetings.

IVDUs. An evaluation of a risk-reduction program for IVDUs was reported by Dale Chitwood (University of Miami School of Medicine) and colleagues. These researchers found that risk reduction in parenteral and other drug use and in sexual behavior could be achieved even with IVDUs not in treatment programs. In a separate presentation, this same team, led by Carolyn McKay (University of Miami School of Medicine), described a program designed to decrease risk behavior in female sexual partners of IVDUs. They enrolled 148 women in a standard or an enhanced intervention program who were reassessed after a 6-month interval to determine sexual behavior change. Almost half of the subjects reduced sexual risk taking, especially reducing the numbers of sexual partners who used drugs; 10% increased the amount of risk-taking behavior; about 40% made no changes at all. Another study measuring the impact of a risk-reducing intervention with female partners of IVDUs was reported by Marc Weiner and others (University of Texas

Health Science Center, San Antonio). An experimental intervention that reflected the values of Mexican-American culture was given to 107 female partners of male IVDUs and 42 heterosexual, male IVDUs. Results indicated that the women showed increases in HIV/AIDS knowledge, acceptance of personal risk, intention to change risk behavior, and skills to effect change. No changes were observed in the male IVDU subjects.

Dr. Don Des Jarlais and associates (Narcotic and Drug Research, Inc., New York) reported on an attempt to stimulate IVDUs to organize themselves to promote HIV prevention, mutual education, and individual counselling. Ex-addict outreach workers were used to provide IVDUs with HIV/AIDS education. The project led to decreases in drug injection frequencies, but not in unprotected anal or vaginal sex frequencies. Some evidence of increased use of bleach, new needles, and condoms was noted.

Reporting on the effects of an extensive street-based intervention involving 189 IVDUs in Italy, Gianstefano Martin (HIV Screening Section, Verona) and coworkers confirmed what others have also found—interventions have greater impact on reducing risk in drug-related behavior than in reducing risk in sexual behavior.

The need for interventions that do more than give information was underscored by a report by Paul Young (NOVA Research Company, Bethesda, Maryland). Reflecting responses to over 15,000 interviews with IVDUs not in treatment programs, it was found that 85% of IVDUs know that cleaning needles with bleach is an effective way of killing HIV, but despite this knowledge, only 11% actually always clean their needles with bleach before injecting drugs.

Results of a questionnaire administered to 833 street-based addicts was a report by Earl Harris (University of California, San Francisco). Of these, 23% reported that they shared needles with others. A wide variety of drugs were used, particularly heroin (91%) and cocaine (57%). Cleaning needles with bleach was reported by 80%; 90% indicated that they were willing to obtain clean needles from an outreach worker and 86%, from a clinic. These data indicate that addicts are aware of risk behaviors and are interested in reducing their level of risk.

Maura O'Brien (Network for Continuing Medical Education, Secaucus, New Jersey) described a normative framework within which issues relating to needle-exchange policies can be analyzed and recommendations can be formulated. Principles considered were those of human dignity, well-being, personal security, justice, and social solidarity. Ethical issues examined included confidentiality of program participation and HIV antibody test results, access of IVDUs to needle-exchange programs, and proportionality of benefits and harms to individuals and society.

In a major study of 4,007 heterosexual IVDUs in San Francisco (1986–89,) Dr. John Watters and others (University of California, San Francisco) reported that following an intervention including bleach distribution and needles exchanges, no evidence emerged supporting arguments that such programs stimulate drug abuse. These researchers encouraged additional outreach, education, bleach distribution, and needle-exchange programs.

Adolescents. The frequently tragic and ungrounded sense of invulnerability shared by adolescents was summarized in the 1987 Report by the U.S. House Select Committee on Children, Youth and Families: "Adolescence is . . . a period characterized by a sense of invulnerability that often leads to 'a good deal' of experimentation with sex and drugs." The report concludes that "If we fail to act now to limit the widening spread of AIDS, the public health threat of the '80s will become the public health and economic disaster of the '90s." Education programs for teenagers are seen as the only recourse.

Even before the House Select Committee Report, in 1986, the CDC institutionalized six initiatives to assist schools and other agencies working with youth to develop and implement programs to reduce HIV infection. Lloyd Kolbe and associates (CDC, Atlanta) reported on the activities to date. State policies for HIV education in schools have been

implemented in 41 of 50 states; an equal number have adopted a curriculum for such education; 35 have conducted personnel training programs, and 33 states assist agencies in HIV programs for school dropouts.

A number of presenters at the Conference described educational programs designed specifically for adolescents. The United States General Accounting Office assessed the status of AIDS education for youth, both in and out of school, as reported in a study by Cheryl Oros and associates (GAO, Washington, D.C.). The General Accounting Office contacted state and local education agencies, national organizations, and local community-based organizations by telephone interview. It was found that two-thirds of the U.S. public schools offer formal AIDS education, but not at all grade levels. Few schools have monitored the results of their programs. Typical teacher training for the programs are insufficient in time and coverage. Programs for high risk out-of-school youth, such as runaways, homeless, and incarcerated youth, were found to be particularly inadequate.

One program specifically designed to target high risk adolescents was reported on by Mary Jane Rotheram-Borus (New York State Psychiatric Institute and Columbia University, New York). The 10-session intervention, based on a cognitive-behavioral model, focused on AIDS knowledge, coping skills, and access to resources for runaways and gay male youths. Results indicated that youths receiving the program were significantly more likely to use condoms, or to practice abstinence than youths who were in a typical AIDS intervention of 1 to 2 sessions.

A program to increase adolescents' knowledge, to build decision-making, problem-solving and communication skills, and to prevent or modify risk behaviors was implemented with 544 ethnically diverse urban high school students and reported by Cherrie Boyer (University of California School of Medicine, San Francisco). Data indicated that the intervention was effective at increasing knowledge and facilitating problem-solving and decision-making skills. Interactive teaching methods, such as using persons with AIDS (PWAs) in the presentation of HIV/AIDS information, was reported to be more effective than non-interactive programs by Kevin Cranston (Massachusetts Department of Education, Quincy) and colleague. Compared to high school students participating in similar HIV/AIDS education programs, but without PWAs, greater changes in attitude scores were obtained.

Recognizing that AIDS education programs can only be as good as the preparation of the program teachers, CDC has funded a special training program that trains the trainers to cover facts, feelings, perceived risk, and skills for teachers and students. The program, described by Patti Britton and coworkers (ETR Associates, Scotts Valley, California) conducted 7 HIV prevention education trainings and 5 trainer trainings for almost 800 educators from 9 states. Evaluation of these training sessions was positive, and the project was said to have had a major impact in the nine states.

College Students. Several Conference reports dealt with the evaluation of AIDS education programs made for college students. Ross Conner and colleagues (University of California, Irvine) showed that a college course that included lectures and small-group discussions about a range of HIV/AIDS topics (biomedical aspects to psychology to legal aspects) was more effective than a traditional human sexuality class in terms of increasing knowledge about HIV/AIDS and bringing about risk-reduction behaviors such as condom use. A study comparing the impact of an intensive AIDS program (with peer risk-reduction education, free condoms, and frequent multimedia reinforcement) with typical AIDS education programs in 11 Northeastern colleges was reported by Steven Weinstein and others (University of Massachusetts Medical School, Worcester). Self-reports of risk behaviors revealed that 57% of college students participating in the intensive AIDS program (285 of 500) were more likely to report reduction in risk behavior than their traditional AIDS education counterparts, and 63% of them reported a reduction in the numbers of sexual partners.

Women. Andrew Box (Michael Reese Hospital, Chicago) and associates reported results of a team of indigenous and professional women educators conducting 437 one-to-one and group sessions in various clinics and wards at Cook County Hospital and Cook County Jail (Illinois). Statistically significant improvement on 83% of the test items was noted when pre- and post-test scores were compared. Of 232 women who were tested for HIV, 23% were HIV-positive. The authors concluded that culturally relevant AIDS education designed for specific high-risk populations can significantly modify attitudes and improve knowledge about HIV.

In an African study measuring the effectiveness of HIV testing and a single follow-up counselling session with 182 randomly selected HIV-positive and HIV-negative women, D. Kiragu and others (University of Nairobi, Kenya) found no significant changes in condom use, oral contraception, and subsequent pregnancy, 12 to 15 months after counselling.

COUNSELLING HIV-INFECTED PERSONS

Counselling individuals represents an intensive way of attempting to change the behaviors of HIV-positive persons (to curtail the potential for transmitting the virus to others) as well as of persons at risk for HIV infection. According to Stephen Bowen and associates (CDC, Atlanta) counselling and testing services are now provided at public expense in 5,013 locations in the U.S. More than 2.5 million people have been tested, and more than 160,000 have been diagnosed as HIV-positive.

Dr. Thomas Coates (University of California, San Francisco) presented an overview of HIV testing and counselling in a comprehensive AIDS prevention strategy. Two major points made in Dr. Coates' plenary lecture were: testing for antibodies to HIV can only ba a part, and not the major part, of a comprehensive AIDS prevention program and that HIV testing could be a more effective strategy in AIDS prevention if it were not used to discriminate, stigmatize, or deny human rights.

A diagnosis of AIDS, ARC, or HIV seropositivity is a significant, frequently life-altering event for the individual. Even though treatments for HIV infection are becoming available, and the implication for the condition is changing from that of imminent death to that of a chronic but manageable disease, a diagnosis has devastating consequences for the patient. Several important questions have been asked about the effect of learning that one is HIV seropositive. What is the psychological impact? How does such knowledge change an individual's risk behavior? And how can at-risk populations be encouraged to seek HIV testing?

Many individuals whose behaviors place them at high risk of infection will avoid testing opportunities if they feel no cure is available and a positive result will socially jeopardize them. Consequently, those at-risk are, in effect, driven "underground"—undermining the ultimate public health objective of curtailing the spread of HIV through education and counselling of persons at high risk of infection.

COUNSELLING APPROACHES

As would any group confronted with terminal illness, those infected with HIV generally have great difficulty coping with their disease. For this reason, psychological counselling can be quite beneficial for many HIV-infected persons. Counselling of high-risk (but uninfected) individuals can also have a profound influence on reducing risky behaviors. Numerous counselling programs have been established, with varying degrees of effectiveness.

Donna Higgins and associates (CDC, Atlanta) reviewed the effects reported in 45 studies of HIV antibody counselling and testing on risk behaviors in homosexual men, intravenous drug users, and high-risk heterosexuals. Most of the 26 studies involving gay men showed a decrease in the frequency of high-risk behavior for both HIV-positive and HIV-negative

men, with larger effects for the former. All 9 studies dealing with counselling of IVDUs showed reduction of high-risk drug related behavior, but only 3 showed changes in sexual behavior. The 10 studies dealing with heterosexuals had inconclusive results.

Dr. Brian Mulhall (University of Sydney, Australia) described a counselling approach used with gay men to help reduce the spread of HIV. A cohort of gay men has been followed since 1983; all cohort members received counselling at each 6-month clinical evaluation. The numbers of sexual partners of these men has fallen significantly since 1983; condom use has increased, and there has been a significant decrease in the frequency of insertive and receptive anal intercourse. The finding that there have been no seroconversions in this group since 1985 suggests that counselling may indeed be effective in reducing high-risk behaviors.

PARTNER NOTIFICATION

Informing sexual partners of one's positive HIV status is viewed by many as crucial to the curtailment of sexually transmitted HIV infection. Nevertheless, the issue of notifying contacts of those found to be HIV infected remains controversial for several reasons. Should the testing organization be responsible for tracing and notifying individuals about their possible seropositivity? Should this be legally binding? How should the counselling of these persons be handled? Confidentiality and anonymity are of paramount importance.

Surveys of different HIV-infected groups reveal that only a small percentage actually inform their sexual contacts. For this reason, many public health units provide a HIV partner notification service. One such service in Florida was described by B. Jones and [illegible] (Florida Department of Health and Rehabilitative Services, Tallahassee). Over a period of 9 months, 1,497 HIV-positive persons named 2,087 partners for field follow-up. Almost 75% of these (1,467) were actually located and counselled. Of the 1,286 persons who were subsequently tested, 30.3% were found to be HIV-positive. Such a high seroprevalence rate has essentially mandated that Florida's STD bureau consider HIV partner notification to be a top priority issue.

CONSEQUENCES OF HIV DIAGNOSIS

A diagnosis of HIV seropositivity is of major consequence. Previous research has shown that there are a number of behavioral consequences of HIV infection. Some of these are:

- Most persons prefer to be told their HIV-antibody status when they have the opportunity.
- Most studies showed that an awareness of serostatus decreases risk behaviors (that is, more change is noted among HIV-positive persons than among HIV- negative individuals); inappropriate "binge" behavior has generally not been reported.

Two studies at the Conference reported on the behavior of blood donors who have been notified that they are HIV-positive. Carol White and others including investigators from 20 U.S. blood centers (CDC, Atlanta) attempted to determine why HIV-positive heterosexuals who are aware of their partner's risk do not defer from donating blood. The researchers identified 28 males and 61 females who were sexual partners of IVDUs (3 females were partners of bisexual men) and who knew of their partner's risk before the date of donation, and 94% reported reading self-deferral information provided by the blood bank. Reasons for donation included: not believing they belonged to a "risk group," feeling pressure to donate, not thinking they were at risk, wanting to be tested, and not understanding educational materials. Debra Kessler and associates (Greater New York Blood Program) reported that HIV-positive, repeat donors may be using the blood center to reconfirm their original HIV-positive status. More counselling of HIV-positive donors is urged.

Heidi Wayment and associates (University of California, Los Angeles) reported on the "survivor syndrome" of gay men who know they are seronegative. It was found that among 129 HIV-negative gay men, those who had more sexual partners prior to 1985 felt more guilty about their HIV status than those who had fewer sexual partners. Those who had more friends with AIDS felt more vulnerable to HIV infection than those with fewer such friends. Social support from gay friends was seen as helpful to gay men experiencing survival guilt and vulnerability to AIDS.

PSYCHOSOCIAL CONSEQUENCES

Eduard Coene and others (Innovatieprojekt, Amsterdam) reported on a project to increase the knowledge about AIDS-related issues among PWAs and to provide guidelines and information on self-care techniques so that PWAs can remain independent as long as possible. The program was seen as successful in assisting PWAs in self-care skills and self-confidence.

Hemophiliacs. Man-Chiu Poon and others (University of Calgary) reported on a study to analyze risk behaviors in 53 HIV-positive hemophiliacs. Married couples in which the male was HIV-positive (4 females were also HIV-positive) were separately given questionnaires regarding sexual behavior and other potentially high-risk behavior. Of the men, 25% reported no condom use during intercourse as did 44% of women. Other high-risk sexual behavior was reported, as was razor and toothbrush sharing. These couples had 13 children born to them in the previous 4 years; 2 of the women were currently pregnant. It was concluded that "safer sex" counselling has not been successful with this group. In a study on the same topic, Charles Roy (Montreal Children's Hospital) found that in 20 couples with a hemophiliac male, the women expressed a greater need and interest than did the men in all forms of psychosocial support services. "Safer sex" was selected as a topic of interest to 65% of the women, compared with only 14% of the men.

A study of the stigmatization of 212 Danish HIV-positive and HIV-negative hemophiliacs was reported by Jakob Bjorner and colleagues (University of Copenhagen). While the objective data indicated that the two groups had equally intact social networks, HIV-positive men were less sure whether they were accepted by their friends and whether their doctors were open with them, thus reflecting a greater social vulnerability.

Men. A comparison of the psychosocial adjustment of HIV-positive and HIV-negative high-risk men was reported by Varda Soskolne (Hadassah Medical Organization, Jerusalem) and colleagues. Of the 46 HIV-positive and 40 HIV-negative men in the study, support from friends was reported to be more common than support from family. Both groups encountered psychological distress at the psychopathological level. HIV-positive men had less support from friends than HIV-negative men (44% versus 82%), and more psychosocial adjustment problems. K. Frasier and others (Henry Ford Hospital, Detroit) reported on a pilot study of 20 black and 20 white seropositive gay men attending a major infectious disease clinic. Physical examinations and interviews were conducted to assess social resources and coping strategies, specific stressors and vulnerabilities. Significant racial differences in tension-reduction expectancies and sexual disinhibition associated with the use of alcohol and marijuana were reported. Racial differences were also noted in the reporting of primary versus steady sex partners, and in the use of drugs in the sexual setting. These findings have implications for the devising of culturally and behaviorally appropriate interventions in these populations.

A comparison of coping responses between gay men who know and do not know their HIV status was reported by Richard Rodrigues and others (University of California, Los Angeles). Men who had chosen not know their antibody status (169) and men who know they are HIV-negative (312) reported less engagement in various active coping processes such as helping others, personal growth, health promotion behaviors as compared to HIV-

positive men (238). Those who do not know, but who *believe* themselves to be HIV-positive worry about developing AIDS as much as the men who *know* they are HIV-positive. It was concluded that choosing to know one's HIV status and finding that it is HIV-positive may promote active coping efforts.

The finding that persons who communicate their feelings about stressful situations and who participate in religious/spiritual activity reduce their risk behavior was reported by Constance Phillips (University of California, San Francisco). In a study of 540 HIV-positive, HIV-negative, and untested gay men, it was found that subjects who cope by keeping their feelings to themselves are more likely to engage in unprotected anal sex, and that subjects who take part in religious/spiritual activity are less likely to engage in this risk behavior.

A high, negative correlation was found between coping skills and reported levels of depression and anxiety in 60 HIV-positive gay men, as reported by Barbara Hedge and J. Green (St. Mary's Hospital, London). These researchers found that a short-term focused cognitive behavioral intervention led to increased levels of self-esteem, self-efficacy and greater problem-solving skills, as well as less depression and anxiety.

Women. Laurie Hauer and associates (San Francisco General Hospital) found that HIV-positive pregnant women have complex psycho/socio/economic problems both before and after birth, such problems often contributing to lack of compliance with research protocols and treatment plans. Obstacles to compliance include receipt of public assistance (92%); domestic violence (85%); IV drug use (77%); care of children other than index pregnancy (46%); and homelessness (38%). It was found that client compliance can be enhanced by assistance with these problems, as well as use of a multi-ethnic, bilingual staff, on-site nonmedical services, availability of consistent care providers, interagency case coordination, small cash reimbursements, and emphasis on integrity of the mother-infant unit.

FAMILY AND CARE GIVER NEEDS

While much research focuses on the needs of the HIV-infected patient, a few studies are beginning to look at the needs of the patients' families and care givers.

Jacqueline Y.Q. Mok and associates (City Hospital, Edinburgh, Scotland) described an investigation of the psychosocial needs of 16 HIV-infected mothers of 18 children. These women reported the following sources of support: health professionals (75%), partner (68.8%), parent or sibling (68.8%), and friends (43.8%). Only 53.9% discussed HIV-related problems with other parents, even though 81.3% knew other parents of HIV-positive children. Less than half wanted to meet other similar parents.

Rheba Adolph and E. Schere (Toronto) described a module to facilitate bereavement among AIDS-affected populations (care givers, lovers, buddies/friends, families, PWAs and HIV positives). An "AIDS language" of grieving was constructed and is now spoken in the AIDS affected community. Bereavement modules have been developed to enable individuals to discern and label responses that are normal to grief and bereavement.

APPENDIX A: CDC DEFINITION OF AIDS

Acquired immunodeficiency syndrome (AIDS) is an aggregate of signs and symptoms and illnesses resulting from a compromised immune system. A diagnosis of AIDS requires the definitive or presumptive diagnosis of one or more "indicator diseases" and, depending on certain criteria, may or may not require laboratory evidence of HIV infection. The following outline is used by physicians in the United States to arrive at an AIDS diagnosis.

Note: The original case definition for AIDS, and a subsequent revision in 1985, set forth by the Centers for Disease Control (CDC) failed to recognize several disease entities that are now known to be the direct result of infection with the AIDS virus (human immunodeficiency virus, or HIV) or an indirect result of immunodeficiency caused by HIV. Below is an adaptation of the latest revision, August 1987.

I A diagnosis of AIDS can be made if laboratory evidence of HIV infection has been established and a definitive diagnosis of any of the following indicator diseases has been made—regardless of the presence of other causes of immunodeficiency.

- Candidiasis of the esophagus, trachea, bronchi, or lungs
- Coccidioidomycosis, disseminated
- Cryptococcosis (extrapulmonary)
- Cryptosporidiosis with diarrhea persisting more than 1 month
- Cytomegalovirus disease of an organ other than the liver, spleen, or lymph nodes in a patient older than 1 month
- Herpes simplex virus infection causing a mucocutaneous ulcer (e.g., in eyes, nose, mouth, and genitoanal areas) that persists for more than 1 month; or bronchitis, pneumonitis, or esophagitis caused by herpes simplex virus in a patient older than 1 month.
- Histoplasmosis, disseminated
- HIV encephalopathy; also called subacute encephalopathy due to HIV; also referred to as HIV dementia or AIDS dementia complex (ADC), which is clinically defined as a disabling cognitive or motor dysfunction interfering with the patient's occupation or activities of daily living, or loss of behavioral developmental milestones in the absence of a concurrent illness or condition
- HIV wasting syndrome, defined as involuntary weight loss of greater than 10% of body weight plus chronic diarrhea or chronic weakness and fever in the absence of a concurrent illness or condition; also referred to as "slim disease"
- Isosporiasis with diarrhea persisting for longer than 1 month
- Kaposi's sarcoma
- Lymphoma (primary) of the brain
- Lymphoid interstitial pneumonitis (LIP) and/or pulmonary lymphoid hyperplasia affecting a child under 13 years of age
- Mycobacterial (other than *Mycobacterium tuberculosis*) disease, disseminated
- *M. tuberculosis* disease, extrapulmonary (may have concurrent pulmonary involvement)
- Non-Hodgkin's lymphoma
- *Pneumocystis carinii* pneumonia
- Progressive multifocal leukoencephalopathy
- *Salmonella* septicemia, recurrent
- Toxoplasmosis of the brain in a patient older than 1 month
- Any combination of at least two of the following bacterial infections within a 2-year period affecting a patient less than 13 years of age: septicemia, pneumonia, meningitis, bone or joint infection, or abscess of an internal organ or body cavity caused by *Haemophilus*, *Streptococcus*, or other fever-inducing bacteria

II A diagnosis of AIDS can be made if laboratory evidence of HIV is positive and any of the following indicator diseases is diagnosed *presumptively*. (A presumptive diagnosis is generally made in situations in which the patient's condition does not permit the performance of definitive testing.)

- Candidiasis of the esophagus
- Cytomegalovirus retinitis with loss of vision
- Kaposi's sarcoma
- Lymphoid interstitial pneumonitis and/or pulmonary lymphoid hyperplasia affecting a patient less than age 13
- Mycobacterial disease, disseminated
- *Pneumocystis carinii* pneumonia
- Toxoplasmosis of the brain in a patient older than 1 month

III A diagnosis of AIDS can be made if laboratory evidence of HIV infection is LACKING or INCONCLUSIVE *but* a definitive diagnosis of any of the following indicator diseases is made, provided other known causes of immunodeficiency are ruled out.

- Candidiasis of the esophagus, trachea, bronchi, or lungs
- Cryptococcosis, extrapulmonary
- Cryptosporidiosis with diarrhea persisting longer than 1 month
- Cytomegalovirus disease of an organ other than the liver, spleen, or lymph nodes in a patient older than 1 month
- Herpes simplex virus infection causing a mucocutaneous ulcer than persists longer than 1 month; or bronchitis, pneumonitis, or esophagitis affecting a patient older than 1 month
- Kaposi's sarcoma affecting a patient below age 60
- Lymphoma of the brain (primary) affecting a patient less than 60 years of age
- Lymphoid interstitial pneumonitis and/or pulmonary lymphoid hyperplasia affecting a patient less than age 13
- *Mycobacterium avium* complex or *M. kansasii* disease, disseminated
- *Pneumocystis carinii* pneumonia
- Progressive multifocal leukoencephalopathy
- Toxoplasmosis of the brain in a patient older than 1 month

IV A diagnosis of AIDS can also be made when laboratory evidence of HIV infection is negative. If all other causes of immunodeficiency are excluded and the patient has had either a definitive diagnosis of *P. carinii* pneumonia or a definitive diagnosis of any of the indicator diseases of AIDS and a CD4 (T4) cell count less than 400/mm^3.

CRITERIA FOR ARC

The CDC does not provide a precise definition of AIDS-related complex (ARC), but rather classifies it as an AIDS-like syndrome, noting that it is synonymous with pre-AIDS and prodromal AIDS. The medical community has adopted its own definition based on exclusion clauses in the CDC definition of AIDS.

Persons with HIV infection who have "constitutional symptoms," or clinical abnormalities, that accompany lymphadenopathy are described as patients with ARC. These include patients with fever persisting for more than 1 month, involuntary weight loss of greater than 10% of baseline, or diarrhea lasting more than 1 month in the absence of other conditions that could explain these findings; patients with hairy leukoplakia, multidermatomal herpes zoster, recurrent *Salmonella* bacteremia, nocardiosis, tuberculosis, or oral candidiasis; and patients with chronic lymphoid interstitial pneumonitis. Note that ARC is not synonymous with persistent generalized lymphadenopathy (PGL).

ARC patients present with a spectrum of immunologic abnormalities ranging from minimal alterations to severe cellular immunodeficiency comparable to those of patients with frank AIDS. An absolute CD4 cell count cannot be used as a diagnostic measure since some asymptomatic HIV-infected individuals have had low CD4 cell counts while other patients with constitutional symptoms have had relatively high counts. Many ARC patients are anergic, and many exhibit hypergammaglobulinemia, hypersplenism, and sometimes a diminution in primary antibody responses. Some ARC patients also have mild anemia, leukopenia, or thrombocytopenia. Patients with PGL or ARC generally progress to full-blown AIDS, usually within a 3-year period.

APPENDIX B: CLASSIFICATION OF PEDIATRIC HIV INFECTION (UNDER AGE 13)

CLASS PO: INDETERMINATE INFECTION

This class encompasses infants and children up to age 15 months who cannot be classified as definitely infected according to any of the definitions below but who have antibody to HIV.

CLASS P1: ASYMPTOMATIC INFECTION

Pediatric patients who have documented HIV infection but who have had no previous signs or symptoms. Subclassifications of asymptomatic HIV-infected children are based on immunological test findings (tests should included quantitative immunoglobulins, complete blood count with differential, and T lymphocyte subset counts):

Subclass A. Normal immune function
Subclass B. Abnormal immune function
Subclass C. Immune function not tested

CLASS P2: SYMPTOMATIC INFECTION

Pediatric patients who have proven HIV infection and who have signs and symptoms of immunodeficiency, for which other causes have been excluded, fall into this category. Subclasses are defined according to signs and symptoms: patients may be classified in more than one subclass.

Subclass A. Nonspecific findings
Subclass B. Progressive neurological disease
Subclass C. Lymphoid interstitial pneumonitis
Subclass D. Indicator diseases
- **Category D-1.** Specified indicator diseases listed in the (adult) CDC AIDS definition
- **Category D-2.** Recurrent serious bacterial infections
- **Category D-3.** Other indicator diseases:, including oral candidiasis persisting for 2 months or more, 2 or more episodes of herpes stomatitis within 1 year, or multidermatomal or disseminated herpes zoster infection

Subclass E. Secondary cancers
- **Category E-1.** Specified secondary cancers listed in the (adult) CDC AIDS definition
- **Category E-2.** Other cancers possibly secondary to HIV infection

Subclass F. Other diseases possibly due to HIV infection, such as hepatitis, cardiopathy, nephropathy, hematologic disorders (anemia, thrombocytopenia), and dermatologic diseases

APPENDIX C: STAGING CLASSIFICATION SYSTEMS FOR HIV INFECTION

CDC Classification

STAGE	DESCRIPTION
I	Acute HIV infection (at time of exposure)
II	Asymptomatic HIV infection (but may have some signs of immune dysfunction)
III	Progressive generalized lymphadenopathy (PGL)
IV	HIV disease
IVa	Constitutional disease: Having one or more of the following symptoms: fever for longer than 1 month, 10% weight loss, diarrhea lasting 1 month—and no other conditions explain findings.
IVb	Neurological disease: AIDS dementia complex (ADC), myelopathy, or peripheral neuropathy—and no other conditions explain findings.
IVc	Secondary infectious diseases
c-1	Opportunistic infections (one of the following): *Pneumocystis carinii* pneumonia; cryptococcosis; cryptosporidiosis; toxoplasmosis; strongyloidiasis (extra-intestinal); isosporiasis; candidiasis (esophageal, bronchial, or pulmonary); histoplasmosis; mycobacterial disease (disseminated); cytomegalovirus; herpes simplex (disseminated); progressive multifocal leukoencephalopathy
c-2	Symptomatic or invasive disease (one of the following): oral hairy leukoplakia, multidermatomal herpes zoster, *Salmonella* bacteremia (recurrent), nocardiosis, tuberculosis (extrapulmonary), or oral candidiasis
IVd	Cancers (one of the following): Kaposi's sarcoma, non-Hodgkin's lymphoma, primary brain lymphoma
IVe	Other conditions: lymphoid interstitial pneumonitis, constitutional symptoms not meeting subgroup IVa, infectious diseases not meeting subgroup IVc, cancers not meeting subgroup IVd

Walter Reed Staging

This classification system uses specific immune parameters to establish patients' disease status: from WR0, or HIV exposure, through progressive immune dysfunction, to WR5 (ARC) and WR6 (AIDS).

Stage	HIV status (antibody or virus)	Lymphadenopathy (chronic)	CD4 count (#/mm^3)	Skin Tests (delayed hypersensitivity)	Oral thrush	Opportunistic infections
WR0	negative	no	>400	normal	no	no
WR1	positive	no	>400	normal	no	no
WR2	positive	yes	>400	normal	no	no
WR3	positive	yes or no	< 400	normal	no	no
WR4	positive	yes or no	<400	partial defect	no	no
WR5	positive	yes or no	<400	complete defect *and/or*	yes	no
WR6	positive	yes or no	<400	partial or complete	yes or no	yes

APPENDIX D: UNIVERSAL PRECAUTIONS

In the Health Care Setting & Precautions for Special Settings

The Centers for Disease Control (CDC), Atlanta, published recommendations for the prevention of transmission of HIV and other blood-borne pathogens in the health care setting in Morbidity *and Mortality Weekly Report*: 37(24): June 24, 1988. In this update, the CDC emphasized the need to treat blood and other body fluids from all patients as potentially infective. Changes or additions to the 1983 edition ("Guidelines for Isolation Precautions in Hospitals") and 1987 edition ("Recommendations for Prevention of HIV Transmission in Health-Care Settings") are indicated. This year, the CDC added new recommendations for health care workers and developed specific guidelines for guidelines for fire-service personnel, emergency medical technicians, paramedics, and law-enforcement and correctional-facility personnel: "Guidelines for Prevention of Transmission of HIV and Hepatitis B Virus to Health-Care and Public-Safety Workers," *Morbidity and Mortality Weekly Report*: 38(S-6): June 23, 1989.

I. Universal Precautions
(also referred to as "universal blood and body-fluid precautions")

Blood and body-fluid precautions are to be used for all patients, regardless of their infection status (i.e., seropositive or seronegative). This recommendation serves to protect health care workers from unsuspected specimens that are in fact infected and specimens encountered in emergency medical care situations. (The 1983 guidelines called for adoption of precautions only when a patient was known or suspected to be infected.) Universal precautions are intended to supplement rather than replace recommendations for routine infection control, such as handwashing and using gloves to prevent gross microbial contamination of hands.

- "Exposure" is defined as contact with blood or other body fluids through percutaneous inoculation or contact with an open wound, nonintact skin, or mucous membrane.
- Extra attention should be paid when using needles, scalpels, and other sharp instruments or devices; when handling sharp instruments after procedures; when cleaning used instruments; and when disposing of used needles.
- Used needles should not be recapped by hand; used needles should not be removed from disposable syringes by hand; used needles should not be bent, broken, or otherwise manipulated by hand. Used disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers (as close to the use area as is practical) for disposal.
- Reusable needles should be left on the syringe body and should be placed in a puncture-resistant container for transport to the reprocessing area.
- Health-care workers who have exudative lesions or weeping dermatitis should refrain from all direct patient care and from handling patient-care equipment until the condition resolves.
- Hands and other skin surfaces should be thoroughly washed immediately after contamination with blood or other body fluids. Hands should be washed immediately after gloves are removed, even if gloves appear to be intact.
- When hand-washing facilities are available, wash hands with warm water and soap. When hand-washing facilities are not available, use a waterless antiseptic hand cleanser.
- Body fluids to which universal precautions apply:
  - — Semen
  - — Vaginal secretions
  - — Cerebrospinal fluid (CSF)
  - — Synovial fluid
  - — Pleural fluid
  - — Peritoneal fluid
  - — Pericardial fluid
  - — Amniotic fluid
  - — Feces, nasal secretions, sputum, sweat, tears, urine, and vomitus (only when they contain visible blood). However, when emergency medical and public-safety workers encounter body fluids under uncontrolled emergency circumstances in which differentiation between fluid types (contaminated or noncontaminated) is difficult, they should treat all body fluids as potentially hazardous.
  - — Breast milk in special setting: breast milk banking
  - — Saliva, only in the dental setting, where it is likely to be contaminated with blood
- Protective barriers to reduce the risk of exposure to blood and other body fluids. Masks, protective eyewear, and face shields should reduce the incidence of contamination of mucous membranes of the mouth, nose, and eyes.
  - — Gloves should reduce the incidence of contamination of hands, but they cannot prevent penetrating injuries due to needles or other sharp instruments.
  - — Gowns

— Masks
— Protective eyewear
— Face shields

- Special note on gloves: The Food and Drug Administration regulates the medical glove industry, both sterile surgical gloves and nonsterile examination gloves made of vinyl or latex. Intact latex gloves have been shown to have greater barrier effectiveness.
  — Sterile gloves should be used for procedures involving contact with normally sterile areas of the body.
  — Examination gloves should be used for procedures involving contact with mucous membranes and for other patient care or diagnostic procedures that do not warrant the use of sterile gloves.
  — Change gloves between patient contacts.
  — Do not wash or disinfect gloves for reuse.
  — General-purpose utility gloves (rubber household gloves) can be used for housekeeping chores involving potential blood contact and for instrument cleaning and decontamination procedures. Utility gloves may be decontaminated and reused but should be discarded if they are peeling, cracked, or discolored, or if they have punctures, tears, or other evidence of deterioration.
  — Gloves should be worn for performing phlebotomy (drawing blood samples) when the health-care worker has cuts, scratches, or other breaks in their skin.
  — Gloves should be worn in situations where the health-care worker judges that hand contamination with blood may occur, for example, when performing phlebotomy on an uncooperative patient.
  — Gloves should be worn for performing finger and/or heel sticks on infants and children.
  — Gloves should be worn when persons are receiving training in phlebotomy.

II. Precautions for Invasive Procedures

Universal precautions apply to all surgical procedures in the delivery room, operating room, emergency room, physicians' and dentists' offices, or any outpatient setting. Surgery includes:

- Cardiac catheterization and angiographic procedures
- Vaginal or cesarean delivery or other invasive obstetrical procedure. (All health care workers who perform or assist in vaginal or cesarean deliveries should wear gloves and gowns when handling the placenta or the infant until blood and amniotic fluid have been removed from the infant's skin and should wear gloves during post-delivery care of the umbilical cord.)
- Manipulation, cutting, or removal of any oral or perioral tissues.

III. Precautions for Dentistry

Universal precautions apply to blood, saliva, and gingival fluid from all dental patients.

- Rubber dams, high-speed evacuation, and proper patient positioning, when appropriate, should be utilized to minimize generation of droplets and spatter.
- Handpieces should be sterilized after use with each patient; handpieces that cannot be sterilized should at least be flushed, the outside surface cleaned and wiped with a suitable germicide, and rinsed. Handpieces should be flushed at the beginning of the day and after use with each patient.
- Materials that have been used in the mouth—bite registration materials, for example—should be thoroughly cleaned of blood and saliva. Contaminated materials should be cleaned and disinfected before being handled in the dental laboratory and before placed in the patient's mouth.
- Dental equipment and surface that may become contaminated and are difficult to disinfect—x-ray heads, for example—should be wrapped with impervious-backed paper, aluminum foil, or clear plastic wrap. The coverings should be removed and discarded, and clean coverings should be put in place after use with each patient.

IV. Precautions for Autopsies or Morticians' Services

- Instruments and surfaces contaminated during postmortem procedures should be decontaminated with appropriate chemical germicide.
- All persons performing or assisting in postmortem procedures should wear gloves, masks, protective eyewear, gowns, and waterproof aprons.

V. Precautions for Dialysis

- Disinfecting dialysis fluid pathways of the hemodialysis machine after use by all patients are based on conventional infection-control strategies: formaldehyde or sodium hypochlorite (household bleach) for 30-40 minutes overnight.
- Individual dialyzers must never be used on more than one patient.
- HIV-infected patients on dialysis need not be isolated from other patients.

VI. Precautions for Clinical Laboratories

- All specimens should be put in a well-constructed container with a secure lid to prevent leaking during transport. Contamination of the outside of the specimen container should be avoided when collecting the specimen. (Implementation of universal blood and body-fluid precautions eliminates the need for warning labels on specimens.)
- All personnel handling blood and body fluid specimens should wear gloves. Gloves should be changed and hands washed after processing each specimen.
- Masks and protective eyewear should be worn if mucous-membrane contact is anticipated.
- Biological safety cabinets should be used for procedures that have a high potential for generating droplets, including blending, sonicating, and vigorous mixing.
- Mouth pipetting should never be permitted. Mechanical pipetting devices should be used for all liquids.
- Laboratory work surface should be decontaminated with appropriate germicide after a spill of blood or other body fluid and when work is completed each day.
- Contaminated materials used in laboratory tests should be

decontaminated before reprocessing or disposal. (See infective waste disposal outline below; institutional policies for disposal of infective wastes are generally guided by state and local regulations.)
- Laboratory equipment that has been contaminated with blood or other body fluids should be decontaminated and cleaned before being repaired in the laboratory or transported anywhere for service.
- All personnel should wash their hands after completing laboratory activities and should remove protective clothing before leaving the laboratory.

VII. Sterilization & Disinfection
- Instruments or devices that enter sterile tissue or the vascular system of any patient or through which blood flows should be sterilized before reuse. Devices or instruments that contact intact mucous membranes should be sterilized or receive high-level disinfection. (Chemical germicides registered with the EPA as "sterilants" may be used either for sterilization or for high-level disinfection depending on contact time.)
- Contact lenses used in trial fittings should be disinfected after each fitting by using a hydrogen peroxide contact lens disinfecting system, or if permissible, with heat (78° to 80° C) for 10 minutes.
- Medical devices or instruments that require sterilization or disinfection should be thoroughly cleaned before being exposed to the germicide, and the manufacturer's instructions for the use of the germicide should be followed.
- In addition to commercially available chemical germicides, a solution of sodium hypochlorite (household bleach) prepared daily is an effective germicide. Concentrations ranging from approximately 500 to 5,000 ppm (parts per million) are effective depending on the amount of blood or other body fluid present on the surface to be cleaned and disinfected. Commercially available chemical germicides may be more compatible with certain medical devices that might be corroded by repeated exposure to sodium hypochlorite.

VIII. Cleaning & Decontamination Spills of Body Fluids
- Chemical germicides that are approved for use as "hospital disinfectants" and are tuberculocidal when used at recommended dilutions can be used to decontaminate spills of blood and other body fluids.
- In patient-care settings, visible material should first be removed and then the area should be decontaminated with an appropriate germicide. Gloves should be worn during cleaning and decontaminating. If splashing is anticipated, protective eyewear should be worn along with an impervious gown or apron which provides an effective barrier to splashes.
- Where there is massive blood contamination on floors, the use of disposable impervious shoe coverings should be worn. Gloves should be worn to remove contaminated shoe coverings. The coverings and gloves should be disposed of in plastic bags.
- Large spills of cultured or concentrated infectious agents in the laboratory require that the contaminated area be flooded with a liquid germicide before cleaning, then decontaminated with fresh germicide. Gloves should be worn during cleaning and decontaminating.
- Soiled cleaning equipment should be cleaned and decontaminated or placed in an appropriate container and disposed of properly. Plastic bags should be available for removal of contaminated items from the site of the spill.

IX. Infective Waste
- Wastes with the potential for causing infection during handling and disposal should be identified; policies for defining, collecting, storing, decontaminating, and disposing of infective waste are generally determined by institution in accordance with state and local regulations.
- Hospital wastes for which special precautions appear prudent include microbiology laboratory waste, pathology waste, and blood specimens or blood products.
- Infective waste, in general, should either be incinerated or should be decontaminated before disposal in a sanitary landfill.
- Bulk blood, suctioned fluids, excretions, and secretions may be carefully poured down a drain connected to a sanitary sewer.

X. Housekeeping
- Cleaning and removal of soil from surfaces such as walls and floors should be routine.
- Horizontal surfaces—bedside tables and flooring—in patient-care areas should be cleaned when soiling or spills occur and when a patient is discharged.
- Disinfectant-detergent formulations registered by EPA can be used for cleaning surfaces, but the actual physical removal of microorganisms by scrubbing is equally important.

XI. Laundry
- Hygienic and common-sense storage and processing of clean and soiled linen are recommended.
- Soiled linen should be handled as little as possible and with minimum agitation to prevent gross microbial contamination of the air and of persons handling the linen.
- All soiled linen should be bagged at the location where it was used; it should not be sorted or rinsed in patient-care areas.
- Linen soiled with blood or body fluids should be placed and transported in bags that prevent leakage.
- Linen should be washed with detergent in water (at least 71° C) for 25 minutes.
- Protective clothing and work uniforms should be washed and dried according to manufacturer's recommendations.
- Boots and leather goods may be brush-scrubbed with soap and hot water to remove contamination.

XII. Precautions for Fire and Emergency Medical Personnel
- All protective attire—including disposable gloves, gowns, masks, and protective eyewear—should be put on before

beginning patient care. For multiple trauma victims, gloves should be changed between patient contacts, if the emergency situation allows.

- Structural fire-fighting gloves should be worn in any situation where sharp or rough surfaces are likely to be encountered.
- While wearing gloves, avoid handling personal items that could become soiled or contaminated. Gloves that have become contaminated with blood or other body fluids should be removed as soon as possible, taking care to avoid skin contact with the exterior surface. Contaminated gloves should be disposed of or, in the case of reusable gloves, cleaned and disinfected properly.
- Masks and eyewear and impervious gowns or aprons should be used prior to any situation where splashes of blood or other body fluid are anticipated. An extra change of work clothing should be available at all times.
- Disposable airway equipment or resuscitation bags should be used during artificial ventilation with trauma victims. Disposable resuscitation equipment and devices should be used once and disposed of or, if reusable, thoroughly cleaned and disinfected after each use according to the manufacturer's recommendations.
- Pocket mouth-to-mouth resuscitation masks (i.e., double lumen systems) designed to isolate emergency response personnel from contact with victims' blood and blood-contaminated saliva, respiratory secretions, and vomitus should be provided to all emergency personnel.

XIII. Precautions for Law-Enforcement and Correctional-Facility Officers

- Caution must be used in dealing with the suspect or prisoner if there is any indication of assaultive or combative behavior. When blood is present and a suspect or an inmate is combative or threatening to staff, gloves should always be put on as soon as conditions permit. In case of blood contamination of clothing, an extra change of clothing should be available at all times.
- Protective masks or resuscitation airways should be available to officers, along with special training in their proper use. Devices with one-way valves to prevent the patients' saliva or vomitus from entering the care giver's mouth are preferable.
- Protective gloves should be worn if exposure to blood is likely to occur. For very large spills (e.g., a homicide crime scene), aprons, boots, or protective shoe covers should be donned. If torn or soiled, they should be changed and removed prior to leaving a scene.
- While wearing gloves, avoid handling personal items that could become soiled or contaminated.
- Protective gloves should be worn for all body cavity searches.
- If cotton gloves are to be worn when working with evidence of potential latent fingerprint value at a crime scene, they can be worn over protective disposable gloves when exposure to blood may occur.
- Use puncture-proof containers to store sharp instruments and clearly mark plastic bags to store other possibly contaminated items.
- Face masks and eye protection or a face shield are required for laboratory and evidence technicians whose jobs which entail potential exposures to blood via a splash to the face, mouth, nose, or eyes. It is recommended that protective masks and eyewear or face shields be worn by laboratory or evidence technicians when removing the blood stain for laboratory analyses. (Airborne particles of dried blood may be generated when a stain is scraped.)
- When handling a deceased person, all personnel should wear gloves and cover all cuts and abrasions to create a barrier and carefully wash all exposed areas after contact with blood—regardless of whether the person(s) is known or suspected to be infected. Precautions should be used for everyone. These precautions should also be followed when handling amputated limbs, hands, or other body parts.
- In forensic laboratories, blood from all individuals should be considered infective. All blood specimens should be put in a well-constructed, appropriately labelled container with a secure lid to prevent leaking during transport.Care should be taken when collecting each specimen to avoid contaminating the outside of the container and of the laboratory form accompanying the specimen.
  - All persons processing blood specimens should wear gloves. Masks and protective eyewear or face shields should be worn if mucous-membrane contact with blood is anticipated. Hands should be washed after completion of specimen processing.
  - Mechanical pipetting devices should be used for manipulating all liquids in the laboratory. Mouth pipetting must not be done.
  - Laboratory work surfaces should be cleaned of visible materials and then decontaminated with an appropriate chemical germicide after a spill of blood, semen, or blood-contaminated body fluid and when work activities are completed.
  - Contaminated materials used in laboratory tests should be decontaminated before reprocessing or be placed in bags and disposed of in accordance with institutional and local regulatory policies for disposal of infective waste.
  - All persons should wash their hands after completing laboratory activities and should remove protective clothing before leaving the laboratory.
  - Area posting of warning signs should be considered to remind employees of continuing hazard of infectious disease transmission in the laboratory setting.

PRESENTER INDEX

Abramowitz, Al, 305
Abrams, Elaine, 110
Achoar, Ammar, 120
Adeodato Filho, Sergio, 291
Adib, Maurice, 302
Adjorlolo, Georgette, 84
Adolph, Rheba, 319
Aggleton, Peter, 313
Alary, Michel, 53
Allain, Jean-Pierre, 85, 99
Allen, David, 64
Altman, Sidney, 158
Alvarez, Perez, 76
Alves, Carlos, 80
Amemiya, Kei, 13, 14
Ampel, Niel, 205
Anand, Rita, 236
Andersen, Robert, 179
Anderson, Donna, 278
Ankrah, E. Maxine, 293
Antinori, Spinello, 73
Aoki, Shizuko, 187
Aoun, Hacib, 283
Apte, S., 60
Araneta, Maria Rosario, 196
Archibald, Chris, 207
Arco, Massimo, 88
Armitage, Peter, 219
Armstrong, John, 160
Arno, Peter, 217, 222
Arnold, Wendy, 295
Aronow, Howard, 244
Arshanoff, Rena, 304
Artigas, Juan, 248
Atkinson, Hampton, 255, 255f
Augustyniak, Linda, 70
Baba, Masanori, 153
Baende, Ekungola, 195
Balanon, Angelita, 310
Ball, Judy, 215
Bandura, Albert, 289
Barchi, Peter, 312
Bard, M., 295
Barnes, Donna, 219
Barry, Anita, 195
Bartholow, Brad, 302
Bartlett, John, 165t
Bash, Margaret, 225
Battjes, Robert, 64, 66
Batungwanayo, Jean, 195
Bauer, Stanley, 273
Baum, M., 212
Beach, Richard, 54
Beekmann, Susan, 272, 273
Behets, Frieda, 108
Beirnes, Denise, 310
Beldescu, Nicolae, 57
Bell, David, 271, 272
Belman, Anita, 263
Bendet, I., 108
Benito-Garcia, Ana, 83
Benson, Constance, 196
Berger, Edward, 150, 161t
Berger, Joseph, 245
Bergmann, John, 8
Berkelman, Ruth, 65
Berman, Phillip, 132
Bertozzi, Stefano, 214
Berzofsky, Jay, 116, 128
Beuscart, Claude, 295
Bez, Gabriel, 213
Bhave, Geeta, 72, 73f
Biberfeld, Gunnel, 128, 133
Bigley, Joseph, 261
Bird, Graham, 87
Birx, Deborah, 19
Bjorner, Jakob, 318
Blatt, Stephen, 211
Blumenfeld, Walter, 189
Boccher-Lattimore, Daria, 309
Bohlin, Ann-Britt, 79
Bomboko, Bombelenga, 300
Bornstein, Robert, 259
Boucher, Charles, 152, 184
Boudinot, Douglas, 153
Boue, Francois, 79, 79t
Boulos, Reginald, 82
Bowen, Stephen, 316
Box, Andrew, 316
Boyer, Cherrie, 315
Bozzette, Samuel, 185
Brito, Germania, 294
Britton, Patti, 315
Broadhead, Robert, 297
Brunkhorst, Ulrike, 165t
Brust, Stefan, 94t
Bryce, Jennifer, 291
Buchanan, Thomas, 128
Buck, David, 130
Bustan, Muhammad, 299
Butler, Karina, 227
Caine, Virginia, 277
Calabrese, Leonard, 276
Calabrese, Teresa, 294
Caldwell, Blake, 77
Callahan, Larry, 148
Camara, Carmen S., 195
Campbell, Ian, 293, 294
Campbell, Scott, 270
Canas-Ferreira, Wanda, 15
Canavaggio, Michel, 84
Canessa, Andrea, 245
Canick, Jonathan, 258
Capella. Fausto, 189
Capellan, Mirtha, 55, 84, 85
Capon, Daniel, 149
Carey, John, 164t
Carles, Michel, 183
Carrow, Emily, 37, 122
Casarigo, Zulema, 205
Catania, Joseph, 298
Caussy, Deoraj, 226
Cech, Thomas, 158
Celum, Connie, 100
Cereda, Paolo Marino, 148
Cerigo, Helene, 195
Chaisson, Mary Ann, 71
Chamberland, Mary, 272
Chase, Cynthia, 262
Chehimi, Jihed, 34
Chen, Yao-Tseng, 242
Cheng, Sheau-Mei, 133
Cheng-Mayer, Cecilia, 46
Cheynier, Remi, 12, 110
Chitwood, Dale, 313
Choi, Kyung-Hee, 304, 311
Chopard, Pierre, 190
Chou, Ting-Chao, 161t, 165t
Christeff, Nevena, 178
Chu, Susan, 82
Chuang, Henry, 253
Cimoch, Paul, 212
Ciricillo, Samuel, 250
Clark, Wayne, 305
Clarke, Sian, 76
Clarkson, Robert, 175
Clay, Charlton, 310
Cleghorn, Farley, 55
Clifford, David, 258
Closen, Michael, 292
Coates, Randall, 179
Coates, Thomas, 316
Coberly, Jacqueline, 75, 311
Coene, Eduard, 318
Coffin, John, 12
Cohen, Bruce, 246
Cohen, Eric, 12
Cohen, Mitchell, 290
Coleman, Patrick, 95t
Collier, Ann, 187
Collman, Ronald, 32
Coltin, Kathryn, 218
Cone, Lawrence, 205
Conner, Ross, 315
Connor, Edward, 227
Connors, Margaret, 304
Conway, Brian, 105
Cooke, Molly, 276, 283
Cooley, Timothy, 187
Coombs, Robert, 161t, 183
Cooney, Elizabeth, 124
Cooper, E.C., 261
Corbeil, Jacques, 44
Corey, Lawrence, 136
Cornuet, Pierre, 31, 32
Costagliola, Dominique, 69, 86, 226
Costanzi, Giulio, 260-261
Coste, Joliette, 84
Cottone, James, 278
Couland, Jean-Pierre, 197
Coulston, Ann, 212
Couturier, Elisabeth, 206
Cowan, Jane, 310
Cowen, Edward, 281
Coyle, Molly, 216
Cranston, Kevin, 315
Crisell, Paul, 158
Crombleholme, William, 228
Crooks, Levinia, 294, 295
Cross, David, 113
Crowe, Suzanne, 174
Culbert, Arthur, 277
Dabis, Francoise, 224
Dadaglio, Giles, 120
Dalakas, Marinos, 242
Dallabetta, Gina, 68
Dandekar, Satya, 210
Darrow, William, 291, 297
Davenny, Katherine, 175
Davey, Richard, 165t
Davey, Victoria, 164t, 185-186
Davies, Peter, 301
Davis, Arlene, 297
Davis, Brian, 31
Davis, Iris, 195
De Clercq, Erik, 147, 151
De Jose, Maria Isabel, 227
de Gans, Jan, 244-245
de la Fuente Luis, 65
Deckmann, Michael, 94t
DeCock, Kevin, 82
Del-Rio, Aurora, 299
Delgado-Rodriquez, Miguel, 64
Denning, David, 205
Denomme, Nicole, 174
Des Jarlais, Don, 314
Desgranges, Claude, 136
Desrosiers, Ronald, 126
Detels, Roger, 177
DeVico, Anthony, 8
Dickie, Peter, 21
DiClemente, Ralph, 307
Dieterich, Douglas, 193
Dietrich, Ursula, 15
Dittmann, Sieghart, 56
Doerr, Hans, 72
Dohin, Elisabeth, 192
Dolan, Katie, 66
Dolin, Raphael, 153, 186
Dominique, Mathez, 177
Dondero, Dale, 105

f = figure; t = table

Donegan, Patrick, 78
Dorn, Henry, 234
Downs, Angela, 58, 88
Drew, Lawrence, 193
Drexler, Karen, 256
Dumas, Michel, 84
Durnan, Richard, 280
Duzgunes, Nejat, 40
Egan, Vincent, 260
Eichberg, Jorg, 132
Einhaüpl, Karl, 250f, 251
Eke, Tukumbe, 295
Eliaswicz, Murielle, 194
Elifson, Kirk, 73
Ellerbrock, Tedd, 310
Ellison, Viola, 8
Elmslie, K., 291
Emery, Vincent, 14
Ensoli, Barbara, 44
Eriki, Peter, 194
Escaich, Sonia, 111
Evans, D.L., 255
Evans, Louise, 33, 34
Evans, Nancy, 295
Fabrizio, Manca, 123
Fahey, John, 175
Falutz, Julian, 189
Farnet, Christopher, 8
Farzadegan, Homayoon, 46
Fassil, Ketema, 108
Fauci, Anthony, 40, 42
Feijoo, Rosa, 291
Feinberg, Judith, 192
Feingold, Lisa, 298
Ferguson, Leona, 294
Fernandez, Isabel, 279
Fernandez, M. I., 293
Fernandez, Maria E., 307
Fetro, J., 295
Feuerstein, Irwin, 190-191
Finberg, Robert, 150
Fischl, Margaret, 182, 183
Fishbein, Martin, 312
Fisman, Noemi, 290
Flam, Lisa, 296
Flanigan, Timothy, 204
Fleetwood, Debbie, 189, 217
Fong, Kei-Lai, 148
Foreman, Maeve, 311
Fowler, Arnold, 103
Francioli, Patrick, 269, 269f
Frasier, K., 318
Freedberg, Kenneth, 218
Friedman, Wolfgang, 209
Friedman-Kien, Alvin, 207
Fultz, Patricia, 19
Fung, Michael, 129, 130
Fust, George, 34
Fyfe, James, 7
Gabiano, Clara, 110
Gail, Mitchell, 86
Galavotti, Christine, 313
Galli, Richard, 100
Ganzales-Canali, Gustavo, 208
Garcia, Homer D.C., 293
Gardiner, Ben, 295
Gardner, Murray, 19, 133, 134
Garraffo, Rodolphe, 227
Garry, Robert, 113
Gatti, Giorgio, 188
Gavazzeni, Giovanna, 214
Gendelman, Howard, 163
Gerberding, Julie, 268, 273
Gianni, Guiseppe, 39, 40
Gibson, David, 297
Gilson, Ian, 292
Giuckman, Jean-Claude, 132
Gohchi, Kengo, 161t
Goldberg, David, 64
Golden, Eve, 305
Goldstein, Allan, 127, 136
Goldstein, Andrew, 102
Goldstein, Mark, 292
Gonzalez, Roxane, 55
Gopinath, Nanda, 191
Gothua, Samuel, 195
Gottlinger, Heinrich, 7
Goudsmit, Jaap, 120, 122
Graham, Neil, 217
Granato, Celso, 72
Grant, Igor, 256-257
Green, J., 319
Green, Jesse, 217, 220f, 221f, 222
Green, Timothy, 53
Griffin, Diane, 238
Griffin, John, 241
Grose, Robert, 294
Gross, Elaine, 279
Grund, Paul, 305
Grunfeld, Carl, 211
Guermonprez, G., 208
Guerraro, Ernesto, 75, 311
Guimaraes Dias, Martha, 206
Gurtman, Alejandra, 204
Gutierrez, Rosben, 256
Gwinn, Marta, 52
Habicht, Sabine, 246
Haffer, Omar, 129
Hall, Christopher, 296
Hankins, C., 295
Hansell, David, 294
Hansen, Bente, 295
Hansen, John-Erik, 121, 148
Hardy, David, 192
Harouse, Janet, 33
Harris, Earl, 314
Harrison, Janet, 301
Harrison, Lee, 83
Hart, Graham, 67
Hartgers, Christina, 65
Hartman, Neil, 187
Hasbrouck La Mar, 305
Haseltine, Peter, 188
Hauer, Laurie, 319
Hausser, Dominique, 299
Havlik, Joseph, 196
Hayes, Melvyn, 235
Hayes, Robbie, 306
Hays, Robert, 301
Healy-Chidekel, Jane, 280
Hedge, Barbara, 319
Heise, Walter, 193
Henderson, Lee, 34
Hendriks, Jan, 88
Hengelein, Hans, 294
Henin, Yvette, 69
Henrion, Roger, 111
Herbert, Pamela, 280
Hersh, Bradley, 81
Hersh, Evan, 166
Herskowitz, Ahvie, 39
Hewitt, Ross, 164t
Hewlett, Indira, 160
Higgins, Donna, 316
Hill, Todd, 94t
Hingson, Ralph, 308
Hinkin, Charles, 258
Hittleman, Joan, 263
Hodges, Teri, 187
Hojlyng, Niels, 204
Hollander, Harry, 252
Holodnly, Mark, 178
Holtzman, Deborah, 307
Hooker, Malcolm, 217
Horsburgh, Robert, 196
Houff, Sidney, 237-238, 241
Hoxie, James, 15
Hu, Shiu-Lok, 124
Huet, Thierry, 16, 17
Hughes, Gail, 62
Humes, Steven, 281
Hunter-Young, Nancy, 215
Hurtrel, Bruno, 235
Iguchi, Martin, 65
Ikuta, Kazuyoshi, 12
Indyk, Leonard, 295
Ippolito, Giuseppe, 271f, 272, 274
Isaacman, Scott, 292
Ishida, Takuo, 20
Izaguirre, Carlos, 104
Izazola, Jose, 303
Jacobson, Lisa, 207
Jacobson, Mark, 193
Jadresic, Danitza, 253
Jagger, Janine, 268-269
James, William, 158
Jason, Janine, 290
Jeang, Kuan-Teh, 14
Jennens, Ian, 193
Jewett, Don, 252
Jimenez, Antonio, 306
Jimenez, Richard, 292
Jindau, M. K., 290
Jingu, Mwana, 300
Johnson, Margaret, 190
Johnson, Paul, 121
Johnson, Victoria, 161t, 165t
Johnston, Susan, 212
Jones, B., 317
Jones, David, 190, 294
Jordan, Elaine, 235
Joshi, Shriram, 108
Jouault, Thierry, 4, 32
Jun, Li Jian, 105
Justement, J. S., 41
Kabagabo, Uwa, 262
Kahn, James, 188, 215
Kaldor, John, 60
Kaleebu, Pontiano, 46
Kall, Kerstin, 73
Kamps, Bernd Sebastian, 295
Kaneshima, Hideto, 21
Kang, Edward, 96t
Kannagi, Mari, 38
Kapiga, Saidi, 308
Kapita, Bila, 82
Kaplan, Jonathan, 85
Kaplan, Lawrence, 209
Karon, John, 87
Karpas, Abraham, 160
Kashamuka, Mwandagalirwa, 108
Kass, Nancy, 274-275
Katabira, Elly, 220
Katlama, Christine, 193
Keet, Ireneus, 61
Keffelew, Atnaf, 66
Kelen, Gabor, 277
Keller, Steven, 76, 308
Kelley, Patrick, 78f
Kellogg, Timothy, 61
Kemeny, Margaret, 173
Kemp, Maurice, 166
Kemp, Sharon, 152
Kemper, Carol, 197
Kent, Charlotte, 197
Kent, Karen, 134
Kerndt, Peter, 303
Kessler, Debra, 80, 317
Kestens, Luc, 103, 176f
Khaitov, Rakhim, 96t
Khayam-Bashi, Hassan, 105
Kilbourne, Barbara, 82
Kingsley, Lawrence, 61
Kiragu, D., 316
Klee, Hilary, 304
Kleiber, Dieter, 304
Knupp, C.A., 186
Koch, Matthew, 184
Koch, Thomas, 263
Kocsis, Agnes, 258, 259t
Koff, Wayne, 115
Kojouharoff, Georgi, 30
Kolbe, Lloyd, 307, 314
Koopman, Cheryl, 308
Koralnik, Igor, 251f, 252
Kotloff, Karen, 309
Kouri, Yamil, 214
Kovacs, Andrea, 225
Kowalewski, Mark, 305
Kozhich, Alexander, 95t
Kraft, Pal, 308
Kramer, Alexander, 175
Krell, Pamela, 259
Krown, Susan, 165t, 208
Kuiken, Carla, 119
Kukla, Michael, 153
Kumagai, Kazuo, 149
Kurth, Reinhard, 19
Kuznetsova, Irina, 75
Kyungu, [illegible], 290
LaBoy, Felicity, 306
Lacomte, Isabelle, 204
Lacroix, Martial, 94t

f = figure; t = table

Laga, Marie, 58, 72
Lagakos, Steven, 174
Lallemant, Marc, 82
Lamptey, Peter, 69, 80
Lane, Clifford, 163, 164t, 181, 187
Lang, William, 217
Laporte, A., 189
Larder, Brendan, 146, 152, 184
Laurent, Anne, 11
Laurian, Yves, 70
Lavilla, P., 205
Lee, Francis, 111, 112
Lee, Helen, 85
Lee, Jacalyn, 294
Leibman, Bryan, 311
Leigh, Timothy, 191
Lemp, George, 81
Leonard, John, 282
Leoro-Monroy, Galo, 109
Lepage, Philippe, 82, , 225
Leport, Catherine, 190, 192, 196, 197, 204
Lerche, Nicholas, 19
Levin, B.E., 259
Levine, Alexandra, 126, 136
Levy, Jay, 29, 42, 46, 122
Levy, Robert, 247, 249f
Lewis, Charles, 276
Lewis, Diane, 304
Lhomond, Brigitte, 298
Li, Jui-Lien, 142
Lichter, Saralyn, 206
Liekweg, Richard, 215, 216t
Liesnard, Corinne, 194
Lifson, Alan, 52, 171, 172f
Lima, Moreira, 281
Lindholm, Merete, 295
Link, W., 294
Littell, Cary, 271
Liuzzi, Grazia, 241
Lo, Shyh-Ching, 44, 197
Longhini, Ira, 87
Longiaru, Mathew, 85
Longo, Paulo, 291, 313
Loring, Keith, 277
Louie, Leslie, 46, 47, 172
Louis, Jean Paul, 300
Loveday, Clive, 103
Low, Loretta, 151
Luna, G. Cajedtan, 308
Luo, Nkandu, 108
Macguire, Bryan, 219
MacKenzie, Betsey, 291
Mackewicz, Carl, 38
Maeda, Yosuke, 130
Magi, Carlos, 54
Magruder, Charles, 299
Mahari, Maxwell, 194
Malcolm, John, 212
Malek, Adel, 225
Malkin, Jean-Elie, 83
Manhalter, Josef, 132
Mann, Dean, 206-207
Manning, Donna, 254
Marasco, Canio, 159
Marchisio, Paola, 227
Marcus, Ruthanne, 267
Marder, Reggi, 215
Margolis, David, 13
Marin, Barbara, 306
Mariner, Wendy, 117
Markoulatos, Panayotis, 105
Marra, Christina, 259
Martin, Alex, 241
Martin, Gianstefano, 314
Martin, Luisa, 73
Martin, Malcolm, 13
Martin, Natasha, 111
Martinex-Maza, Oto, 43, 44
Masiero, Janet, 68
Masur, Henry, 164t
Matheny, Samuel, 222
Matheron, Sophie, 193-194
Mayer, Kenneth, 188
Mbidde, Edward, 206
McAllister, Ruth, 252
McArthur, Justin, 260
McBride, Evelyn, 279
McCray, Eugene, 70
McCullough, Laurence, 282, 283
McCune, Joseph, 21, 142
McDaniel, Reg, 166
McGrail, Michael, 188
McGrath, Michael, 154
McGreevy, P. B., 97
McKay, Carolyn, 304, 313
McKinney, Ross, 227
McKusick, Leon, 302
McManus, Thomas, 302
McMichael, Andrew, 36, 42
Meng, Tze-Chiang, 161t, 185
Merigan, Thomas, 153, 185, 186
Merino, Fernando, 58
Merrill, Pauline, 252
Merson, Michael, 49, 58
Merzel, Cheryl, 213
Messenheimer, John, 252
Meyerhans, Andreas, 38
Michel, Frederique, 121
Middlestadt, Susan, 312
Miedzinski, Lil, 218
Mikol, Jacqueline, 247
Miller, Kim, 291
Minns, Daniel, 306
Mishu, Ban, 274
Mitchell, Charles, 226
Mitchell, Janet, 79
Mitsuya, Hiroaki, 185
Moas, Carlos, 56
Mohri, Hiroshi, 153
Moien, Mary, 213
Mok, Jacqueline Y.Q., 319
Molye, Graeme, 193
Moncany, Maurice, 105
Montagnier, Luc, 44, 45, 197
Montaner, Julio, 152, 153
Montgomery, Bruce, 189
Montgomery, Kathleen, 276
Monzon, Ofelia, 109
Mora, Jose Luis, 54
Moran, Patricia, 104
Moriyama, Etsuko, 16
Morse, Gene, 164t
Mosier, Donald, 21, 134, 135
Mozharova, Galina, 308
Mposo, Ntumbanzondo, 281
Muga, Roberto, 64
Mulanga, Kabeya, 107
Mulder, Jan, 161t
Mulhall, Brian, 60, 62, 317
Mullen, M., 244
Muller. Werner, 155
Murphy-Corb, Michael, 126
Musagara, Maako, 300
Muska, Susan, 295
Mwale, Clement, 79
Mwilu, Roy, 294
Myers, Ted, 313
Naber, Dieter, 253, 259
Nadeau, Denise, 275
Narayan, Bill, 18
Nau, Charles, 285
Ndugwa, Christopher, 75
Nease, Jr. R.F., 275
Nelson, Kenrad, 66
Neumann, Peter, 178
Ngcongco, Vuyelwa Ndiki, 278
Nicholas, Peter, 245
Nicolosi, Alfredo, 69
Noble, Anita, 204
Noskin, Gary, 190
Ntaba, H.M., 217
Nyamathi, Adeline, 311
O'Brien, Joan, 279
O'Brien, Maura, 314
O'Brien, Thomas, 73
O'Bryen, Barbara, 97, 98f
O'Farrell, Nigel, 71
O'Reilly, Kevin, 302
Odero, Theresa, 295
Ogden, Ann, 279
Ogunyankin, O., 290
Ohki, Kohji, 130
Ohlsson-Wilhelm, Betsy, 104, 177
Orchi, Nicoletta, 214
Ordonana, Juan, 308
Orenstein, Jan, 205
Ornelas, Gloria, 294
Oros, Cheryl, 315
Osmond, Dennis, 179
Ostrow, David, 254
Otten, Mac, 71
Owens, Douglas, 267
Packard, Mark, 174
Padgug, Robert, 222
Padian, Nancy, 67, 69
Page, Iris, 295
Pando, Jose, 188
Panicali, Dennis, 129
Panlilio, Adelisa, 267-268
Pappas, Les, 304
Para, Michael, 174-175
Parenti, David, 192
Park, Lawrence, 172
Parry, John, 102
Patrone-Reese, Janine, 278
Paul, David, 34
Pauwels, Rudi, 8, 153
Payanzo, Ntsomo, 300
Pedersen, Court, 174
Peeters, Martine, 16, 20
Pelagi, Micaela, 130
Pemberton, Iain K., 8
Penny, Ronald, 32, 44, 45f
Peralta, J., 83
Pereira, Rosemarie, 31, 40
Perkins, Diana, 255
Perl, Daniel, 240
Perno, Carlo-Federico, 42
Pert, Candace, 150
Peterman, Thomas, 78
Peters, Barry Stephen, 82
Phillips, Andrew, 172
Phillips, Constance, 319
Picard, Odile, 137
Plata, Fernando, 38, 123
Plotkin, Stanley, 125
Pokrovsky, Vadim, 56, 308
Poli, Guido, 41
Pollack, Lance, 301
Pollard, Richard, 162
Polsky, Bruce, 140
Poon, Man-Chiu, 318
Porter, Anne, 95t
Pottage, John, 196
Prakesh, Kesh, 125
Price, Richard, 239-240
Prudent, Nicole, 295
Pulliam, Lynn, 240
Puro, Vincenzo, 273
Pursell, Kenneth, 205
Putney, Scott, 36, 37, 119f, 120, 122
Quieffin, Jean, 191
Quinhoes, Eleonora, 194
Quinn, Thomas, 80
Quinnan, Gerald, 122
Quintos, M. Lourdes L., 310
Rabin, Linda, 21
Robkin, Charles, 209
Rader, Alison, 294
Rakusan, Tamare, 111
Ramah, Michael, 289
Rankin, Michael, 292
Rasmussen, Bjarne, 308
Rautenberg, Ellen, 216
Reade, Russell, 310
Recker, David, 113
Redfield, Robert, 135
Redick, Mary, 292
Reyes, Oswaldo, 299
Reynes, Jacques, 206
Rhodes, Andrew, 158
Rich, Kenneth, 112
Rich, Lynne, 261
Rico, Blanca, 291
Rieckmann, Peter, 39
Riera, Anna, 212
Robbins, Susan, 309
Roberts, Jeff, 225
Roberts, Noel, 158
Robertson, Kevin, 259
Robertson, Roy, 172
Robinson, Edward, 122
Rodrigues, Lair, 54

f = figure; t = table

Rodrigues, Richard, 318
Rodriquez, Evelyn, 84
Rogers, Mari, 280
Rohde, Iris, 190
Roithamann, Sergio, 208
Romo, Javier, 194
Rose, David, 194
Rose, Thanne, 102
Rosenberg, Philip, 88
Rosenberg, Steven, 160
Rosenblum, Mark, 209, 247, 250
Rotheram-Borus, Mary Jane, 315
Roy, Charles, 318
Royce, Rachel, 172
Rubini, Norma P.M., 226
Rubio, Rafael, 209
Ruf, Bernhard, 244, 245
Rukarangira, N.W., 291
Ruprecht, Ruth, 134
Rusche, James, 11
Russell, Michele, 306
Rwakagiri, Francis, 281
Saag, Michael, 177
Sakaguchi, Mamoru, 32
Sakai, Koji, 12
Sakuntanaga, Pralom, 67
Salk, Jonas, 125
Salmon, Dominique, 183
Sandfort, Theo, 297
Santana, Rosemarie, 310
Santos Esperanca Abreu, 272
Sarver, Nava, 158
Scaravilli, F., 248
Schattenkerk, Eeftinck, 204
Scheibel, Steven, 190
Schere, E., 319
Scherer, Wolfgang, 163
Schiltz, Marie-ange, 62
Schinaia, Nicola, 70, 172
Schindler, Stephen, 293
Schmid, P., 238
Schmitt, Frederick, 260
Schnittman, Steven, 29
Schoenbaum, Ellie, 63
Schouten, Jeffrey, 294
Schroder, Heinz, 155
Schuman, Paula, 228
Schurmann, Dirk, 247
Schwartz, David H., 165t
Scott, Charles, 37
Sczakiel, Georg, 157, 158
Seaton, Timothy, 211
Selik, Richard, 214
Selnes, Ola, 260
Seppo, Sarna, 313
Sepulveda, Jaime, 54, 55t
Shafferman, Avigdor, 133
Shah, Kiran, 226
Sharer, Leroy, 236
Shaunak, Sunil, 238
Sheppard, Haynes, 178
Sherr, Lorraine, 289
Shields, Anne, 214
Shih, Yihshing, 99
Shin, Diana, 277
Shioda, Tatsuo, 29
Shirasaka, Takuma, 152
Shum, Archie, 13
Sia, Dwo, 126
Siegal, Frederick, 196-197
Siliciano, Robert, 135
Siller, Karen, 101
Simpson, David, 243
Sinclair, Anne, 123
Singer, Elyse, 240, 241
Sirianni, 35
Skinner, Harvey, 283
Skowron, Gail, 161t, 185
Slavin, Monica, 191
Smit, Lia, 157
Smith, Elmer, 222
Smith, Gale, 135
Smith, Jaime, 253
Smith, Mark, 218
Snyder, Frederick, 305
Soave, Rosemary, 204
Soda, Kenji, 60
Sodrosky, Joseph, 4
Solomon, Liza, 290
Soskolne, Varda, 318
Sparger, Ellen, 20
Sperduto, Andrea, 40
St. Clair, Marty, 184
St. Lawrence, Janet, 302
Stall, Ron, 302
Stanley, Ann, 88
Stanton, David, 217
Stapinski, Andrzej, 58
Stark, Klaus, 64
Steimer, Kathelyn, 122
Stein, Michael, 194
Stewart, Karen, 192
Stille, W., 295
Stimson, Gerald, 63, 303
Stocking, Carol, 282
Stramer, Susan, 102
Strauss, Ronald, 292
Strayer, David, 154
Stricof, Rachel, 76
Strunin, Lee, 277, 307
Sugarman, Jeremy, 195
Sutor, Christopher, 130
Swanton, Katherine, 81
Sweeney, Patricia, 74
Syndulko, Karl, 256
Tacconi, Federica, 68
Takeda, Akira, 42
Tamalet, Catherine, 83
Temoshok, Lydia, 258
Tenant-Flowers, Melinda, 245
Terwilliger, Ernest, 11
Thomas, Pauline, 53
Thompson, P. Imania, 293
Ticc, Jeffrey, 300
Tokars, Jerome, 272, 273
Toma, Emil, 190
Torres, Ramon, 191
Torrey, David, 38
Tortem, Michael, 20
Tozzi, Valerio, 260
Tristem, Michael, 15
Tsubota, Hiroshi, 150
Tucker, Tarvez, 246
Tuite, Michael, 253
Tunstall, Chrystal, 311
Tuur, Sylvana, 197
Uittenbogaart, Christel, 178
Ungar, Wendy, 186
Updike, Marcia, 246
Urbain, Daniel, 205
Valle, S. L., 313
Van den Abeele, Anne, 95t
Van der Wouw, Poll, 164t
van Griensven, Godfried J.P., 173
Vanham, Guido, 38
Vanhems, Phillipe, 214t
Vazeux, Rosemary, 261-262
Velin, Robert, 259-260
Vercautern, Gaby, 97
Vermund, Sten, 209
Viale, Giovanna, 120
Vilde, Jean-Louis, 211
Viteri, Fernando, 212
Vogt, Guillaume, 133
Volberding, Paul, 182
Volker, Daniel, 39
Volkow, Patricia, 81
Volsky, David, 140
von Laer, Dorothee, 30, 31
Vujcic, Luba, 119
Vyakarnam, Annapurna, 41
Wadhawan, D., 196
Wagle, Usha, 73f
Wagner, Ralf, 129
Walker, Christopher, 38
Wang, Richard, 177, 178
Ward, John, 103
Waskin, Hetty, 191
Watters, John, 64, 304, 314
Wayment, Heidi, 318
Weber, Jonathan, 46
Weber, Rainer, 172
Weber, Thomas, 246
Weerts, Danielle, 164t, 166
Weiblen, Barbara, 111
Weiner, Marc, 313, 314
Weinstein, Steven, 315
Weinstock, Hillard, 298
Weintrub, Peggy, 228
Weiss, Stanley, 172
Wells, James, 297
Werdegar, David, 292
Weslowski, Virginia, 215
White, Carol, 80, 317
White-Scharf, Mary, 37
Whitt, Kenneth, 263
Widman, Mindy, 215
Wiebel, Wayne, 65
Wiley, Clayton, 248
Wilhelm, James, 129
Williams, Kenneth, 294
Willoughby, Brian, 62
Wilson, Mary T., 292
Winkelstein, Warren, 172, 207
Wolfe, Hannah, 65
Wolitski, Richard, 62
Wolters, Pamela, 227, 263
Wong, A.Y., 277
Wong, Philipe, 95t
Wong-Staal, Flossie, 12
Wood, Lauren, 38, 121
Woods Joycelyn Sue, 291
Wu, Ping, 94t
Yamamoto, Janet, 134
Yarchoan, Robert, 153, 184, 185, 187, 208
Yeoh, Eng-kiong, 60
Young, Mary, 188
Young, Paul, 314
Zaccarelli, Mauro, 64
Zackin, Robert, 166
Zagury, Daniel, 136
Zamora, Laura, 196
Zarling, Joyce, 147
Zerboni, Roberto, 71
Zessler, Laurent, 54
Zuccotti, G., 79
Zurlo, John, 192

AFFILIATION INDEX

Abbott Laboratories, Chicago, 84, 85, 94t, 99, 102, 103, 106, 107t, 108, 109, 158
Academic Medical Center, Amsterdam, 119, 120, 122, 152, 157, 164t, 184, 204, 244-245
Advanced BioScience Laboratories, Kensington, Maryland, 8
Agency for Health Care Policy and Research, Rockville, Maryland, 215
AIDS Committee of Toronto, 313
AIDS Control Programme/Rakai Project, Uganda, 300
AIDS Info BBS, San Francisco, 295
AIDS Secretariat, Frederiksberg, Denmark, 308
AIDS Unit USL, Rome, 64
AIDSCOM/Porter Novelli, Washington, D.C., 289
Albert Einstein College of Medicine, New York, 196-197, 217, 222
American Red Cross, 100
Amsterdam Municipal Health Service, 88, 173
Applied bioTechnology, Cambridge, Massachusetts, 129
Armed Forces Institute of Pathology, Washington, D.C., 44, 177, 178, 197
Association of State and Territorial Public Health Laboratory Directors, 100
Auguste-Viktoria Hospital, Berlin, 193 248
Bangkok Metropolitan Administration, 67
Bar Ilan University, Israel, 166
Baylor College of Medicine, Houston, 282, 283
Bayview-Hunter's Point Foundation, San Francisco, 305
Becton Dickinson Monoclonal Center, Mountain View, California, 130
Behringwerke AB, Marburg, FRG, 94t
"Being Alive," Los Angeles, 295
Bernard Nocht Institute for Tropical Medicine, Hamburg, FRG, 31, 166
Beth Israel Medical Center, New York, 273
Biocine, 137
Boston City Hospital, 78, 218, 262
Boston Department of Health & Hospitals, 195
Boston University Schools of Medicine & Public Health, 187, 195, 277, 307
Boston University, 117, 295, 308
Brazilian Army Institute for Biology, Rio de Janeiro, 97, 108
Bristol Polytechnic, Bristol, U.K., 313
Bristol-Myers Squibb Company, New York, 153, 186
Bristol-Myers Squibb Pharmaceutical Group, Ottawa, 186
British Biotechnology, Ltd., Oxford, U.K., 129, 137
Brown University, Providence, Rhode Island, 161t, 311
Burroughs Wellcome Company, Research Triangle Park, North Carolina, 147, 163, 261
Cabrini Medical Center, New York City, 244
California Department of Health Services, Berkeley, 105, 178
California Primate Research Center, Davis, 19
California State University, Long Beach, 62, 312
Cambridge BioScience Corporation, Worcester, Massachusetts, 100, 106, 107t, 108
Cambridge University, U.K., 160
Canadian AIDS Society, 294
Caremark Inc., Lincolnshire, Illinois, 180
Case Western Reserve University, Cleveland, 164t, 204
Cedars-Sinai Medical Center, Los Angeles, 153
Center for Special Immunology, Ft. Lauderdale, Florida, 212
Centers for Development and Health, Port-au Prince, 82
Centers for Disease Control, Atlanta, 52, 52f, 53, 58, 63f, 64, 65, 70, 71, 72, 74, 77, 78, 80, 81, 82, 83, 85, 87, 100, 103, 108, 113, 194, 224, 214, 267, 270, 271, 272, 273, 274, 279, 290, 291, 292, 293, 294, 297, 298, 301, 302, 307, 310, 312, 313, 314, 316, 317
Central Bureau of AIDS Coordination Programme, Kinshasa, Zaire, 295
Central Institute of Hygiene, Microbiology, & Epidemiology, Berlin, 56
Central Research Institute of Epidemiology, Moscow, 56, 75, 308
Centre Hospitalier de Kigali, Rwanda, 82, 195, 225
Centre Hospitalier de l'Universite Laval, Quebec, 275
Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, 269, 269f
Centre Regional de Transfusion Sanguine, Montpellier, France, 84
Centro de Orientacion e Investigacion Integral, Santo Domingo, Dominican Republic, 75, 311
Centro Medico Entre Amigos, Quito, Ecuador, 294
Cetus Corporation, Emeryville, California, 99
Charing Cross and Westminster Hospital, London, 63, 66
Children's Hospital of New Jersey, Newark, 227, 279
Children's Hospital of Philadelphia, 34, 125
Children's Hospital of San Francisco, 217
Children's National Medical Center, Washington, D.C., 111
Chiron Corporation, Emeryville, California, 38, 122, 137
Christ Church Cambridge AIDS Concern Committee, Cambridge, Massachusetts, 292
Chulalongkorn University Hospital, Bangkok, 102
Ciba-Geigy, 137
Citizens' Commision on AIDS, New York City and Northern New Jersey, 284
City Health Department, Durban, South Africa, 71
City Health, Harare, Zimbabwe, 194
City Hospital, Edinburgh, 260, 319
City of Hope Medical Center, Duarte, California, 158
Cleveland Clinic Foundation, Ohio, 276
Clinical Partners, Boston, 215
Clonatec, Paris, 107t
CNRS, France, 62, 298
Columbia University College of Physicians & Surgeons, New York, 8, 140, 211
Columbia University, New York, 308, 315
Communicable Disease Surveillance Centre, London, 76
Community Health Network, Rochester, New York, 190
Connaught Centre for Biotechnology Research, Ontario, 126
Cook County Hospital, Illinois, 316
Cook County Jail, Illinois, 316
Cornell Medical Center, New York, 204
Cornell University Medical College, New York, 101, 242
Coulter Immunology, Hialeah, Florida, 103
Dallas-Fort Worth Medical Center, Grand Prairie, 166
Dana-Farber Cancer Institute, Boston, 4, 7, 8, 11, 12, 134, 150
Danderyds Hospital, Stockholm, 79
Decision Focus Inc., Los Altos, California, 275
Denver Disease Control, Colorado, 280, 302
Department of Disease Control, Akure, Nigeria, 290
Department of Health Research Institute for Tropical Medicine, Philippines, 109, 310
Department of Public Health, San Francisco, 171, 172f
Department of Veterans Affairs, Washington, D.C., 280
Deutsche AIDS-Hilfe, Berlin, 294
Deutsches Krebsforschungszentrum, Heidelberg, 158
Direccion General de Salud, Murcia, Spain, 308
Disease Detection International, Irvine, California, 107t
Duke University Medical Center, Durham, North Carolina, 165t, 174, 191, 195, 215, 216t, 227, 238
DuPont, Wilmington, Delaware, 100, 107, 107t, 108
Ecole de Sante Publique, Zaire, 300
Ecuadorian Red Cross, Quito, 109, 299
Edinburgh Drug Addiction Study, 172
Eisenhower Medical Center, Rancho Mirage, California, 205
Elmhurst Hospital, New York, 204, 245
Emilio Ribas Hospital, São Paulo, Brazil, 272
Emory University School of Medicine, Atlanta, 111, 196
Emory University, Atlanta, 19, 87
Empire Blue Cross & Blue Shield, New York, 222
Epitope, Inc., Beaverton, Oregon, 102
Erasme Hospital, Brussels, 194
ETR Associates, Scotts Valley, California, 315
European Addiction Research Institute, Rotterdam, 305

f = figure; t = table

Fairfield Hospital, Melbourne, 191, 192, 193
Family Health International, Durham, North Carolina, 69, 80, 109, 109f
Federal Centre for AIDS, Ottawa, 53f, 102, 104, 178, 291, 295
Federal University of Bahia, Salvador, 80
Federation of Uganda Employers, 281
Fenway Community Health Center, Boston, 188
Finlay Medical Society, Miami, 56
Florida Department of Health and Rehabilitative Services, Tallahassee, 317
Florida Psychological Association AIDS Network, Ft. Lauderdale, 261
Food & Drug Administration, Bethesda, Maryland, 37, 100, 119, 122, 142, 143, 147, 148, 160, 192, 236, 261
Free Hospital and School of Medicine, London, 190
Free University, Berlin, 244, 245, 246
Fujirebio Inc., Japan, 106, 107, 108, 109
Fukushima Medical College, Japan, 153
Fundacao Oswaldo Cruz, Rio de Janeiro, 83
G.E.E.P., Villeneuve-Saint-Georges, Burkina Faso, 83
Gaffrée & Guinle University Hospital, Rio de Janeiro, 195, 206
Gay Men's Health Crisis, New York, 291, 294
Gen-Probe, Inc., San Diego, 105
Genelabs, Inc., Redwood City, California, 154
Genentech, Inc., San Francisco, 132, 149, 149f
General Accounting Office, Washington, D.C., 315
Genetic Systems, Inc., Seattle, 95t, 106, 107t
Geneva University Hospital, 190
Georg-Speyer-Haus, Frankfurt, 15, 30
George Washington University Medical Center, Washington, D.C., 127, 136, 188, 192, 205
George Washington University, Washington, D.C., 274-275
Georgia State University, Atlanta, 73
Glaxo, Inc., North Carolina, 152
Governor's Committee on AIDS, Honolulu, 294
Grady Memorial Hospital, Atlanta, 196
Greater New York Blood Program, 80, 317
Grupo Argentino de Tratamiento de la Leucemia Aguda, Buenos Aires, 290
Hadassah Medical Organization, Jerusalem, 318
Hahnemann University, Philadelphia, 154, 309
Hannover Medical School, Germany, 165t
Harlem Hospital Center, New York, 74, 79
Harvard Community Health Plan, Boston, 218
Harvard Institute for International Development, Cambridge, Massachusetts, 214
Harvard Medical School, Boston, 32
Harvard School of Public Health, Boston, 291, 303
Harvard University, Boston, 161t, 165t, 174
Hasanuddin University, Indonesia, 299
Hawaii Medical Association, 294
Health Care Financing Administration, Baltimore, 222
Health Department, Madrid Council, 76
Health Resources and Services Administration, Rockville, Maryland, 222
HEM Research, Inc., Philadelphia, 154
Henry Ford Hospital, Detroit, 318
Henry M. Jackson Foundation, Rockville, Maryland, 163, 258
HIV Immunology Unit, Edinburgh, 175
HIV Screening Section, Verona, 314
Hoffmann-LaRoche & Company, Nutley, New Jersey, 153, 162
Hoffmann-LaRoche, Basel, 94t
Hokkaido University, Sapporo, Japan, 12, 130
Hong Kong Department of Health, 60
Hôpital Antoine Beclare, Clamart, France, 79, 79t
Hôpital Bichat-Claude Bernard, Paris, 161t, 183, 190, 192, 193-194, 196, 197, 204, 211
Hôpital Cantonal Universitaire, Geneva, 214
Hôpital Cochin, Paris, 111
Hôpital de la Timone, Marseilles, 83
Hôpital du St. Sacrement, Quebec, 53
Hôpital Fernandez, Buenos Aires, 205
Hôpital Lariboisiere, Paris, 247
Hôpital Paul-Brousse, Villejuif, France, 31, 32
Hôpital Pitie-Salpètriére, Paris, 192, 193, 208
Hôpital Raymond Poincare, Garches, France, 177
Hôpital Saint-Antoine, Paris, 189. 208
Hospital Clinic, Barcelona, 196
Hospital de la Santa Creu i Sant Pau, Barcelona, 212
Hospital Gaffrée a Guinle, Rio de Janeiro, 194
Hospital Germans Trias i Pujol, Barcelona, 64, 212
Hospital Infantil la Paz, Madrid, 227
Hospital PEMEX, Mexico City, 81
Hôtel-Dieu de Montréal, 190
Hunter College Center for Community Action to Prevent AIDS, 294
Hvidovre Hospital, Denmark, 121, 148
IAF BioChem International, Inc., Montreal, 94t, 100
Il Universita, Rome, 88
Illinois State University, Normal, 297
Immune Products, Ltd., Horsham, Pennsylvania, 126, 136
Immune Response Corporation, San Diego, 136
ImmunoAG, Vienna, 132
Imreg, Inc., New Orleans, 166
Indiana University School of Medicine, Indianapolis, 190
Innogenetics, Antwerp, 95t
Innovatieprojekt, Amsterdam, 318
INSERM, Lyon, France, 111, 136
INSERM, Paris, 69, 86
INSERM, Universite de Bordeaux, France, 224
INSERM, Universite Paris, 226
INSERM, Villejuif, France, 31, 290
Insituto Brasileiro de Inovacoes em Saude Social, Rio de Janeiro, 313
Institut de Neurologie Tropicale, CHU Limoges, France, 84
Institut fur Physiologische Chemie, Universitat Mainz, 155
Institut Pasteur Hellinesque, Athens, 105
Institut Pasteur, Paris, 11, 12, 16, 17, 38, 44, 69, 105, 110, 120, 121, 123, 197, 235, 261-262
Institute Adolfo Lutz-Sao Paulo, 72
Institute of Immunohaematology, Bombay, 60, 108
Institute of Medical and Veterinary Science, Adelaide, Australia, 31, 40
Institute of Public Health, Oslo, 308
Institute of Tropical Medicine, Antwerp, 16, 20, 38, 72, 97, 103, 108, 176f
Institute of Venereology of Medical Academy, Warsaw, 58
Instituto Nacional de Saude, Lisbon, 83
Instituto Superiore di Sanita, Rome, 172
International Hemophilia Training Center, Kremlin-Bicetre, France, 70
IPN and Universite de Kinshasa, Zaire, 300
Israel Institute of Biological Research, Ness-Ziona, 133
Italian Cooperative Hemophilia-HIV Study Group, 70
Italian Partners' Study, 68
Italian Study Group on Occupational Risk of HIV Exposure, 273
Janssen Research Foundation, Spring House, Pennsylvania, 153
Johann Wolfgang Goethe Universitat. Frankfurt, 295
Johannes Gutenberg Universitat, Mainz, 39
John Marshall Law School, Chicago, 292
John Radcliffe Hospital, Oxford University, U.K., 36
Johns Hopkins Hospital, Baltimore, 39, 246
Johns Hopkins School of Hygiene & Public Health, Baltimore, Maryland, 217, 290
Johns Hopkins University School of Medicine, Baltimore, 18, 135, 206, 260
Johns Hopkins University, Baltimore, Maryland, 46, 66, 68, 75, 189, 216, 217, 218, 238, 241, 260, 277, 311
Jornal Do Brasil & the Instituto Brasileire de Inovacoes em Saude Social, Rio de Janeiro, 291
Kabalikat ng Pamilyang Philipino Foundation, Manila, 310
Karolinska Institute, Sundbyberg, 73
Katholieke Universiteit Leuven, Belgium, 8
Keith Medical Group, Los Angeles, 244
Kenya Medical Research Institute, Nairobi, 195
King's College Hospital, London, 302
Kingston General Hospital, Ontario, 279
Kumamoto University Medical School, Japan, 38, 130
Laennec Hospital, Paris, 208
Landesinstitut fur tropenmedizin, Berlin, 64
Lazzaro Spallanzani Hospital, Rome, 271f, 272, 273, 274
Life Foundation, 294
Lincoln Hospital, New York Medical College, Valhalla, 226
London Medical College, 8
Long Island Jewish Medical Center/Albert Einstein College of Medicine, New York, 196-197
Los Alamos National Laboratory, New Mexico, 88

f = figure, t = table

Los Angeles County & University of Southern California Medical Center, 225
Los Angeles County Department of Health Services, 102, 303
MacFarlane Burnet Centre for Medical Research, Melbourne, 174
MACS. *See* Multicenter AIDS Cohort Study
Makerere University, Kampala, Uganda, 75, 293
Mama Yemo Hospital, Kinshasa, 82
Manchester Polytechnic, U.K., 304
Massachusetts Department of Education, Quincy, 315
Massachusetts General Hospital, Boston, 105, 121, 161t, 165t
Massachusetts Institute of Technology, Cambridge, 214, 292
Massachusetts State Laboratory, Newton, 111
Max von Pettenkofer-Institut, Munich, 129
Medical Biology Institute, La Jolla, California, 21, 134
Medical Research Council Clinical Research Center, Harrow, U.K., 123
Medical School of Hannover, 130
Memorial Sloan-Kettering Cancer Center, New York, 140 161t, 165t, 205, 208
Merck, Sharp, & Dohme Research Laboratories, West Point, Pennsylvania, 120, 158
Merieux Institute, France, 166
Mexico City General Hospital, 194
Michael Reese Hospital, Chicago, 316
Michigan Department of Public Health, Lansing, 291
MicroGeneSys, Inc., West Haven, Connecticut, 124, 135
Mid-Atlantic AIDS Education & Training Center, Washington, D.C., 281
Middlesex Hospital, London, 252
Middlesex School of Medicine, London, 41
Midwest AIDS Biobehavioral Research Center, Chicago, 306
Ministerio Justicia, Madrid, 73
Ministry of Health, Brazil, 54, 281
Ministry of Health, Kenya, 295
Ministry of Health, Malawi, 217
Ministry of Health, Mexico, 54, 55t, 294, 299, 303
Ministry of Health, Paris, 189, 213
Ministry of Health, Romania, 57
Ministry of Public Health, Ecuador, 294
Ministry of Public Health, Moscow, 96t
Montefiore Medical Center, New York, 63, 175, 217, 222
Montreal Children's Hospital, 318
Montreal General Hospital, 189
Mount Sinai Hospital, New York, 240, 243
Mount Sinai School of Medicine, New York, 194, 295
Mount Zion Hospital and Medical Center, San Francisco, 193, 308
Mulago Hospital, Kampala, 75, 220
Multicenter AIDS Cohort Study, Bethesda, 172, 207
Multicenter AIDS Cohort Study, Los Angeles, 175, 177
Municipal Health Services, Amsterdam, 61, 65, 161t
Municipal School System, Copenhagen, 295
Murex Corporation, Norcross, Georgia, 107t
Narcotic and Drug Research Inc., New York, 291, 314
National Bacteriological Laboratory, Stockholm, 128, 133
National Cancer Institute, Bethesda, Maryland, 42, 44, 86, 88, 116, 128, 152, 153, 160, 184, 185, 187, 206-207, 208, 209, 226, 227, 263
National Center for Health Statistics, Hyattsville, Maryland, 213
National Centre in HIV Epidemiology & Clinical Research, Sydney, 60
National Council of Research, Milan, 69
National Hemophilia Foundation, New York, 70, 296
National Hospital, London, 248
National Institute for Biological Standards & Control, Potters Bar, U.K., 134
National Institute of Allergy & Infectious Diseases, Bethesda, Maryland, 13, 14, 21, 29, 38, 39, 40, 41, 42, 80, 84, 113, 115, 121, 125, 150, 158, 161t, 163, 164t, 165t, 181, 185-186, 187, 192, 209
National Institute of Genetics, Mishiwa, Japan, 16
National Institute of Hematology and Blood Transfusions, Heidelberg, 34
National Institute of Mental Health, Bethesda, Maryland, 150, 187, 235, 241
National Institute of Neurological Disorders and Stroke, Bethesda, 13, 14
National Institute on Drug Abuse, Rockville, Maryland, 64, 66, 294
National Institutes of Health, Bethesda, Maryland, 190-191, 192, 234, 235, 237-238, 241, 242, 272, 273
National Medical Association, Washington, D.C., 277
National Public Health and Hospital Institute, Washington, D.C., 215
National Research Institute of Health, Addis Ababa, Ethiopia, 108
National Task Force on AIDS Risk Reduction, San Francisco, 306
National Veterinary Institute, Uppsala, Sweden, 124
Neptune Computer Group, Stamford, Connecticut, 295
Network for Continuing Medical Education, Secaucus, New Jersey, 314
Nevada Brothel Association, 310
New England Behavioral Health Study, Providence, Rhode Island, 298
New England Deaconess Hospital, Boston, 32, 187
New England Regional Primate Center, Massachusetts, 126, 150
New Jersey Medical School, Newark, 236
New University of Lisbon, Portugal, 15
New York Business Group on Health, Inc., 281
New York City Department of Health, 53, 71, 196, 216, 310
New York City Perinatal HIV Transmission Collaborative Study Group, 110
New York Hospital-Cornell Medical Center, 195, 254, 306
New York Medical College, Valhalla, 211
New York State Department of Health, Albany, 76
New York State Psychiatric Institute, 308, 315
New York University Medical Center, 105, 207, 217, 220f, 221f, 222
New York VA Medical Center, 195
Nippon Veterinary & Zootechnical College, Tokyo, 20
North American Biologicals, Inc., Miami, 112
North Carolina AIDS Service Coalition, Chapel Hill, 294
North York Public Health Department, Ontario, 295
Northwestern University School of Medicine, Chicago, 209, 246, 247, 249f
NOVA Research Company, Bethesda, Maryland, 305, 314
Obafemi Awolowo University, Nigeria, 290
Ohio State University, Columbus, 174-175, 259
Oncogen Corporation, Seattle, 104, 124, 129, 135, 136, 147
Ontario Ministry of Health, Toronto, 100
Organisation de Coordination pour la Lutte contre les Endemies en Afrique Centrale, Cameroon, 300
Organon Teknika, 109
ORSTOM S/URB, Paris/Brazzaville, 82
Ortho Diagnostics Systems, Raritan, New Jersey, 94t
Ospedali Riuniti, Bergamo, 214
Ottawa General Hospital/University of Ottawa AIDS Study Group, 174
Oxford University, U.K., 36, 42, 129, 158, 219
Pan American Health Organization, Mexico City, 291
Pan American Health Organization, Washington, D.C., 54, 55, 55t
Paul Ehrlich Institute, Langen/Frankfurt, 19
PB Diagnostics Systems, Westwood, Massachusetts, 95t, 107t
Pennsylvania State University College of Medicine, Hershey, 104, 177
Peoria City/County Health Department, Illinois, 297
Pharmaceutical Manufacturers Association, 143, 147
PHLS Virus Reference Laboratory, London, 102
Pitie-Salpétrière Medical School, Paris, 4, 32, 132
Pitzer College, Claremont, California, 293
Planned Parenthood, Alameda/San Francisco, 310
Population Services International, Washington, D.C., 290
Professional Standards Review Council of America, Inc., New York, 279
Project HOPE Center for Health Affairs, Chevy Chase, Maryland, 297
Projet CONNAISSIDA, Lubumbashi-Zaire, 291
Projet RETRO-CI, Abidjan, Ivory Coast, 82, 84, 95t
Projet San Francisco, Kigali, Rwanda, 225, 295
Projet SIDA, Kinshasa, Zaire, 107, 108, 195, 262, 281, 300

f = figure; t = table

Public Health Department, Miami, 68
R.O.W. Sciences, Inc., 306
Rega Institute for Medical Research, Leuven, Belgium, 147, 151, 153
Regional Epidemiology Unit, Rome, 214
Repligen Corporation, Cambridge, Massachusetts, 11, 37, 119f, 120, 122
Research Triangle Institute, North Carolina, 184
Rhode Island Hospital, Providence, 194
Roche Diagnostic Systems, Inc., Nutley, New Jersey, 85
Roche Products, Ltd., U.K., 8, 158
Rochester Medical Center, New York, 186, 253
Rorer-Rhone Poulec, Fort Washington, Pennsylvania, 136
Royal Free Hospital School of Medicine, London, 14
Royal Hospital & School of Medicine, London, 172
Royal Newcastle Hospital, Australia, 212
Royal Postgraduate Medical School, London, 46
Ruchill Hospital, Glasgow, 64
Rudolf Virchow University Hospital, Berlin, 163, 209, 247
Rush-Presbyterian-St. Luke's Medical Center, Chicago, 34, 196
Rutgers University, New Brunswick, New Jersey, 213, 309
Rutgers University, Newark, New Jersey, 309
S. Martino Hospital, Genoa, 123
Saint-Antoine Hospital, Paris, 194
Salk Institute, La Jolla, California, 125
Salvation Army Chikankata Hospital, Zambia, 293, 294
San Francisco AIDS Foundation, 304
San Francisco Community College District, 292
San Francisco Department of Public Health, 52, 61, 62, 81, 292, 295, 301, 305
San Francisco General Hospital, 65, 154, 179, 188, 193, 209, 263, 271, 273, 319
Santa Clara Valley Medical Center, San Jose, California, 197, 204, 205
Sanyo-Kokusaku Pulp Company, Yamaguchi, Japan, 152
Schering Plough, 162
Seattle-King County Department of Public Health, 282
Seth G.S. Medical College & KEM Hospital, Bombay, 72, 73f
SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania, 148, 158
South Bank Polytechnic, London, 301
Southern Illinois University School of Medicine, Springfield, 279
Southwest Foundation for Biomedical Research, San Antonio, Texas, 132
Sozialpaedogogisches Institut Berlin, 304
Spallanzani Hospital, Rome, 260
Spectrum House, Amherst, Massachusetts, 304
SRA Technologies, Inc., Rockville, Maryland, 103
Srikant Tripathy of Indian council of Medical Research, New Deli, 73f
SSA/DPS/SPE/CEA, Fontenay aux Roses, France, 133
St. James's Hospital, Dublin, 311
St. Luke's/Roosevelt Hospital Center, New York, 12, 140, 193
St. Mary's Hospital and Medical School, London, 82, 258, 259t, 289, 319
St. Paul's Hospital, Vancouver, 62, 152, 191
St. Paul-Ramsey Medical Center, St. Paul, Minnesota, 270
St. Pierre University Hospital, Brussels, 164t, 166, 205
St. Stephen's Clinic, London, 217, 253
St. Vincent's Hospital and Medical Center, New York, 191
St. Vincent's Hospital, Sydney, Australia, 44, 245
Stanford University Medical Center, California, 31, 153, 165t, 212, 267
Stanford University School of Medicine, California, 178, 185, 197
Stanford University, California, 8, 186
State Office for National Plan on Drugs, Madrid, 64, 65
State University of New York Brooklyn, 263
State University of New York, Buffalo, 164t
State University of New York, Stony Brook, 263
Statens Seruminstitut, Copenhagen, 204
Sumitomo Chemical Co. Ltd., Hyogo, Japan, 149
Syntex Corporation, Palo Alto, California, 285
SyStemix, Inc., Palo Alto, California, 21, 142
Tanox Biosystems Inc., Houston, 129
Tenon Hospital, Paris, 204
Texas A & M University, College Station, 105
Transfusion Related AIDS Support Unit, Sydney, Australia, 81
Triton Biosciences, California, 162
Tucson VA Medical Center, University of Arizona College of Medicine, 205
Tufts University, Boston, 12
Tulane School of Medicine, New Orleans, 34, 113
U.S. Conference of Mayors, Washington, D.C., 293
Uganda Cancer Institute, Kampala, 206
Uganda Virus Research Institute, Entebbe, 46
Unified School District, San Francisco, 295
Union of American Hebrew Congregations Committee on AIDS, New York, 292
United Biomedical, Inc., Lake Success, New York, 96t, 106
Universidad Autonoma, Madrid, 205
Universidad del Pais, Vasco, 58
Universite de Lille, France, 295
Université de Montréal, 190
Université Pierre et Marie Curie, Paris, 120, 137
University College & Middlesex School of Medicine, London, 67, 103
University Hospital, Berlin, 190
University Hospital, Geneva, 251f, 252
University Hospital, Nice, 183
University Hospitals of Cleveland, Ohio, 246
University Medical College, Chicago, 190
University of Alabama, Birmingham, 177
University of Alberta, Edmonton, 218
University of Arizona, Tucson, 166, 307
University of Bari, Italy, 241
University of British Columbia, Vancouver, 62, 207, 253
University of Calgary, Alberta, 253, 313
University of California,
Berkeley, 46, 67, 69, 172, 197, 207, 212
Davis, 12, 19, 20, 134, Davis, 210
Irvine, 315
Los Angeles, 276, 277, 305, 311, 318
San Diego, 161t, 185, 255f, 256, 259-260
San Francisco, 29, 40, 42, 46, 64, 65, 66, 105, 111, 122, 182, 189, 193, 211, 215, 219, 228, 240, 250, 252, 263, 271, 272, 276, 280, 297, 298, 300, 301, 302, 304, 305, 307, 311, 314, 316, 319
Santa Cruz, 304
University of California, School of Medicine, Davis, 133
Los Angeles, 18, 40, 43, 44, 173, 178, 192 258
San Diego, 248, 256-257
San Francisco, 38, 315, 252, 258
University of California, School of Pharmacy, San Francisco, 188
University of Cambridge, U.K., 15
University of Chicago, 282
University of Colorado Health Sciences Center, Denver, 278
University of Colorado, Boulder, 158
University of Connecticut, Storrs, 297
University of Copenhagen, 174, 318
University of Dar es Salaam, Tanzania, 308
University of Edinburgh, 87
University of Frankfurt, 72
University of Genoa, 245
University of Georgia, Athens, 153
University of Göttingen, 246
University of Hawaii, 294
University of Heidelburg, 39
University of Helsinki, Finland, 313
University of Illinois, Champaign-Urbana, 312
University of Illinois, Chicago, 65, 112, 215, 306
University of Kentucky School of Medicine, Lexington, 260
University of Lausanne, Switzerland, 299
University of Maryland School of Medicine, Baltimore, 309, 310
University of Massachusetts, Worcester, 42, 315
University of Medicine & Dentistry of New Jersey, 65, 76, 172, 215, 227, 279, 308
University of Miami School of Medicine, 54, 68, 182, 183, 212, 226, 304, 313, 259, 280
University of Miami, Florida, 245, 278
University of Michigan, Ann Arbor, 254, 255, 302
University of Milan, 71, 73, 79, 120, 130, 227, 260-261
University of Minnesota, Minneapolis, 239-240
University of Mississippi Medical Center, Jackson, 302
University of Montpellier, France, 206
University of Munich, 250f, 251, 253, 259
University of Nairobi, Kenya, 316
University of New South Wales, Sydney, Australia, 32, 33, 34, 44

f = figure; t = table

University of North Carolina School of Medicine, Chapel Hill, 259
University of North Carolina School of Pharmacy, Chapel Hill, 159
University of North Carolina, Chapel Hill, 252, 255, 263, 292
University of Pavia, Italy, 68, 148
University of Pennsylvania Medical Center, Philadelphia, 32
University of Pennsylvania School of Medicine, Philadelphia, 33
University of Pennsylvania, Philadelphia, 15
University of Pittsburgh, 38, 61, 160
University of Rio de Janeiro, 226
University of Rome, 35
University of San Francisco, 306
University of Southern California School of Medicine, Los Angeles, 126, 136
University of Southern California, Los Angeles, 188
University of Sydney, Australia, 60, 62, 317
University of Texas Health Science Center, San Antonio, 278, 313, 314
University of Texas Medical Branch, Galveston, 162
University of the West Indies/CAREC, Port of Spain, 55
University of Toronto, 179, 191, 283, 304
University of Turin, Italy, 110
University of Utrecht, The Netherlands, 297
University of Virginia, Charlottesville, 268-269
University of Washington, Seattle, 100, 124, 128, 136, 161t, 183, 187, 259, 310
University of Wollonong, Australia, 294
University of Zaragiza, Spain, 291
University of Zurich, 172
University Teaching Hospital, Lusaka, Zambia, 79, 108, 196, 225
University Technologica de Santiago, 55, 84, 85
University Tubingen, 175
Upjohn Company, Kalamazoo, Michigan, 158
USSR Academy of Sciences, Moscow, 95t
VA Medical Center, San Diego, 255, 255f, 256, 259-260
VA Medical Center, University of Miami, Florida, 188
VA Medical Center, West Los Angeles, 256
Vanderbilt University, Nashville, Tennessee, 122
Veterans Administration Medical Center, San Francisco, 259
Vical, Inc., San Diego, California, 142
Viral Technologies, Inc., Washington, D.C., 127, 136
ViRx, Inc., San Francisco, 179
Walter Reed Army Institute of Research, Rockville, Maryland, 19
Walter Reed Army Institute of Research, Washington, D.C., 74, 78, 78f, 97, 98f, 135
Walter Reed Army Medical Center, Washington, D.C., 100, 225, 258
Washington Biolab, Inc., Creston, Iowa, 154
Washington State Office on HIV/AIDS, Seattle, 214
Washington University School of Medicine, St. Louis, 252, 258
Wayne State University of Medicine, Detroit, 228
Wellcome Diagnostics, 108
Wellcome Research Laboratories, Beckenham, U.K., 146, 152, 184
Wellcome Research Laboratories, Research Triangle Park, North Carolina, 7, 184
Wellcome, Inc., Paris, 227
West Los Angeles Veterans Administration Medical Center, 238, 240, 241
Westmead Hospital, Sydney, Australia, 151
Westminster Hospital, London, 191, 193
Westminster Medical School, London, 303
WHO Collaborating Centre on AIDS, Paris, 206
Wilford Hall U.S. Air Force Medical Center, San Antonio, 211, 256
Womack Hospital, Ft. Bragg, North Carolina, 299
World Health Organization Collaborating Centre on AIDS, Paris, 58, 88
World Health Organization, Geneva, 49, 51, 52, 57f, 58, 59f, 67, 74, 108, 179, 194, 291, 294, 299
World Health Organization, Regional Office for Africa, Congo, 278
Wyeth-Ayerst Research Laboratories, Pennsylvania, 129, 133
Yale University, 158
Yerkes Regional Primate Center, Georgia, 126
Yokohama City University School of Medicine, 60

INDEX

A

acemannan, 166
  AZT with, 164t
acriflavine derivatives, 144t
acrylic acid, 151
acupuncture, 73, 266
acyclouridine derivatives, 144t, 153
acyclovir, 144t, 183
  AZT with, 161t
ADC. *See* AIDS dementia complex
ADCC. *See* antibody-dependent cellular cytotoxicity
adenovirus, in recombinant vaccine, 126
adjustment disorders, 253
adjuvants
  defined, 123
  vaccines based on, 127t, 128-129
adolescents, 75-77
  AIDS incidence among, 77
  alcohol use among, 76, 308
  education of, 314-315
  ethnic/cultural variables among, 76, 305, 308
  homeless and runaway, 76, 308, 315
  homosexual, 308, 315
  knowledge, attitudes, and behavior of, 307-308
  risk behaviors among, 75-76, 305, 307-308
  seroprevalence among, 53, 76
Africa
  AIDS cases in, 51, 58-59, 59f, 69, 80
  blood supply in, 80
  clinical trials in, 220-221
  heterosexual transmission in, risk factors for, 68
  HIV-2 in, 83
  HIV-associated mortality in, 82
  HTLV-I in, 84
  Kaposi's sarcoma in, 206
  nursing care in, 278
  pediatric HIV infection in, 223
  perinatal transmission in, 79
  religious organizations in, 293
  transfusion-associated AIDS cases in, 69, 80
  tuberculosis and HIV in, 194
  women in, 75, 88-89
Afro-Americans. *See* ethnic/cultural variables
age, disease progression and, 172
agglutination tests, 106. *See also* specific tests (e.g., Serodia)
  on pooled sera, 109
AIDS
  clinical aspects of, 168-228. *See also* specific aspects (e.g., opportunistic infections)
  diagnosis of
    criteria for, 170-171, 320
    financial implications of, 171
  education. *See* education
  epidemiology. *See* epidemiology; specific groups at risk (e.g., IV drug users; women) and specific geographic regions
  incidence, worldwide, 49-50. *See also* specific geographic areas
  mortality in, 81-82
  occupationally acquired, 270. *See also* occupational risks
  pediatric. *See* pediatric AIDS
  progression of. *See* disease progression
  risk factors for. *See* risk factors
  transfusion-associated, 69-70. *See also* transfusion-associated AIDS
AIDS dementia complex (ADC), 239-241, 322
  defined, 239
  prevalence of, 256
AIDS policies, corporate, guidelines for, 284
AIDS-related complex (ARC)
  criteria for, 321
  in HIV disease progression, 171f
  passive immunotherapy for, 160
alcohol, high-risk sexual behavior and, 76, 301-302, 305, 308, 318
alpha interferon. *See* under interferon(s)
aluminum, 233f, 240
amebiasis, 198t
American Dental Association, HIV-related recommendations by, 284
American Medical Association, HIV-related recommendations by, 284
amikacin, 196
amniotic fluid, 268, 323, 324
ammonium trichlorotellurate. *See* AS-101
amphotericin B, 145t
  for cryptococcosis, 244
Ampligen, 141f, 144t, 154-156, 155f
anal intercourse, 61, 62, 68, 73, 75, 301, 305, 319
androgens, disease progression and, 178
anginlyc, 145t
animal models, 18-21. *See also* specific animals (e.g., mice)
  drug evaluation using, 142
  in vaccine development, 130-135
  of neurological aspects, 235-236
ansamycin. *See* rifabutin
antibody(-ies)
  detection of. *See* antibody tests
  enhancing
    in HIV infection, 42-43
    vaccine development and, 122
  idiotype/anti-idiotype, 129-130, 131f
  intrathecal, 237-238
  in neonates, 110-111, 223f, 224
  monoclonal. *See* monoclonal antibodies
  neutralizing
    cross-neutralization capabilities of, 122
    defined, 36
    in response to HIV infection, 36-37, 37f
    vaccine elicited, 121-122
  to gp41. *See* gp41
  to gp120. *See* gp120
  to gp160. *See* gp160
  to p24. *See* p24
  to "self." *See* autoantibodies
antibody response. *See* immune response
antibody tests, 94-96t, 98-102. *See also* diagnostic tests; specific tests (e.g., ELISA)
  algorithms for, 91, 91f, 109f
  antigens used in, 99
  for HIV-2, 94-96t, 100
  problems with, 92
  rapid, 106, 107t
antibody-dependent cellular cytotoxicity (ADCC)
  defined, 38
  epitopes eliciting, 121
  in autoimmune neurological disease, 241
  in response to HIV infection, 37f, 38
  vaccine elicited, 121, 123
    in chimpanzees, 132
antidepressants, 254
antigen (HIV), 36-37f. *See also* specific antigens (e.g., p24)
  conformation of, 116, 118, 121, 122, 123
  detection of. *See* antigen tests
  mass production of, 129
antigen presentation
  cells involved in, 26
  in vaccine development, 116, 118, 123-124
antigen recognition, 27, 28, 33f
  by B cells, 24, 28
  by T cells, 26, 123
  in normal immune response, 27
antigen tests, 93t, 102-104
antigenemia, disease progression and, 175-177, 176f, 179
antigenic determinants. *See* epitope(s)
antigenic drift, 28, 116, 119, 121. *See also* strain variation
antigenic sites. *See* epitope(s)
antigen-presenting cells, 26
antigen-specific memory, 28
anti-idiotypes, 129-130, 131f
antisense DNA, 156, 156f
antisense molecule(s), 156-158, 156f
antisense oligonucleotide(s), 141f, 144t, 157
  alpha interferon with, 165t
  AZT with, 161t
antisense RNA, 141f, 144t, 156-157, 156f, 157-158
antiviral agents, 147-159, 181-188. *See also* specific agents (e.g., Ampligen; AZT)
  combination therapies using, 146-147, 161t, 167
  immunomodulators with, 147, 164-165t, 167
  target sites of, 140, 141f
appetite stimulation, 212
ARC. *See* AIDS-related complex

f = figure; t = table

Argentina, 55t, 290
AS-101 (ammonium trichlorotellurate), 145t, 166
AZT with, 164t
Asia, AIDS cases in, 51, 60
aspergillosis, 205
Australia
AIDS cases in, 60
heterosexual transmission in, 67
homosexual/bisexual men in, 62, 317
AuT (1-aurothioglucose), 145t
autoantibodies
defined, 39, 148
in HIV infection, 39
in CNS, 233f
in inflammatory demyelinating polyneuropathy, 242
to CD4 receptor, 39, 148
autoimmune disease, 27. *See also* autoantibodies
cross-reacting antibodies in, 101
in HIV infection, 39
neurological, 241-243
autoradiography, in PCR testing, 105
azidouridine (AZddU), 144t
AZT (azidothymidine or zidovudine), 152-153, 181-186
acemannan with, 164t
ACTG trials of, 182-183
acyclovir with, 161t, 183
alpha interferon with, 163, 164t, 165t, 185-186
Ampligen synergism with, 155
antisense oligonucleotide with, 161t
AS-101 with, 164t
CD4-exotoxin hybrid with, 150, 161t
combination therapy with, 161t, 164-165t, 185-186
cost effectiveness of, 218
ddC with, 161t, 185
ddI with, 161t
depression and, 255
disease progression, effect on, 81-82, 86, 181-182, 216
doses and dosing schedules for, 182-183
dsRNA with, 155, 161t
during pregnancy, for prevention of pediatric HIV infection, 228
erythropoietin with, 164t
evaluation of, 152
in humans, 181-186
in SCID-hu mice, 142
for Kaposi's sarcoma, 208
for pediatric HIV infection, 226-227
G-CSF with, 164t
GM-CSF with, 164t, 165t
IgG with, 165t
in early HIV disease, 182
interleukin-2 with, 165t
length of treatment with, 184
lipid analogue synergism with, 159
liposome-encapsulated vs. standard, evaluation in mice, 142
mechanism of action of, 8, 141f, 144t, 152
myopathy due to, 183, 184, 242-243
neuropsychological performance and, 260-261
post-exposure prophylaxis with, 273-274
resistance to, 152-153, 184-185
survival time and, 81-82
toxicity of, 152, 183-184
underuse of, in pre-AIDS patients, 217

B

B cells, 24, 25f
activation of, in HIV infection, 39, 237
cytokine secretion by, 39
HIV-specific antibody-secreting, detection of, 111-112
in CNS, 237
baculovirus, recombinant vaccine using, 135
BCG (bacille Calmette-Guérin), recombinant vaccine using, 126
behavior. *See* behavior change; knowledge, attitudes, and behavior; specific behaviors (e.g., condom use)
behavior change. *See* also counselling; education; specific behaviors (e.g., condom use)
among adolescents, 307
among cocaine users, 305-306
among female partners of IVDUs, 313-314
among homosexual/bisexual men, 62, 302
among IVDUs, 65, 66-67, 305-306, 314
in needle sharing, 66-67, 304-305
induction of. *See* education
relapse, 62, 302
strategies for, 312-313
benzodiazepines, 153, 254
bereavement, 319
beta interferon. *See* under interferon(s)
beta-2-microglobulin
disease progression and, 175, 179
in children, 226
in cerebrospinal fluid, 237, 238
Betaseron, 162. *See* also interferon(s), beta
BHC-89, 144t
bioavailability, defined, 142
biologic response modifiers. *See* immunomodulators; specific agents
biopsy, brain. *See* brain biopsy
bisexual men. *See also* homosexual/bisexual men
and heterosexual transmission, 54, 67
blacks
adolescents, 76, 305, 308
AIDS education, effectiveness of, 306
condom use among, 298, 299, 305, 309
crack use among, 305
homosexual/bisexual men, 61, 306, 318
IVDUs, 63, 74, 290-291
knowledge surveys among, 306
in U.S. military, 77-78, 78f
seroprevalence (HIV) among, 52-53, 74
seroprevalence (HTLV-I) among, 84
survival time in, 81
women
seroprevalence among, 52-53, 74
IVDUs, 63
in U.S. military, 78
mortality in, 82
risk behaviors among, 298, 309, 311
blastocystis, 198t
blastomycosis, 198t
bleach (in IV needle reuse), 56, 67, 305
bleomycin, 208
blood/blood products. *See also* transfusion-associated AIDS
occupational exposure to. *See* occupational risk
screening of. *See* under blood supply
transmission via. *See* under transmission (HIV)
universal precautions for, 323-326
blood supply
HIV screening of, 97, 98f, 109f
in developing countries, 107-108
in India, 60
safety of, 80, 96-97
HTLV-I/II screening of, 85
transmission via. *See* under transmission (HIV); transfusion-associated AIDS
blood-brain barrier, 233f, 236-237
body fluids, universal precautions for, 323-326
bone marrow toxicity, AZT and, 183
bovine colostrum, hyperimmune, 204
bovine immunodeficiency virus (BIV), 17f
bovine leukemia virus (BLV), 17f
bovine syncytial virus (BSV), 17f
bowel epithelial cells, 32, 34
brain. *See also* central nervous system
cell types infected by HIV in, 232-234
model of HIV infection in, 233f
tumors in, 247
brain biopsy
image-guided, 247
indications for, 249f, 250
Brazil
AIDS cases in, 51, 54, 55t
blood donors in, 80
education by mass media in, 291
heterosexual transmission in, 67
HIV-2 in, 83
IV drug users in, 64
Kaposi's sarcoma in, 206
tuberculosis and HIV in, 194
breastfeeding
antibody transfer via, 110
child-to-mother transmission via, 75
mother-to-child transmission via, 79
bronchospasm, pentamidine-induced, 191
Bulgaria, AIDS cases in, 56, 57f
Burkitt's lymphoma, 208
Burundi
AIDS cases in, 59f
tuberculosis and HIV in, 194
business and industry
education in, 280-282
ethical and legal considerations in, 285
guidelines for corporate AIDS policies, 284

C

cachexia, 41, 211
California
AIDS cases in, 52f
mortality in children in, 82
women in, 74
campylobacter enteritis, 197, 200t

Canada
AIDS cases in, 52, 53-54, 53t
heterosexual transmission in, 67
HIV-associated mortality in, 82
needle/syringe exchange programs in, 66
cancer, HIV-associated, 43-44, 166, 206-209, 320, 321, 322
candidiasis, 198t, 205
as AIDS-defining illness, 320, 321, 322
treatment of, 205-206
caprine arthritis encephalitis virus (CAEV), 17f
capsid. *See* p24
carbohydrate epitope, 121
carboxyclic dideoxyguanosine (Carbovir), 144t
cardiomyelopathy, HIV-associated, 39
care givers, 319. *See also* health care workers
Caremark Connection, 180
Caribbean, AIDS cases in, 55-56, 55t, 69
cat(s), as animal model, 20, 134
cat scratch disease, 197
CD4 cell(s), 24, 25f, 26
abnormal function of, in HIV infection, 34
defined, 29, 146, 170
depletion of, in HIV infection, 34
depletion of precursor cells for, 40
HIV entry into, 32, 33f
in central nervous system, 234
preferential infection of, 29-30
regulatory functions of, 25f, 29
CD4 cell counts
AZT efficacy and, 181-184, 227
disease progression and, 174, 174t, 179, 322
in children, 226
epidemiological model based on, 87
in in vivo drug studies, 146
normal range of, 174
CD4 immunoadhesin, 144t, 149-150, 149f, 187
CD4 Immunotoxin, 141f, 144t
CD4 receptor, 4, 170
antibodies to, 32, 141f, 144t, 147
autoantibodies to, 148
in enhancement of HIV infection, 42
in HIV entry into cell, 31-33, 33f
interventions aimed at, 7-8, 144t, 147-151
microglial cell infection and, 234
on non-T cells, 32-33
CD4, soluble (sCD4), 141f, 144t, 148-150, 149f, 187
bacterial toxin conjugation with, 150, 161t
ddI with, 161t
for pediatric HIV infection, 228
IgG fusion with, 144t, 149-150, 149f, 187
liposome conjugation with, 149
CD4-toxin hybrid, 150, 161t
CD8 cells, 24-26, 25f. *See also* cytotoxic T cells; suppressor T cells
CD8, soluble, disease progression and, 179
CDC. *See* Centers for Disease Control
cell therapy, 160
cell tropism
of HIV, 29-34, 117, 232-234
of lentiviruses, 18
Centers for Disease Control (CDC). *See also in Affiliation Index*
criteria for AIDS diagnosis, 171, 320
staging classifcation system, 322
Central America, 55t. *See also* Latin America
central nervous system (CNS)
cell types infected by HIV in, 232-234
defined, 232
HIV entry into, 237
immune surveillance in, 236-237
immunological changes in, 237-238
model of HIV infection in, 233f
opportunistic infections of, 243-246
cerebrospinal fluid (CSF)
antibody levels in, 237-238
HIV detection in, 238-239
JC virus detection in, 246-247
quinolinic acid levels in, 235
cervical cancer, 209
cervical ectopy, as risk factor, 68
cervix, as HIV reservoir, 78
Chagas' disease, 198t
Challenge-HIV, 112
chancroid, 68, 198t
children. *See* adolescents; entries beginning with "pediatric" and "perinatal"
chimpanzee(s)
lentivirus infections in 19-20
vaccine trials involving, 130-132
chimpanzee immunodeficiency virus (CIV), 16, 17f
chlamydia, 72, 198t
ciprofloxacin, 196
circumcision, 68, 89
cladosporiosis, 198t
clarithromycin, 196
clindamycin, for toxoplasmosis, 245
clindamycin/primaquine, 190
clinical management, 168-228. *See also* patient care; specific aspects of (e.g., nutrition, opportunistic infections; therapeutic agents)
clinical trials, 143
ACTG trials, 182-183
changes in methodology of, 219-221
community-based, 220
in children, 226-228
in developing countries, 220-221
of AZT, 181-185, 226-227
of AZT combination therapy, 185-186
of Compound Q, 188
of ddC, 186
of ddI, 186-187, 227-228
of peptide T, 188
of soluble CD4, 228
parallel track, 220
clofazimine, 196
CMV. *See* cytomegalovirus
cocaine, 65-66, 71, 75, 291, 305-306
coccidioidomycosis, 199t, 205, 320
cognitive impairment. *See* neuropsychological deficits
cohort study, defined, 171
colitis, cytomegalovirus, 193
college students
AIDS education of, 292, 315
high-risk behavior among, 300
knowledge, attitudes, and behavior of, 296, 309
seroprevalence among, 309
colony stimulating factors (CSF), 27t, 163-166
defined, 163
granulocyte (G-CSF), 163
AZT with, 164t
therapeutic use of, 145t
granulocyte-mononuclear (GM-CSF), 163
alpha interferon with, 165t
AZT with, 164t, 165t
for Kaposi's sarcoma, 208
ganciclovir with, 192
HIV replication and, 41
therapeutic use of, 145t
virus culture stimulation using, 104
mononuclear (M-CSF), 163
therapeutic use of, 145t
virus culture stimulation using, 104
therapeutic use of, 145t
colostrum, hyperimmune bovine, 204
combination therapy, 167
advantages of, 146-147
AZT in, 185-186
with antiviral agents, 161t, 167
with antiviral agents and immunomodulators, 164-165t, 167
community-based organizations (CBOs), in AIDS education, 293-295
complement protein(s), defined, 149-150
Compound Q, 188. *See also* GLQ223
computed tomography (CT)
for neurological diagnosis, 249-250, 249f, 250f
single photon emission (SPECT), 250-251, 250f
computers, in AIDS education, 295-296
condom use
among adolescents, 307, 308
among blacks, 298, 299, 305, 309
among Hispanics, 306-307
among homosexual/bisexual men, 61, 62, 301-302
among STD clinic attendees, 298-299
among women, 310, 311-312
factors affecting, 297-298
in developing countries, 300
in general population, 299
in heterosexual transmission, 68
knowledge and beliefs and, 297-298
oral sex and, 305
condyloma acuminatum, 200t
confidentiality
duty to warn and, 285
HIV status of health care workers and, 283-284
partner notification and, 317
patient's right to, 282
Congo
AIDS cases in, 59f
pediatric AIDS in, 82
perinatal transmission in, 79
core protein. *See* p24
correctional facility officers, 266, 281, 326
corticosteroids. *See also* steroid hormones
for myopathy, 243
cortisol
disease progression and, 178-179
in pathogenesis of AIDS dementia complex, 240

Costa Rica, 54, 55t
costs, HIV-related, 221-223
duration of AZT therapy and, 184
early intervention and, 217-218
of hospital-based care, 213-215, 214t, 216t
of outpatient care, 180, 180f, 215-216, 216t
public sector financing of, 220f, 221f, 222
co-trimoxazole, 190, 245. *See also* trimethoprim/sulfamethoxazole
counselling. *See also* behavior change; education
for HIV-infected persons, 316-319
training health care workers for, 280
CPFs (synthetic peptides), 141f, 144t, 150
crack, 65-66, 71, 75, 291, 305-306
cross-species transmission theory, 16-17
cryptococcosis, 199t, 204, 243-244, 320, 322
cryptosporidiosis, 199t, 204, 320, 322
CSF. *See* cerebrospinal fluid; colony stimulating factors
CTLs. *See* cytotoxic T cells
Cuba, AIDS cases in, 55t, 56
cytokine(s), 26-27, 27t. *See also* interferon(s); specific cytokines
defined, 19, 26, 162
feedback loop, 41-42, 43f
functions of, 40-41
in neuropathogenesis, 233f, 240
inappropriate secretion of, 40-42
B cell activation and, 39
cellular damage due to, 36
in syncytia formation, 35, 35f
therapeutic use of, 145t, 162-163
cytomegalovirus (CMV)
as HIV cofactor, 46
encephalitis due to, 246
gastrointestinal illness due to, 193
HIV LTR transactivation by, 13
infection, 191-193, 198t, 320, 322
retinitis due to, 191-193
cytopenia, defined, 41
cytotoxic T cells (CTLs), 24, 25f, 37-38, 37f
autologous, cell therapy using, 160
epitopes recognized by, 120-121
HIV-specific response of, 37-38, 37f, 121
in infants of HIV-positive mothers, 110
in autoimmune neurological disease, 241, 242
in normal immune response, 28
killing of infected cells by, 34, 37f
vaccine elicited, 120, 123
Czechoslovakia, AIDS cases in, 56, 57f

D-E

d4T (dideoxydidehydrothymidine), 141f, 144t, 153
dapsone, 191, 202t
ddA (dideoxyadenosine), 144t
ddC. *See* dideoxycytidine
ddI. *See* dideoxyinosine
dehydroepiandrosterone (DHEA or EL-10), 145t
delivery room
exposure risk in, 267-268
universal precautions for, 324
dementia. *See* AIDS dementia complex
dendritic cells, 32
dentists
ADA recommendations to, 284
attitudes, beliefs, and practices of, 277-278
HIV transmission and, 266, 274, 283
universal precautions for, 324
deoxynojirimycin (DNM), 144t, 159
depression, 253
AZT and, 255
immunodeficiency and, 173
dermatitis, seborrheic, 203t
developing countries
clinical trials in, 220-221
diagnostic tests for use in, 107-109, 109f
knowledge, attitudes, and behavior in, 299-300
dextran sulfate, 151
dsRNA complexed with, 154
DHEA (dehydroepiandrosterone or EL-10), 145t
diabetics, HIV infection among, 66
diagnosis
of AIDS. *See* under AIDS
of ARC, CDC criteria for, 321
of HIV infection, 170. *See also* diagnostic tests
consequences of, 317-318
diagnostic tests, 90-113, 93t. *See also* specific tests (e.g., ELISA)
algorithms for, 97, 98f, 107-108, 109f
confirmatory, 93t, 97, 98f, 100-101, 101f, 102
direct, 92
false positives and false negatives on, 80, 96-97
for developing countries, 107-109, 109f
for HIV-1/HIV-2 dual infection, 94-96t
for HIV-2, 94-96t, 100
in developing countries, 108
specificity of, 96
for HTLV-I/II, 85
for research use, 93t
indirect, 92
interfering factors in, 113
quality control for, 112-113
rapid, 106, 107-108, 107t, 109f
sensitivity and specificity of, 92-96
using dried blood spots, 108
dialysis
patients as sentinel population, 70
universal precautions for, 324
diarrhea, chronic, 210, 320, 322
in cytomegalovirus infection, 193
in crytosporidial enteritis, 199t, 204
in HIV-infected children, 225
in microsporidia, 204-205
diclazuril, 204
dideoxyadenosine (ddA), 144t
dideoxycytidine (ddC), 141f, 144t, 153, 186
AZT with, 161t, 185
dideoxydidehydrothymidine (d4T), 141f, 144t, 153
dideoxyinosine (ddI), 141f, 144t, 153, 186-187
alpha interferon with, 165t
AZT with, 161t
CD4, soluble (sCD4), with, 161t
CD4-toxin hybrid with, 150, 161t
cognitive impairment and, in children, 263
evaluation of, 153, 186-187
in SCID-hu mice, 142
for pediatric HIV infection, 227-228
mechanism of action of, 141f, 144t
diethyldithiocarbamate (DTC or Imuthiol), 145t, 166-167
discordant couples, 68-69
disease progression, 171-179
AZT affecting, 81-82, 86, 181-182
CTL activity and, 123
duration of, 170, 172f
early intervention and, 216
factors affecting, 46-47, 86, 171-173
in children, 225-226
vs. adult, 82, 86
in hemophiliacs, 70
laboratory markers of, 173-179, 174t
in children, 226
neuropsychological deficits and, 258-260
stages in, 169-170, 171f, 322
therapy targeted to, 146, 162
DMAC (disseminated M. avium complex infection), 196-197
DNA, 3
antisense, 156, 156f
circularization of, 6
diagnostic tests based on, 93t, 104-106. *See also* polymerase chain reaction
integration of, 6-7
interventions aimed at, 8
nucleic acid sequence of, 3f
proviral, 4-5f
defined, 7
detection of, using PCR, 104
DNM (deoxynojirimycin), 144t, 159
Dominican Republic
AIDS cases in, 52, 55-56, 55t
HTLV-I/HIV dual infection in, 84-85
donovanosis, 199t
dot blot assay, 100
drug(s). *See* therapeutic agents; specific agents
drug abuse. *See* IV drug use; specific agents of abuse
drug resistance
of cytomegalovirus to ganciclovir, 193
to AZT, 152-153, 184-185
DTC (diethyldithiocarbamate or Imuthiol), 145t, 166-167
duty to treat, 282-283
duty to warn, 285

economic impact. *See* costs, HIV-related
education, AIDS, 286-319. *See also* counselling
channel variables in, 295-296
in business and industry, 280-282
in workplace, 279-282
international information exchanges in, 291
message variables in, 288-289
of adolescents, 314-315
of college students, 315
of health care workers, 279-280
of homosexual/bisexual men, 313
of IVDUs, 313-314
of male prostitutes, 313
of women, 316

education, AIDS *(continued)*
receiver variables in, 296
social marketing approach to, 288-296
source variables in, 290-295
training health care workers to provide, 280
EIA (enzyme immunoassay). *See also* ELISA
for HIV antibody, 93t
for HIV antigen, 92, 93t, 103-104
for HIV-1/HIV-2 antibodies, 94t, 95t
EL-10 (dehydroepiandrosterone or DHEA), 145t
electroencephalography (EEG), 251f, 252
electrophysiological testing, 251-252, 251f
ELISA (enzyme-linked immunosorbent assay)
for HIV antibody, 93t, 99-100
in saliva, 102
in urine, 102
synthetic peptide-based vs. recombinant-based, 100
for HIV-1/HIV-2 antibodies, 94t, 95t, 96t
for HIV-2 antibody, 94t, 100
immunofiltration, 107t
in combination screening, 97
on pooled sera, 109
rapid, 106
ELISPOT (enzyme-linked immunospot), 111-112
emergency response personnel, 266, 276, 325-326
emergency room personnel, 266, 267
universal precautions for, 325-326
encephalitis, 246, 248
encephalopathy, HIV-associated, 231, 320
in children, 261-262, 321
psychosis and, 253
endonuclease. *See* integrase
endothelial cells of CNS, 234, 233f
enteritis, 199t
bacterial, 197
fungal, 204-205
in children, 225
env gene, 9f, 10, 11f
PCR detection of, 105
envelope, 2f, 3, 7, 10
envelope glycoproteins, 2f, 3. *See also* gp41; gp120; gp160
antigenic variation in, 28, 119
monoclonal antibodies specific to, 141f, 148
enzyme immunoassay. *See* EIA
enzyme-linked immunosorbent assay. *See* ELISA
enzyme-linked immunospot (ELISPOT), 111-112
eosinophil(s), immature, HIV infection of, 31, 32-33
epidemiological modelling, 86-89
epidemiology, 48-89. *See also* specific aspects (e.g., risk factors; risk groups; transmission)
epithelioid angiomatosis, 197, 199t
epitope(s)
ADCC, 121
carbohydrate, 121
CTL, 120-121
defined, 118
enhancing, 122
identification of, 118-121
neutralizing, 119-120, 119f
PND, 119f, 120
Epstein-Barr virus (EBV), 13, 46, 247
equine infectious anemia virus (EIAV), 17f
erythropoietin (EPO), 163
AZT with, 164t
therapeutic use of, 145t
estrogen, disease progression and, 178-179
ethambutol, 196
ethical and legal issues, 117-118, 282-285
in business and industry, 285
in health care setting, 282-285
in vaccine development, 117-118
partner notification, 317
ethnic/cultural variables
among blacks. *See* blacks
among college students, 309
among Hispanics. *See* Hispanics
among homosexual/bisexual men, 61
among U.S. military, 77-78, 78f
among women, 53, 74
commitment to civil rights and, 293
in knowledge, attitudes, and behavior, 306-307
in mortality, 82
in survival time, 81
in Trinidad, 55
in U.S. AIDS cases, 52-53
needle sharing and, 63
Europe
AIDS cases in, 51, 56-58, 57f
epidemiological modelling in, 88
heterosexual transmission in, 67
HIV-2 in, 83
perinatal transmission in, 79
evoked potentials, 251-252, 251f
auditory brainstem response, 252

F-G

false negative
contamination of blood supply due to, 80, 96-97
defined, 96
false positive, defined, 96
Fansidar (pyrimethamine/sulfadoxine), 244-245
Fc receptor
in enhancement of HIV infection, 42-43
in HIV entry into cells, 4, 34
Fc region, of IgG, 149-150, 149f
feline immunodeficiency virus (FIV), 17f, 20
vaccine trials, 134
feline leukemia virus (FeLV), 17f, 20
feline syncytium-forming virus (FSFV), 17f
firefighters, 278, 325-326
first-response personnel, 266, 278, 325-326
flow cytometric assay, 93t
for diagnosis in infants of HIV-positive mothers, 112
for HIV antigens, 93t, 104
fluconazole
for candidiasis, 205-206
for cryptococcosis, 204, 244
flucytosine, 244
fluoroquinolone(s), 145t, 151
folinic acid, for toxoplasmosis, 245
folliculitis, 199t
foscarnet (phosphonoformic acid or PFA), 144t, 151
for cytomegalovirus retinitis, 192, 193, 198t
for systemic cytomegalovirus infection, 246
frameshift, 10
France
AIDS cases in, 51, 57f, 58
AIDS education by mass media in, 290
HIV infection in health care workers in, 271f
HIV-2 in, 83
hospital stays in, 213
transfusion-associated AIDS cases in, 69
Freund's adjuvant, 124, 128
fungal diseases, 204-205. *See also* specific diseases
fusion peptides, vaccines using, 126-127
fusion proteins, antibody tests using, 99

gag gene, 9f, 10, 11f
PCR detection of, 105
in infants of HIV-positive mothers, 111
gag proteins. *See also* specific proteins (e.g., p24)
epitope recognition by CTLs specific to, 121
gag-pol polyprotein, 9f, 10
gamma interferon. *See* under interferon(s)
ganciclovir
cytomegalovirus resistance to, 193
for cytomegalovirus gastrointestinal illness, 193, 198t
for cytomegalovirus retinitis, 192, 193, 198t
for systemic cytomegalovirus infection, 246
gastrointestinal tract. *See also* specific infectious agents and diagnoses (e.g., diarrhea; enteritis)
HIV infection of, 32, 34, 210
G-CSF. *See* under colony stimulating factors
gene amplification, 104. *See also* polymerase chain reaction
GENIE, 106, 107t
for detection of HIV-1 and HIV-2, 95t
genital ulcer disease (GUD), 199t
genital ulceration, 68, 72
homosexual transmission and, 61
genital warts, 75, 200t
Germany
AIDS cases in, 56, 57f, 58
HIV infection in health care workers in, 271f
IV drug users in, 64
giardiasis, 200t
gingivitis, 200t
glial cell(s), HIV infection of, 33, 233f, 234
GLQ223 (trichosanthin), 144t, 154, 188
glucose tolerance, aerosol pentamidine's effects on, 191
glycoproteins. *See* envelope; specific envelope glycoproteins (e.g., gp120)
glycosylation inhibitors, 141f, 144t, 159
glycyrrhizin, 145t
GM-CSF. *See* under colony stimulating factors
gonorrhea, 68, 72, 200t

gp41, 2f, 3, 9f
ADCC epitopes in, 121
antibodies to, 94t, 96t, 101, 101t, 102, 120
enhancing epitope in, 122
fusion of, with HIV, 6
synthetic peptide vaccine using, 127, 128
TM receptor binding to, 34
gp120, 2f, 3, 9f
antibodies to, 94t, 96t, 101, 101t, 102, 120
disease progression and, 46
in vitro vs. in vivo effectiveness of, 148
antigenic variation in, 119
CD4 binding by, 4, 32, 33f
phenylalanine and, 150
diminished NK cell function and, 35
heat denaturation of, in vaccine development, 123
PND, 119f, 120
recombinant vaccine using, 126
human trials of, 137
response to, in chimpanzees, 132
soluble, toxicity of, 150, 233f
V3 loop of, 36-37, 119f, 120
neutralization epitope outside of, 122
synthetic peptide vaccine using, 128
gp160
antibodies to, 101, 101t, 102
adjuvant-based vaccine using, 128
recombinant vaccine using, 126
human trials of, 135-137
response to, in chimpanzees, 132
synthetic peptide vaccine using, 128
granuloma inguinale, 199t
Great Britain. *See* United Kingdom
Guatemala, 54, 55t
Guillain-Barré syndrome, 242

H

hairy leukoplakia, 200t, 205, 321
Haiti
AIDS cases in, 52, 55-56, 55t
HTLV-I/HIV dual infection in, 85
pediatric AIDS in, 82
Health Belief Model, 288-289, 309
health care workers. *See also* occupational risks; specific professions (e.g., dentists)
attitudes, beliefs, and practices of, 274-278
AZT prohylaxis for, 273-274
disclosure of HIV status of, 283-284
education and training for, 279-280
ethical and legal issues for, 282-285
HIV-infection incidence of, 270-273, 271f
infected, risk to patients of, 274, 283-284
testing of, 284
universal precautions for, 268-270, 323-326
helicobacter (campylobacter) infection, 197, 200t
helper T cells, 25f, 26. *See also* CD4 cell(s)
hemagglutination assay, 96t
hemophiliacs, 69-70
age and disease progression in, 172
in Japan, 60
neuropsychological deficits in children, 263
psychosocial consequences of HIV infection in, 318
hepatitis, 200t
hepatitis B virus (HBV)
as HIV cofactor, 46
infection, 75
occupational risk for, in operating room, 267
HEPT derivatives, 144t, 153
heroin
as risk factor, 65, 73
HTLV-II and, 85
herpes simplex virus (HSV)
as HIV cofactor, 46
HIV LTR transactivation by, 13
infection, 200t, 320
herpes zoster, 200t, 321, 322
heterogeneity. *See also* strain variation
vaccine development and, 116
heterosexual transmission, 50, 51f, 67-69. *See also* specific populations (e.g., adolescents; women)
bisexuals and, 54, 67
dynamics of, 68-69
female-to-male, 68-69
in Americas, 52
in Asia, 60
in Caribbean, 55
in Eastern Europe, 56
in India, 60, 72, 73f
in Latin America, 54
in Mexico, 54
in sub-Saharan Africa, 58
in U.S., 67-68
in Western Europe, 58, 88
IV drug users and, 54, 65-66, 88
male-to-female, 68-69
modelling of, 88-89
Pattern I countries and, 67
Pattern II, 50, 88-89
risk factors for, 68
HGP-30, 127, 136
Hispanics
adolescents, 76, 308
condom use among, 305
crack use among, 305
homosexual/bisexual men, 61, 302
IVDUs, 63, 74
mortality in, 82
seroprevalence among, 52-53, 74
survival time among, 81
women
knowledge surveys among, 306
seroprevalence among, 52-53, 74
IVDUs, 63
risk behaviors among, 309, 311
histocompatibility proteins. *See* HLA antigens
histoplasmosis, 201t, 320, 322
HIV
antibodies to. *See* entries beginning "antibody"
antigenic drift in, 28, 116, 119, 121
antigens. *See* antigen (HIV); specific HIV antigens (e.g., gp120)
capsid. *See* p24
cell tropism, 29-34, 117, 232-234
cell types infectible by, 29-31, 210, 232-234
cofactors in transmission of (suggested), 44-47, 245
components of, 2-3, 2f
detection of. *See* diagnostic tests
DNA, 7, 80, 104-105, 111
drug resistant strains of, 152-153, 184-185
endonuclease. *See* integrase
entry of, into central nervous system, 140, 233f, 237
entry of, into host cell, 4-6
CD4-receptor-mediated, 31-33, 33f
interventions aimed at, 7-8, 144t, 147-151
non-CD4-receptor-mediated, 33-34
envelope, 2f, 3. *See also* specific envelope glycoproteins (e.g., gp120)
exposure to
accidental, self-reporting of, 268
AZT prophylaxis after, 273-274
occupational. *See also* occupational risks
genome, 8-14, 11f. *See also* specific genes
deletion mutants of, 125
in cerebrospinal fluid, 238-239
integrase. *See* integrase
life cycle, 3-8, 4-5f, 141f
maturation of, 7, 8
natural history of, 14-17
protease. *See* protease
proteins, 2-3, 2f, 9f. *See also* specific proteins (e.g., gp41)
replication of. *See* replication
reverse transcriptase. *See* reverse transcriptase
RNA, 2f, 3, 5f, 105-106, 154-155
strain variation of. *See* strain variation
therapeutic agents targeting. *See* antiviral agents; specific agents (e.g., AZT)
transmission. *See* transmission
vaccines for. *See* vaccines
viruses related to, 16-17, 17f
HIV immunogen, 126, 136
HIV infection
acquisition of. *See* transmission (HIV)
active, 7, 31f
cancers associated with. *See* Kaposi's sarcoma; lymphomas
cofactors for (suggested), 44-47, 245
course of, 169-170, 171f. *See also* disease progression
diagnosis of, 170. *See also* diagnostic tests
disease-causing mechanisms in, 38-44
epidemiology of. *See also* specific aspects of (e.g., risk groups; transmission)
immune cell destruction in, 34-36, 117
immunological defenses against, 36-38, 37f
incubation time, 53, 81, 86
initial acute illness due to, 170, 171f
latent, 7, 31f, 110, 116
mortality associated with, 81-82
neurological manifestations of. *See* neurological manifestations
neuropsychological deficits associated with. *See* neuropsychological deficits (HIV-related)
opportunistic infections associated with HIV. *See* opportunistic infections
oral infections and lesions, 205-206
pediatric. *See* pediatric HIV infection
Pneumocystis carinii pneumonia (PCP). *See* Pneumocystis carinii pneumonia (PCP)

HIV infection *(continued)*
prevention of. *See* specific aspects of (i.e., behavior change; condom use; counselling; education; vaccines)
psychosocial consequences of, 318-319
reporting of, 51
restrictive, 30, 31f
risk factors for. *See* risk factors
sentinel populations for, 70-78
seroprevalence. *See also* under specific risk groups
defined, 50
staged model of, 87
staging classification systems for, 322
tests for. *See* diagnostic tests
treatment of. *See* patient care; therapeutic agents; specific diagnoses (e.g., cytomegalovirus infection)
worldwide estimate of, 49
HIV-1. *See* HIV
HIV-2, 15
epidemiology of, 83-84
in Africa, 59
in children, 83-84
origin of, 16-17
relatedness to SIV, 134
tests for, 94-96t, 100
in developing countries, 108
specificity of, 96
vaccine for, trials in macaques, 133
HIVAC-1e, 136
immune system priming with, 124, 135, 136
HIVChek, in combination screening, 97
HIVChek 1 + 2, 107t
in combination screening, 108
HIVChek HIV-1, 107t
in combination screening, 107-108, 109f
HIV-1/HIV-2 dual infection
diagnostic tests for, 94-96t, 108
in Africa, 59, 83-84, 108
in Brazil, 83
in children, 84
HIV/HTLV-I dual infection, 84-85
HIV-related dementia. *See* AIDS dementia complex
HLA antigens (histocompatibility proteins)
CTL recognition of epitopes and, 120
defined, 31-32, 120
disease progression and, 47, 172
in normal antigen recognition, 32, 33f
Kaposi's sarcoma and, 207
Hodgkin's disease, 208-209
homeless persons, 77
adolescent, 76, 308, 315
homosexual adolescent, 308, 315
homosexual/bisexual men, 60-62, 301-303, 318-319
AIDS incidence among, 53, 54, 60, 61
attitudes toward, 275
behavior change among, 62, 302
condom use (increased) among, 62
counselling of, 316-317
denial of care to, 275
education targeting, 313
ethnic/cultural variables among, 61, 302, 306, 318
heterosexual transmission by, 67
incubation time in, modelling of, 86
Kaposi's sarcoma among, 206-207
knowledge, attitudes, and behavior surveys among, 301-303
relapse among, 62, 302
resistance to self-identification as, 306
risk factors for, 61-62
alcohol use, 301-302, 318
anal intercourse, 61, 62
condom use, lack of, 61
drug use (recreational), 301-302, 318
genital ulceration, 61
sex partners, number of, 61, 62
STDs, history of, 61
psychosocial studies among, 318-319
seroprevalence (HIV) among, 53, 61
Honduras, AIDS cases in, 54-55, 55t
Hong Kong, AIDS cases in, 60
hospital-based care, 213-215, 214t, 216t
HTLV-I, 17f, 84
blood screening for, 85
HIV LTR transactivation by, 13
seroprevalence of, 85
HTLV-I/HIV dual infection, 84-85
HTLV-II, 17f, 85
human herpes virus-6 (HHV-6), as HIV cofactor, 46
human nasopharyngeal carcinoma virus (NPCV), 17f
human papilloma virus (HPV), cervical cancer and, 209
human T-lymphotropic virus. *See* HTLV-I; HTLV-II
human trials
of therapeutic agents, 181-186, 219-221
of vaccines, 135-137
Hungary, AIDS cases in, 56, 57f
hypericin, 145t
hyperimmune bovine colostrum, 204
hyperimmunoglobulinemia, in HIV infection, 39

I

Idiotype(s)
defined, 129
vaccines based on, 127t, 129-130
IFA (immunofluorescence assay), 93t, 97, 102
IL-1 (interleukin-1), 27t
IL-2. *See* interleukin-2
IL-3. *See* interleukin-3
IL-4 (interleukin-4), 27t
IL-5 (interleukin-5), 27t
IL-6. *See* interleukin-6
imaging techniques, for neurological diagnosis, 249-251
immune response
antibody, 24, 36-37, 37f
diagnostic monitoring of, 24
vaccine elicited, 121-122
in chimpanzees, 132
in humans, 135
cellular, 24-26, 25f, 37-38, 37f. *See also* antibody-dependent cellular cytotoxicity; cytotoxic T cells
therapy aimed at improving, 160
vaccine elicited, 123
in chimpanzees, 132
in humans, 135
cytokines in, 40-41
individual variation in, disease progression and, 46-47
normal, 27-28
nutritional status and, 211
to early HIV infection, 36-38, 37f
vaccine elicited, 121-123, 135
immune system
cellular components of, 24-26, 25f
changes in, in central nervous system infection, 237-238
function of. *See* immune response
in central nervous system, 236, 237
overview of, 23-28
immune-stimulating complexes (ISCOMs), 124, 124f
vaccines based on, 127t, 128
immunity, passive, in infants, 110
immunization. *See also* vaccines
in HIV-infected children, 224
mechanism of, 28
immunoblot assay, 100
Immunocomb, in combination screening, 97
immunofiltration ELISA, 107t
immunofluorescence assay (IFA), 93t, 97, 102
immunogenicity
defined, 123
vaccine strategies involving enhancement of, 123-124, 124f
immunoglobulin G (IgG)
AZT with, 165t
CD4 fusion with, 144t, 149-150, 149f, 187
immunoglobulin therapy, 159-160
immunological marker(s), 173-175, 174t
immunomodulators, 160-167. *See also* specific agents
antiviral agents with, combination therapies using, 164-165t, 167
experimental, 166-167
natural, 145t, 162
synthetic, 145t
with antiviral activity, 166
immunosomes, 124, 124f
vaccines based on, 127t, 129
immunotherapy, 159-167
active, 160-167. *See also* immunomodulators; specific agents
defined, 181
passive, 159-160
Imreg-1, 145t, 166
Imuthiol (diethyldithiocarbamate or DTC), 145t, 166-167
incubation time, 81, 270-271
for pediatric AIDS, 77, 226
modelling of, 86
India
AIDS cases in, 60
prostitutes in, 72, 73f
transfusion-associated AIDS cases in, 69
tuberculosis and HIV in, 194
inducer T cells, 25f, 26. *See also* CD4 cell(s)

infants. *See also* pediatric AIDS; pediatric HIV infection; perinatal transmission
diagnostic tests for, 109-112
immature immune system in, susceptibility of, 40, 226
maternal antibodies, transient, in, 223f
neuropediatrics, 261-263
inflammatory demyelinating polyneuropathy (IDP), 242
Inno-LIA HIV-1/HIV-2, 95t
integrase, 6-7, 9f
gene for, 10
interventions aimed at, 8, 151
integration, 4-5f, 6-7, 6f, 8
interferon(s) (IFN), 27t, 162-163
alpha, 162-163
antisense oligonucleotide with, 165t
AZT with, 164t, 165t, 185-186
clinical trials with, 181
cognitive function and, 261
ddI with, 165t
for Kaposi's sarcoma, 208
GM-CSF with, 165t
therapeutic use of, 145t
triglyceride clearance and, 211
beta, 162
therapeutic use of, 145t
gamma, 162
HIV replication and, 41
therapeutic use of, 145t
therapeutic use of, 145t
interleukin-1 (IL-1), 27t
interleukin-2 (IL-2), 27t, 145t
AZT with, 165t
cell therapy using, 160
defined, 160, 166
diminished NK cell function and, 34-35
interleukin-3 (IL-3), 27t
virus culture stimulation using, 104
interleukin-4 (IL-4), 27t
interleukin-5 (IL-5), 27t
interleukin-6 (IL-6), 27t
B cell activation and, 39
HIV replication and, 41
in HIV-related Kaposi's sarcoma, 44
intrauterine device, 68
intravenous drug users. *See* IV drug users
Intron A, 162. *See also* interferon(s), alpha
investigational new drug (IND)
defined, 192
treatment using, 143
ISCOMs. *See* immune-stimulating complexes
isoniazid, 196
isosporiasis, 201t, 320, 322
Italy
AIDS cases in, 57f, 58
HIV-infected health care workers in, 271f, 272
hospital stays in, 213, 214
IV drug users in, 64
itraconazole, 244
IV drug users, 62-67, 303-306
AIDS incidence among, 53-54, 58, 63-64, 63f
behavior change among, 65, 66-67, 314
counselling of, 316-317
disease progression in, 172, 175
education targeting, 290-291, 313-314
ethnic/cultural variables among, 53, 63, 74, 290-291
heterosexual transmission and, 54, 65-66, 88
in Brazil, 64
in Canada, 53-54
in Germany, 64
in Italy, 64
in Poland, 58
in Spain, 64
in Thailand, 60
in United Kingdom, 64
in United States, 53, 63f, 64
in Western Europe, 58
knowledge, attitudes, and behavior surveys among, 303-306
needle/syringe exchange programs for, 66-67, 314
prisoners as, 73
prostitutes as, 71-72
risk factors for, 62, 65
cocaine/crack use, 65-66
condom use, lack of, 65
needle sharing, 62, 65
sex partners, number of, 65
occupational risks of intravenous viewers of, 297
seroprevalence (HIV) among, 54, 58, 60, 63-64
seroprevalence (HTLV-I/II) among, 85
Ivory Coast, 59f, 82

J-K-L

Japan, AIDS cases in, 60
JC virus
HIV LTR transactivation by, 13, 14
progressive multifocal leukoencephalopathy and, 246-247
job discrimination, 284, 285

Kaposi's sarcoma (KS), 206-208, 320, 322
cytokine involvement in, 43-44, 45f
defined, 43
epidemiology of, 206
etiology of, 45f, 207-208
immunogenetics of, 206-207
mortality trends with, 81
tat protein as growth stimulant for, 44
tests for etiologic agent for, 44
treatment of, 208
Kenya, AIDS cases in, 59, 59f
ketoconazole, 244
knowledge, attitudes, and behavior, 296-312
in developing countries, 299-300
of adolescents, 307-308
of college students, 309
of ethnic minority groups, 306-307
of general population, 297-299
of health care workers, 274-278
of homosexual/bisexual men, 301-303
of IVDUs, 303-306
of women, 309-312
reliability of surveys of, 296-297
training health care workers to collect data on, 279
workplace education programs' effects on, 281-282
latent infection, 7, 31f
in seroreverting infants, 110
vaccine development and, 116
Latin America
AIDS cases in, 54-55, 55t
changes in transmission pattern in, 52, 54
Latinos. *See* Hispanics
law enforcement officers, 266, 281, 326
legal issues. *See* ethical and legal issues
leishmaniasis, 201t
lentinan, 145t
lentiviruses. *See also* specific viruses (e.g., simian immunodeficiency virus)
characteristics of, 18
classification of, 17f
infections with, in animals, 18-21
leprosy, 201t
leukemia, adult T-cell, HTLV-I and, 84
liability concerns. *See* ethical and legal issues
life cycle. *See* under HIV
lipid analogues, 141f, 144t, 159
liposomes, 124, 124f
conjugation with, sCD4 effectiveness and, 149
defined, 142
drug encapsulation in, 142
ribozyme encapsulation in, 157f
vaccines based on, 127t, 129
lithium, 254
long terminal repeat(s) (LTR), 12-14, 13f
interventions aimed at, 14
lymphadenopathy, 171f, 224, 225, 320, 321, 322
lymphocyte(s), 24-26. *See also* specific types
lymphocyte counts, as marker of disease progression, 174-175. *See also* CD4 cell counts;
lymphogranulomatosis, 202
lymphoid interstitial pneumonitis (LIP), 201t, 320, 321, 322
in children, 224-225, 321
lymphokines, defined, 26
lymphoma, 43, 208-209
AZT therapy and, 184
brain, 247, 320, 322
Hodgkin's disease, 208-209
non-Hodgkins' lymphoma (NHL), 208-209, 247, 320, 322

M

macaques
lentivirus infections in, 18-19
vaccine trials involving, 132-134
macrophages, 25f, 26, 33f
abnormal function of, in HIV infection, 36, 39-40
activation of, HIV activation and, 45-46
CD4 receptor on, 32
defined, 162
HIV infection of, 29, 30, 232-234, 233f
Fc receptor in, 34

macrophages *(continued)*
immature, HIV infection of, 40
in autoimmune neurological disease, 241-242
in neurologic infection, 233f, 234, 237, 238
in normal immune response, 28
in syncytia formation, 35, 35f
killing of infected cells by, 34
reverse transcriptase inhibitor produced by, 113
tumor necrosis factor's effects on, 41
magnetic resonance imaging (MRI), for neurological diagnosis, 249f, 250
major histocompatibility antigens. *See* HLA antigens
Malawi, AIDS cases in, 59, 59t
male-to-female ratio, 50, 54, 55, 55t, 69. *See also* heterosexual transmission
malignancy. *See* cancer
malnutrition. *See* nutrition
marijuana, 76, 308
marketing, social. *See* social marketing approach
mass media, in AIDS education, 290-291
matrix protein. *See* p17
M-CSF. *See* under colony stimulating factors
Medicaid
eligibility for, 222-223
AIDS diagnosis and, 171
policy on ambulatory care, 216
proportion of patients covered by, 220f, 221f, 222
medical students, attitudes, beliefs, and practices of, 277
megakaryocytic cells, HIV infection of, 32-33
megastrol acetate, 212
melanins, soluble, 144t
memory cell(s)
B, 24
CD4, HIV infection of, 29-30
in normal immune response, 28
T, 24
memory deficits, 231, 239, 243t
menaquinone-7, 145t
meningitis, 201t
cryptococcal, 201t, 204
menstruation, sex during, 68
metabolic markers, 174t, 178-179
Mexico
AIDS cases in, 52, 54, 55t
heterosexual transmission in, 67
knowledge surveys in, 299
transfusion-associated AIDS in, 54, 69
tuberculosis and HIV in, 194
MHC antigens. *See* HLA antigens
mice
drug evaluation in, 142
lentivirus infections in, 20-21
SCID, 21
vaccine trials involving, 134-135
SCID-hu, drug evaluation in, 142
transgenic, 21
vaccine trials involving, 134-135
microglial cells
CD4 receptor on, 32
HIV infection of, 234
microsporidiosis, 204-205
Middle East, 51
military personnel, 77-78, 78f
mitochondrial damage, in AZT-related myopathy, 243
modelling, epidemiological, 86-89
molluscum contagiosum, 201t
monoclonal antibodies
defined, 119, 147
enhancing, for vaccine developemnt, 122
envelope glycoprotein-specific, 148
idiotype-based vaccines using, 129-130, 131f
in vaccine development, 119-120
therapeutic use of, 141f, 144t, 147-148
monocytes, 26. *See also* macrophages
abnormal function of, in HIV infection, 39-40
CD4 receptor on, 32
in neurologic infection, 234
monophosphoryl lipid A, vaccine containing, 124
mortality, HIV-associated, 81-82
morticians, 266, 324
mRNA. *See* under RNA
muramyldipeptide (MDP), killed SIV vaccine using, 124, 133, 134
mycobacterial infections. *See also* tuberculosis
nontubercular, 196-197, 201t, 320, 322
mycoplasma
as HIV cofactor, 44-45
nephropathy due to, 197
Mycoplasma incognitus, 197
myelin, defined, 235
myelin basic protein, antibody against, 241
myelitis, 246
myelopathy, 322
HTLV-I associated, 84
vacuolar, 235, 236, 242
myocarditis, 201t
myopathy
autoimmune, 242-243
due to AZT, 183, 184, 242-243
myositis, defined, 235

N

N-9 (nonoxynol-9), 145t
naphthalenedisulfonic acid derivatives, 145t
natural killer cells (NK), 25f, 26
abnormal function of, in HIV infection, 34-35
in normal immune response, 28
in response to HIV infection, 37f, 38
killing of infected cells by, 34, 37f
needle exchange programs, 66-67, 314
needle reuse
bleach in, 66, 67, 305
in Soviet hospital, 56
pediatric AIDS in Romania due to, 57
needle sharing, 303-305
ethnic/cultural variables affecting, 63
transmission by, 62
needlestick injuries, 267, 268-269
nef gene, 10-11, 11f
PCR detection of, 105
negative regulatory element (NRE), 10, 13f, 14
neoplasms, 206-209, 247. *See also* Kaposi's sarcoma; lymphoma
neopterin, 173
AZT efficacy and, in children, 227
disease progression and, 178, 179
in cerebrospinal fluid, 237, 238
neuroimmunology 236-239
neuroleptics, 254
neurological manifestation (HIV-related),. 239-248, 243t, 261. *See also* neuropsychological deficits; psychiatry
AIDS dementia complex, 239-241
autoimmune neurological disease, 241-243
brain tumors, 247
diagnostic tests for, 248-253, 249f, 250f, 251
algorithm for, 249f
CT scan, 249-250, 249f, 250f
electrophysiological function, 251-253, 251f
MRI, 249f, 250, 250f
SPECT, 250-251, 250f
encephalitis, 246, 248
myelopathy (vacuolar), 242
myopathy, 242-243
neuropathies, 241-242
opportunistic infections (CNS), 243-246
pediatric, 261-263
progressive multifocal leukoencephalopathy, 246-247
neuropathogenesis, 232-236, 233f
animal models of, 235-236
cell types (CNS) infectible by HIV and, 232-234
immunological changes and. *See* neuroimmunology
neurons, defined, 232
neuropathy, 231, 241-242, 322
neuropsychological deficits, 256-261
AZT therapy and, 259-260
confounding factors in, 260
disease progression and, 258-260
in asymptomatic HIV-infected persons, 257
in children, 262-263
in uninfected infants of seropositive mothers, 263
manifestations of, 256
neurogenic vs. psychogenic, 256
prevalence of, 256-257
in children, 262-263
rehabilitation of, 261
testing and evaluation for, 258, 259t
in drug evaluation, 261
neurosyphilis, 245-246
neutralizing antibodies. *See* under antibody(-ies)
neutropenia, defined, 166
neutrophils, defined, 39
Nevada
AIDS cases in, 52f
AIDS prevention in brothels in, 310
New York
AIDS cases in, 52f
Medicaid for, 220f, 221f
tuberculosis and HIV in, 194
newborns. *See* infants; pediatric AIDS; pediatric HIV infection; perinatal transmission
NF-kB sequences, 13, 13f 42
Nigeria, 59f, 290

nitrite inhalants, 207
NK cells. *See* natural killer cells
nocardiosis, 202t, 321, 322
nongovernmental organizations (NGOs), in AIDS education, 293-295
nonoxynol-9 (N-9), 145t
North America, AIDS cases in, 52-54, 52f, 53t
nuclear factor kappa B, 13, 13f
nucleoside analogues, 151-153
nurses, 266. *See also* occupational risks
  attitudes, beliefs, and practices of, 278
  care standards, for patients with HIV infection, 279
  universal precautions for, 323-324
    compliance with, 269, 269t
nutrition, 209-212
  assessment of status of, 211-212
  immunologic function and, 211
  in HIV-infected children, 225
  intervention to improve, 212

O-P

occupational risks, 266-273. *See also* universal precautions
  epidemiological studies of, 270-273, 271f
  in delivery room, 267-268
  in emergency room, 267
  in non-health care settings, 265-266
  in operating room, 267
  perceived
    by dentists, 266, 277
    by physicians, 266-268, 275
Oceania, AIDS cases in, 51
oculomotor testing, 252-253
ofloxacin, 145t
oligonucleotides, antisense. *See* antisense oligonucleotide(s)
oncoviruses, classification of, 17f
operating room
  exposure risk in, 267
  universal precautions for, 324
opportunistic infections, 188-205, 198-203t. *See also* specific infections
  diagnosis of, 188
  HIV risk category and, 189
  in HIV-infected children, 224
  of central nervous system, 243-246
  oral, 205-206
OPUS HIV (1 + 2), 95t, 107t
oral infections and lesions, 205-206
oral sex, 207, 305
oral warts, 202t
otoneurological tests, 251f, 252
outpatient care, 215-216, 216t
oxophenarsine, 145t
ozone, 145t

p6, 7, 9f
  gene for, 10
p9, 9f
  gene for, 10
p15, 9f
p17, 2f, 3, 9f
  gene for, 10
  synthetic peptide vaccine using, 127, 136
p24 (capsid), 2-3, 2f, 9f
  antibody to, 94t, 95t, 100, 101, 101f
    disease progression and, 46
    in Sjogren's syndrome, 113
  cell-associated, quantitation of, in monitoring HIV infection, 177
  detection of, 92, 103-104
  enzyme immunoassay for, 92
  flow cytometric assay for, 104, 112
  gene for, 10
  in recombinant vaccine, 126
  in serum, 103
    bound vs. free, 176-177
    disease progression and, 175-177, 176f, 179
  measurement of
    in in vitro drug screening, 140
    in in vivo drug studies, 146
  viruslike-particle-based vaccine, 137
p55, in recombinant vaccine, 126
P300, 252
Panama
  AIDS cases in, 55t
  HTLV-II in, 85
papaverine hydrochloride, 145t
paramedics, 278, 325-326
partner notification, 285, 317
passive agglutination, screening tests using, 93t
patient(s)
  expectations of, for involvement in medical decisions, 219
  of infected health care workers
    disclosure of HIV status to, 283-284
    risk of transmission to, 274
patient care
  access to, 217, 222-223
  changing patterns of, 218-221
  costs of. *See* costs (HIV-related)
  delivery of, 213-223
  early intervention, 216-218
  hospital-based, 213-215, 214t, 216t
  outpatient, 215-216, 216t
  public sector financing of, 220f, 221f, 222
  strategies for, 179-180
patient's rights
  partner notification and, 317
  to confidentiality, 282
    duty to warn and, 285
  to participate in medical decisions, 219
Pattern I, 50, 51f. *See also* transmission
  increasing heterosexual-contact cases in, 67
Pattern II, 50, 51f, 67. *See also* transmission
  epidemiologic models for, 88-89
Pattern III, 50, 51f. *See also* transmission
PCP. *See* Pneumocystis carinii pneumonia
PCR. *See* polymerase chain reaction
pediatric AIDS. *See also* pediatric HIV infection; perinatal transmission
  epidemiology of, 77
  in Romania, 57
  incubation time for, 77
    modelling of, 86
  mortality from, 82
  survival time with, 82
  transfusion-associated
    in Africa, 69
    in U.S., 70
pediatric HIV infection, 223-228. *See also* pediatric AIDS; perinatal transmission
  classification of, 321
  clinical assessment in, 224
  clinical manifestations of, 224-225
  disease progression and prognosis in, 225-226
  drug evaluation in, 146
  incidence of, 223
  neurological aspects of, 261-263
  prevention of, 228
  transmission of, 223
  treatment of, 226-228
pelvic inflammatory disease (PID), 202t
  as indicator disease, 171
pentamidine
  aerosol
    cost effectiveness of, 218
    for Pneumocystis carinii pneumonia prophylaxis, 190, 191
    for Pneumocystis carinii pneumonia treatment, 190
  intravenous, for Pneumocystis carinii pneumonia, 189
  survival time and, 81-82
pentosan sulfate, 151
pepstatin, 144t
Peptest 1 + 2, 95t
peptide(s)
  chimeric, 99, 126-127
  defined, 149
  fusion, vaccines using, 126-127
  synthetic
    anti-HIV protease inhibitors, 144t, 158
    derivatives of, 144t
    therapeutic use of, 150
Peptide T, 141f, 144t, 150, 187-138
Peptilav (1-2), 95t
Peptoscreen-2, 96t
perinatal transmission, 78-79, 79t. *See also* pediatric AIDS; pediatric HIV infection
  defined, 78
  disease progression with, 226
  HIV-2 and, 83-84
  maternal patterns of gene detection by PCR and, 111
  prevention of, 228
  rates of, 110
periodontitis, 202t
peripheral neuritis, defined, 235
peripheral neuropathy, autoimmune, 241-242
PFA. *See* foscarnet
PHA-CHEK, 106
phagocytic cell, defined, 234
phagocytosis, defined, 26
pharmacokinetics, defined, 142
Phase I, II, and III clinical trials, 143
phenylalanine, 150
phosphocholine, lipid analogue containing, 159
phosphonoformic acid. *See* foscarnet
phosphonylmethoxy-ethyl-adenine (PMEA), 144t
phthalocyanines, 145t

physicians
AMA recommendations to, 283-284
attitudes, beliefs, and practices of, 275-277
changes in patients' expectations of, 219
duty to treat, 282-283
plasma cells, 24
platelets, defined, 39
PMEA (phosphonylmethoxy-ethyl-adenine), 144t
Pneumocystis carinii pneumonia (PCP), 189-191, 320, 321, 322
in children, 224, 225
prognostic markers in, 189
prophylaxis of, 190-191
cost effectiveness of, 218
in HIV-infected children, 225
sulfonamides for, toxoplasmosis prophylaxis and, 245
survival time and, 81-82
treatment of, 189-190
pneumocystis disease, extrapulmonary, 190-191
pneumonia, 202t. *See also* Pneumocystis carinii pneumonia
lymphoid interstitial, 201t, 320, 321, 322
in children, 224-225
pokeweed antiviral protein, defined, 147
pol gene, 9f, 10, 11f
PCR detection of, 105
Poland, AIDS cases in, 56, 57f, 58
police. *See* law enforcement
poliovirus, in recombinant vaccine, 126
poly-ICLC, 141f, 144t, 156
polymer(s), sulfated, 151
polymerase, defined, 6
polymerase chain reaction (PCR), 92, 104-106
cerebrospinal fluid evaluation using, 238-239
defined, 38
disease progression monitoring using, 177-178
drug evaluation using, 142
HTLV-I/II screening using, 85
in perinatal evaluation, 111
polymerase inhibitors, 141f, 144t, 151
polynucleotides, therapeutic use of, 144t, 154-156
polyprotein(s)
assembly, budding, and release of, 7
agents blocking, 144t, 159
gag-pol, 9f, 10
processing of, by protease, 7, 9f
synthesis of, 7
polyradiculomyelitis, 246
polysaccharides, sulfated, therapeutic use of, 151
polyvinyl alcohol, sulfated, 151
pooled sera, diagnostic tests on, 108-109
porphyrins, 145t
Portugal, HIV-2 in, 83
precautions. *See* universal precautions
prednisone, 243
pregnancy
cross-reacting antibodies in, 93, 101
drug evaluation during, 146
HIV transmission, prophylaxis for, 228
termination of, 310-311
primaquine, 190
primates. *See also* simian(s); simian immunodeficiency virus
lentivirus infections in, 18-20
vaccine trials in, 130-134
principal neutralizing determinant (PND), 119f, 120
neutralizing antibody induction using, 121-122
prisoners, 73
progesterone, disease progression and, 178-179
progressive multifocal leukoencephalopathy (PML), 246-247, 320
prostitutes
female
in India, seroprevalence in, 60, 72, 73f
knowledge, attitudes, and behavior of, 310
seroprevalence in, 71-73
male, 72-73, 313
protease (proteinase), 7
action of, 7, 9f
as therapeutic target, 8, 141f, 144t, 158
functions of, 158
gene for, 10
inhibitors of, 8, 141f, 144t, 158
protein synthesis, 6f, 7
proteinase. *See* protease
Pseudomonas aeruginosa, exotoxin of, soluble CD4 conjugation with, 150, 161t
pseudovirion. *See* viruslike particles
psychiatry, 253-256
pharmacologic issues in, 254-255
psychological factors. *See also* neuropsychological deficits
disease progression and, 173
psychopharmacologic issues, 254-255
psychosis, 253
psychostimulatory agents, 255
puromycin aminonucleosides, 144t
PWAs (persons with AIDS). *See also* patient care; patient's rights
attitudes toward, factors affecting, 275-278, 293
involvement in medical decisions, 219
in AIDS education, 279, 295
job discrimination affecting, 284, 285,
needs of care givers of, 319
pyrimethamine, 244-245

Q-R

QC-HIV, 112
quality control, in diagnostic testing, 112-113
quinolinic acid
in cerebrospinal fluid, 235
in pathogenesis of AIDS dementia complex, 240-241
quinolizine, 145t
quinolone, inhibition of viral replication by, 45

rabbits, as animal models, 236
radiation therapy, for brain lymphoma, 247
radiation-resistant score, 177
radioimmunoprecipitation assay (RIPA or RIA), 93t, 98
rapid tests, 106, 107t
for developing countries, 107-108, 109f
rats, as animal models, 236
Recombigen, 106, 107t
regulatory genes, 10-12
function of, 8
interventions aimed at, 12
relapse (in behavior change), 62, 302
religious organizations, in AIDS education, 292-293
replication, 5f, 6, 6f
agents aimed at, 144t, 151-158
anti-mycoplasma drugs' effects on, 45
episodic, during disease progression, 177
gamma interferon's effects on, 41
GM-CSF's effects on, 41
interleukin-6's effects on, 41
prevention of, by CTLs, 38
tumor necrosis factor's effects on, 41
reporter genes, 140
reproductive decisions, 310-311
retinitis, 202t
cytomegalovirus, 191-193, 202t
Retrogen D, 154
Retrovir. *See* AZT
retroviral vector, defined, 158
retrovirus, defined, 3
rev gene, 11, 11f
rev responsive element (RRE), 11
reverse transcriptase, 6, 9f
antibodies to, 8
enzyme immunoassay for, 103
gene for, 10
mutations in gene for, AZT resistance and, 152
reverse transcription, 5f, 6, 6f
interventions aimed at, 8, 141f, 144t, 151-153
rhodococcus infection, 202t
RIA (radioimmunoprecipitation assay), 93t, 98
ribavirin, 144t
ribosome inactivators, 151, 154
ribozymes, 14, 141f, 144t, 157f, 158
defined, 158
rifabutin (ansamycin), 196, 197
rifampin, 196
RIPA (radioimmunoprecipitation assay), 93t, 98
risk factors
alcohol use, 76, 301-302, 305, 308, 318
anal intercourse, 61, 62, 68, 73, 75, 78, 301, 305
body fluids
aerosol contact with, 49
mucous membrane contact with, 267, 272
skin, nonintact, contact with, 267, 270, 272
breast feeding, 75, 79
casual contact, lack of risk in, 49, 265, 266
cervical ectopy, 68
circumcision, lack of, 68, 89
cocaine/crack use, 65-66, 71, 75, 305-306
condom use, lack of, 61, 68, 298, 299, 305
drug abuse
IV drug use. *See* IV drug users
non-IV drug abuse, 301-302, 305, 308, 318
genital ulceration, 68, 72
homosexuality, duration of practice of, 61

risk factors *(continued)*
invasive (surgical) procedures, 274, 284
mother, HIV-infected, 77, 78-79
needle reuse, 56, 57, 62, 63, 65, 303-305
needlestick injury, 267, 268-269
nitrate inhalants, 207
occupational. *See* occupational risk factors
oral sex, 207, 305
rectal douching, 61
sex partners, number of, 61, 62, 65, 68, 73, 75, 89
sexual contact
during menses, 68, 75
frequency of, 62, 68
with HIV-infected person, 50t, 76
with homosexual/bisexual man. *See* homosexual/bisexual men
with IV drug users. *See* IV drug users
with person from Pattern II country, 50t, 68, 78
sexually transmitted disease, history of, 58, 61, 68, 70-71, 72, 73, 75-76, 78
transfusion, 69-70, 80
vaginal-penile contact, 68
vaginal tightening agents, 68
risk groups, 50, 50t, 60-70. *See also* specific groups
children of HIV-infected mothers, 77, 78-79
hemophiliacs, 69-70, 313
homosexual/bisexual men, 60-62, 301-303, 318-319
IVDUs, 62-67, 303-306, 313-314
prostitutes, 70-73, 73f
sexual contacts of HIV-infected persons, 67-69
STD clinic attendees, 58, 70-71
transfusion recipients, 69-70
RNA, 2f, 3. *See also* HIV, RNA
agents directed against (HIV), 144t, 154-158
antisense, 141f, 144t, 156-157, 156f, 157-158
double-stranded (dsRNA), 155f
AZT with, 161t
dextran sulfate complexed with, 154
mismatched. *See* Ampligen
messenger (mRNA), 7
formation of, 156, 156f
nucleic acid sequence of, 3f
2,5A synthetase and, 154-155, 154f
RNA viruses, 3
classification of, 17f
Roferon-A, 162. *See also* interferon(s), alpha
Romania, AIDS cases in, 57, 57f
Rous sarcoma virus, 17f
runaways, 76, 308, 315
Rwanda, AIDS cases in, 59f, 82

S

saliva, antibody tests on, 102
salmonella infection, 202t, 320, 321, 322
AZT for prophylaxis of bacteremia in, 197
sarcoidosis, 202t
scabies, 202t
Scotland, 57, 87
screening tests. *See* diagnostic tests
self-efficacy, 289
sepsis, 203t
Serocard, 107t
seroconversion, defined, 92, 267
Serodia-HIV, 106
in combination screening, in developing countries, 107-108, 109f
on pooled sera, 109
Sero-HIV, 112
seroprevalence. *See also* under specific risk groups
defined, 50
seroreversion, 110
sexual behavior. *See also* risk factors
among adolescents, 307-308
among general population, 298
among IVDUs, 304-305
among women, 309-310
in developing countries, 299-300
inclusion of, in medical history, 276
sexual transmission. *See* risk factors; transmission (HIV)
sexually transmitted diseases (STDs). *See also* specific diseases (e.g., chlamydia; trichomoniasis)
as risk factor, 72
HIV seroprevalence in clinic patients, 70-71
shingles, 200t
simian(s). *See also* macaques
as animal models, 130-134, 235-236
lentivirus infections in, 18-19
simian foamy virus (SFV), 17f
simian immunodeficiency virus (SIV), 16, 17f, 18-20
deletion mutants of, 125
gastrointestinal tract infection by, 210
HIV's relationship to, 17, 134
killed vaccine for, 126
trials in macaques, 133
neurological aspects of infection by, 235-236
vaccine trials in macaques, 132-133
Singapore, AIDS cases in, 60
single photon emission computed tomography (SPECT), 250-251, 250f
SIV. *See* simian immunodeficiency virus
Sjogren's syndrome, p24 antibody in, 113
smoking
as risk factor, 75, 311
disease progression and, 172
Social Learning Theory, 289
social marketing approach, 288-296
channel variables in, 295-296
message variables in, 288-289
receiver variables in, 296
source variables in, 290-295
socioeconomic factors, disease progression and, 173
soluble CD4. *See* CD4, soluble
soluble CD8. *See* CD8, soluble
somatosensory evoked potentials (SEPs), 251f, 252
somatostatin, 145t
South America. *See* Latin America
Southern blot analysis, 104-105
Soviet Union, AIDS cases in, 56, 57f
Sp1, 13, 13f
Spain
adolescents in, 76
AIDS cases in, 57f, 58
education by mass media in, 291
HIV-infected health care workers in, 271f
hospital stays in, 213
IV drug users in, 64
SPECT, 250-251, 250f
spumaviruses, classification of, 17f
stem cells
hematopoietic, HIV infection of, 30-31
of immune system, 24
HIV infection of, 30-31
steroid hormones
disease progression and, 178-179
in pathogenesis of AIDS dementia complex, 240
steroid therapy, for myopathy, 243
Stevens-Johnson syndrome, 195-196
strain variation, 14-15, 28-29
AZT sensitivity and, 152-153, 184-185
diagnostic testing and, 93
disease progression and, 46, 173
epitope identification and, 118-119
macrophage-tropic/neurotropic strains, 234, 236
vaccine development and, 116, 120
street-based outreach, 296, 297
stress reduction programs, 280
structural genes, 8, 10
SUDS (single use diagnostic system), 107t
suicide, 255-256, 255f
sulfadiazine, 244-245
sulfadoxine, 244-245
sulfamethoxazole. *See* trimethoprim/sulfamethoxazole
sulfated molecules, 141f, 144t, 150-151
sulfonamides. *See also* trimethoprim/sulfamethoxazole
for Pneumocystic carinii pneumonia, toxoplasmosis prophylaxis and, 245
for toxoplasmosis, 244-245
suppressor T cells, 25f, 26
in normal immune response, 28
surface protein. *See* gp120
survival time, 81-82, 170. *See also* disease progression
survivor syndrome, 318
Sweden
AIDS cases in, 57f
IVDU prisoners in, 73
synapse, defined, 248
syncytia
defined, 35
formation of, 35, 35f
by microglia, 234
in CNS, 233f
soluble CD4's effects on, 148
vaccine development and, 116
syphilis, 203t
history of, as risk factor, 61, 68, 71
HIV infection and, 245
neurological involvement in, 245-246
neuropsychological deficits and, 260

T

T cells, 24-26, 25f
  activated vs. resting, 7
  activation of
    HIV activation and, 45-46
    in central nervous system infection, 238
    NF-kB sequence in, 42
    vaccine strategies involving, 123
  antigen recognition by, 26, 32, 33f, 123
    HIV interference with, 32, 33f
  cytotoxic. *See* cytotoxic T cells
  helper, 25f, 26. *See also* CD4 cell(s)
    in normal immune response, 28
  HIV entry into, 4-6
  immature, HIV infection of, 40
  in normal immune response, 28
  in syncytia formation, 35, 35f
  inducer, 25f, 26. *See also* CD4 cell(s)
  subsets of, 24-26, 25f
  suppressor, 25f, 26
    in normal immune response, 28
T cell antigen receptor (TCR), 24, 26, 33f
TAR sequences, 11-12, 13f
tat gene, 11-12, 11f
tat protein
  and Kaposi's sarcoma, 44, 45f
  transactivation and, 13
tattooing, 73, 266
terbutaline, 191
Testpak 1 + 2, in combination screening, 108
Testpak HIV, 106, 107t
Thailand, AIDS cases in, 60
therapeutic agents, 138-167, 144-145t. *See also* specific agents (e.g., interferon)
  antiviral. *See* antiviral agents; specific agents
  approval process for, in U.S., 142, 143
  combinations. *See* combination therapy
  elements of development of, 140-146
  evaluation of
    in vitro, 140-142
    in vivo, 142-146
    using animal models, 142
  for opportunisitc infections, 198t-202t. *See also* specific OIs (e.g., cytomegalovirus)
  immunomodulating. *See* immunomodulators; specific agents
  long-term therapy with, 146
  physiologic properties of, 140
thiacetazone, 195-196
thiamine (vitamin B1) deficiency, 212
thymocytes, HIV infection of, 31
thymopentin, 145t, 167
thymus, 40
TIBO derivatives, 8, 145t, 153
TM receptor, 34
toxoplasmosis, 193-194, 203t, 244-245
train-the-trainer programs
  in business and industry, 281
  in health care, 280
transactivation, 13-14
  by other viruses, 13-14
  by tat, 11-12
  defined, 13
  interventions aimed at, 14
transactivation responsive elements, 11-12, 13f
transcription, 5f, 6f
  tat as regulator of, 11
transfusion-associated AIDS, 69-70
  in Africa, 69, 80
  in hemophiliacs, 70
  in Mexico, 54, 69
  in Romania, 57
  incubation period for, 81
translation, 5f, 6f
translational frameshift, 10
transmembrane protein. *See* gp41
transmembrane receptor, 34
transmission (HIV)
  children and, modes of, 223
    disease progression and, 226
    mother-to-child. *See also* perinatal transmission
    neurological disorders and, 262
  child-to-mother, 75
  global trends, 50-60
    geographic differences in, 49, 50
    Pattern I, 50, 51f, 67
    Pattern II, 50, 51f, 67, 88-89
    Pattern III, 50, 51f
  health care worker-to-patient, 274
  misconceptions about, 275, 299
  routes
    amniotic fluid, 268
    blood/blood products, 49, 50. *See also* hemophiliacs; IV drug users; needle reuse; tranfusion-assodiated AIDS
    breast milk, 49
    saliva, 49
    semen, 49
    vaginal secretions, 69
  sexual. *See* heterosexual transmission; homosexual/bisexual men; specific HIV-risk behaviors (e.g., anal intercourse)
treatment, 168-228. *See also* patient care; therapeutic agents; specific aspects (e.g., opportunistic infections)
treatment effect, in epidemiological modelling, 86, 87
triazoles, 244
trichomoniasis, 72, 203t
trichosanthin (GLQ223), 144t, 154, 188
triglycerides, alpha interferon and, 211
trimethoprim/sulfamethoxazole. *See also* co-trimoxazole
  cost effectiveness of, 218
  for Pneumocystis carinii pneumonia, 189-190
Trinidad, AIDS cases in, 55, 55t
tropical spastic paraparesis (TSP), 84
tuberculosis, 171, 194-196, 203t
  as indicator disease, 320, 322
tumor necrosis factor (TNF), 27t, 39, 41, 145t
tumors. *See* neoplasms

U

UBI HIV-1,2 Combo PHA, 96t
Uganda
  AIDS cases in, 51, 59, 59f, 82
  tuberculosis and HIV in, 194
United Kingdom
  adolescents in, 76
  AIDS cases in, 57f, 58
  HIV-infected health care workers, 271f
  IV drug users in, 64
United States
  adolescents in, 76-77
  AIDS cases in, 51, 52-53
    by state, 52f
  AIDS projections in, 87-88
  drug approval process in, 142, 143
  education by mass media in, 291
  hemophiliacs in, 70
  heterosexual transmission in, 67-68
  HIV-2 in, 83
  HIV-associated mortality in, 81-82
  homosexual/bisexual men in, 61
  hospital stays in, 213-215
  IV drug users in, 63f, 64
  needle/syringe exchange programs in, 66
  pediatric HIV infection in, 223
  perinatal transmission in, 79
  transfusion-associated AIDS cases in, 70
  tuberculosis and HIV in, 194-195
universal precautions, 268-270, 323-326
  compliance with, 269-270, 269t
urine
  antibody tests on, 102
  PCR test on, 105-106
USSR, AIDS cases in, 56, 57f

V

V3 loop. *See* under gp120
vaccines
  adjuvant-based, 127t, 128-129
  cell, against FIV, 134
  development of, 114-137
    animal models in, 130-135
    ethical and liability concerns in, 117-118
    major obstacles in, 116-117
  human trials of, 135-137
  idiotype-based, 127t, 129-130, 131f
  killed (inactivated) virus, 125-126, 127t
    human trials of, 136
    response to, in chimpanzees, 132
    trials in macaques, 133
  live (attenuated) virus, 125, 127t
    trials in macaques, 133
    using deletion mutants, 125
  pre-exposure vs. post-exposure, 116
  recombinant, 126, 127t
    human trials of, 136-137
    response to, in chimpanzees, 132
    trials in macaques, 133
  recombinant vaccinia/gp160, human trials of, 136-137
  strategies for, 118-124
  subunit, 126-128, 127t
    defined, 118
  synthetic peptide, 126-128, 127t
    animal trials of, 132, 133
    human trials of, 136
  types of, 125-130, 127t
  using viruslike particles, 129, 137

vaccinia virus, recombinant vaccine using, 126
human trials of, 136-137
vaginal secretions, 69
VaxSyn HIV-1
boosting study using, 124, 135, 136
human trials of, 135
ventricles (of brain), defined, 235
vidarabine, 144t
Videx. *See* dideoxyinosine
vif gene, 11f, 12
vinyl alcohol, sulfated, 151
viral assembly, 5f, 7
agents blocking, 144t, 159
viremia, disease progression and, 177-178
virions
defined, 2
immature, 7
virological markers, 174t, 175-178
virulence, strain variation and, 29, 46
virus culture, semi-automated method for, 103-104
virus isolation, 92, 93t
viruslike particles (VLPs), vaccines using, 129
human trials of, 137
visna virus, 17f
vitamin A, 212
vitamin B1 deficiency, 212
vitamin B12 deficiency, 212
vitamin C, therapeutic use of, 145t
vpr gene, 11f, 12
PCR detection of, 105
vpu gene, 11f, 12, 15
PCR detection of, 105
vpx gene, 15

W-X-Y-Z

warts
genital, 200t
oral, 202t
wasting syndrome, 211, 320
weight loss, 212, 320, 321, 322
Wellcozyme, 108
Wellferon, 163. *See also* interferon(s), alpha
Western blot test, 93t, 98f, 100-101, 101f, 109
women, 74-75
AIDS education of, 316
ethnic/cultural variables in, 53
in Honduras, 55
interventions among, 311-312
intrauterine device and heterosexual transmission, 68
IVDUs
needle sharing among, 63
sexual behavior in, 304
knowledge, attitudes, and behavior of, 309-312
psychosocial consequences of HIV infection in, 319
risk factors for, 75, 309-310, 311. *See also* risk factors
vaginal tightening agents, 68
workplace, 264-285
education in, 279-282
risk of infection in. *See* occupational risks

Yugoslavia, AIDS cases in, 56, 57f

Zaire
AIDS cases in, 51, 59, 59f
education by mass media in, 290, 291
HIV-associated mortality in, 82
hospital stays in, 214
pediatric AIDS in, 82
prostitutes in, 72
transfusion-associated AIDS cases in, 69
tuberculosis and HIV in, 194
Zambia
AIDS cases in, 59f
perinatal transmission in, 79
zidovudine (ZDV). *See* AZT
Zimbabwe
AIDS cases in, 59f
tuberculosis and HIV in, 194
zinc deficiency, 212

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