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INTERNATIONAL SYMPOSIUM ON VACCINE DEVELOPMENT AND UTILIZATION

Infant Morbidity and Mortality
Due to Vaccine Preventable Diseases

Sponsored by the
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Pan American Health
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World Health Organization

June 9 and 10, 1986

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"WHAT BIOTECHNOLOGY HAS TO OFFER VACCINE DEVELOPMENT"

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Conventional vaccines have been among the most successful of all biologic products. Smallpox is eradicated. Utilization of vaccines against polio, measles, rubella, and mumps offers the best means for preventing these diseases. In spite of these successes, there have been failures. For a number of viral diseases there are no effective vaccines. Vaccines against bacterial products have on occasion been ineffective or toxic. Vaccines against parisites have only begun to be a reality.

Biotechnology has opened up two major new avenues for vaccine development. First, biotechnology provides tools for understanding virulence and the nature of the microbial immunogens. Secondly, biotechnology offers new strategies for engineering vaccines.

In order to stimulate an effective immune response it is important to know the precise nature of the antigens that stimulate and are recognized by humoral and cellular immunity. The technique of monoclonal antibodies has provided powerful new reagents that have helped define insights into the specific nature of microbiologic surface proteins and the way that they recognize the immune system. The crystallization of viral surface components, intact virions, bacterial toxins, and parasitic surface proteins has

enabled investigators to recognize how the 3-D structure of proteins relates to immunogenic domains. The capacity to clone the genes encoding critical regions of these proteins has allowed the determination of the specific primary structure of these regions. All of these studies have led to an enhanced understanding of the molecular nature of microbial pathogenicity.

A number of new strategies exist for engineering vaccines. When a protein is identified as the major antigen, the gene encoding the immunogenic protein can be inserted into a suitable host (bacteria, yeast, plant), grown up and then used to prepare the immunogenic protein (or the immunogenic portion of the protein). The protein engineered in this manner may be used directly as a subunit vaccine. As an alternative to the protein prepared in this manner, knowing the DNA sequence of the gene provides the information for chemical synthesis of the immunogenic regions of the protein ("synthetic vaccine").

In addition to using isolated genes or portions of genes to make immunogenic proteins, isolated genes can be inserted into vaccinia or herpes viruses. Such hybrid viruses, when inoculated into the host, can stimulate immunity to the inserted "foreign" proteins in addition to stimulating immunity to its own proteins. Hybrid viruses have the potential to stimulate immunity against a number of foreign proteins simultaneously. Manipulation of live microorganisms may also be carried out that can reduce virulence while retaining the capacity to induce immunity. For example a gene can be removed causing loss of virulence. In addition selection of mutants using monoclonal antibodies can also lead to nonvirulent mutants that are immunogenic. These methods illustrate that it is feasible to more efficiently reduce the virulence of live microorganisms resulting in live attenuated vaccine candidates. A special type of attenuated vaccine strain may be generated without the need for bioengineering techniques for segmented genome viruses where new combinations of genes may result in attenuated viruses.

Lastly, it has been possible to mimic the antigenic structure of certain parasites and viruses using anti-idiotypic antibodies. Such "anti-antibodies" have a configuration that resembles the immunogenic protein anti-idiotypes have been shown to prime or actually stimulate an effective immune response.

This brief outline illustrates some of the newer methods of generating vaccines. Although it remains to be seen which will ultimately be useful, the variety of new options suggest that a new era of vaccine development is about to appear.

VACCINES: OBSTACLES AND OPPORTUNITIES FROM DISCOVERY TO USE

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Although vaccine development for disease prevention is a straightforward objective, no simple path exists from discovery of an etiologic agent to publicly accepted vaccination strategies. While cowpox vaccination replaced variolation by 1800, the global eradication of smallpox did not proceed in an easy and straightforward manner from Jenner's initial efforts. As in the case of that first vaccine, unforeseen obstacles often emerge in vaccine development. Further, economic and political realities can be very influential factors.

Widespread vaccination programmes require large scale production of safe and immunogenic antigens. Such an effort is expensive, and the investment in vaccine development depends on the importance of the disease, the potential market size, responsibility for payment, and, perhaps most importantly, liability risks. Fear of liability favours the status quo, thus impeding innovation, adaptation of existing methods, and regulatory decision-making.

For vaccine evaluation, antibody development in vaccinated animals is a basic first step, along with in vitro assays for inhibition of viral replication. Next are challenge experiments, for which non-human primates ultimately should be considered depending on the closeness of disease expression in alternatives. Specifics of numbers and follow-up need to be considered case by case. The potential toxicity of the vaccine must also be investigated.

Human clinical trials are next. Initially a small group of volunteers should be immunized and followed closely. Enlargement of clinical trials will depend on results of earlier stages. Artificial challenge, where acceptable, can be especially informative. Eventually, double-blind, randomized, placebo-controlled trials are warranted. Each successive stage widens the information base available for a decision on vaccine approval for general use. However, as past experience demonstrates, sociopolitical forces can affect the rate of data collection and decision-making.

A basic function of WHO is to establish international norms for the acceptability of vaccines. An expert panel considers realistic vaccine prospects and makes initial suggestions on draft guidelines. WHO Requirements represent an international consensus on minimum elements for acceptability and are issued by the Expert Committee on Biological Standardization. While WHO is not a regulatory agency, and its Requirements are in no way imposed upon national regulatory authorities, many countries voluntarily adopt WHO Requirements.

Concerns for purity and safety need to be addressed along with matters of cost and vaccine availability. For example, the rabies vaccine produced and used in many developing countries is derived from sheep brains, while in industrialized countries production is concentrated on more sophisticated cell culture vaccines. WHO's role, taking due consideration for safety, must be to moderate the pursuit of extreme levels of purity so as to improve existing conditions in the developing world. Rabies vaccine produced from continuous cell lines may be a reasonable substitute for the vaccines based on brain tissue, and the pursuit of extreme purity must not render improvements impossible.

The potential exists to substantially reduce the toll of many infectious diseases. However, realizing this goal through innovation and adaptation will require the cooperation and persistence of manufacturers, the biomedical community, and regulatory authorities.

Feasible Improvements on Existing Vaccines

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This paper will discuss presently available vaccines and some of the ways in which they can be improved. Widely used current vaccines, the schedules for their adminstration, and improved candidate vaccines already available on a limited or research level will all be described. In addition, some of the more obvious drawbacks in present vaccines and administration schedules will be discussed.

For the diphtheria and tetanus components of the DPT vaccine the main considerations involve the optimum dosage schedule. This is because the Expanded Programme in Immunization has suggested a 3-dose regimen for these antigens, and a 2-dose regimen, if adequately immunogenic, would be both cheaper and more easily administered.

For pertussis, the main consideration is the improvement of the vaccine and a possible reduction of the number of doses from three down to two. New vaccines containing semi-purified pertussis toxin and one or another component of the pertussis organism have been developed. These are clearly of lower reactogenicity, but their protective effect is not universally accepted at the present time.

For measles, there is debate as to to the optimal age at immunization as well as the optimal route (subcutaneous or by aerosol) in countries where measles still presents a high incidence and mortality in the first year of life. In addition, the

stability and cost of the vaccine are both targets of possible improvement.

For polio, proponents of the killed vaccine approach have pointed to the improvements in both immunogenicity and cost of newer vaccines and have suggested a 2-dose regimen would be feasible with a high protection rate, comparable cost and high compliance rate. Proponents of the oral vaccine, on the other hand, point to the low cost and the ease of administration, and the impressive past record of immunization campaigns.

Other vaccines such as hepatitis B, hemophilus, meningococcus, and pneumococcus all will admit to some improvement, and these will also be the subject of discussion.

Rotavirus Vaccine

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Rotaviruses have emerged as the single most important etiologic agents of severe diarrhea of infants and young children in developed and developing countries. In developed countries, in long-term cross-sectional studies of infants and young children hospitalized with diarrheal illnesses, rotaviruses are associated with 34-45% of such illnesses (Brandt et al., Kono et al.). In developing areas, diarrhea was ranked first in frequency in the categories of disease and mortality. It was estimated that in Asia, Africa and Latin America during a one year period, there would be 3-5 billion cases of diarrhea and 5-10 million diarrhea-associated deaths (Walsh and Warren). In developing countries, rotaviruses are consistently found to be major etiologic agents of severe diarrhea. In a one year study of patients coming to a treatment center with diarrheal illness in Bangladesh, rotaviruses were the most frequently detected pathogens in children less than 2 years of age, with 46% rotavirus positive (Black et al.).

It is clear that a rotavirus (RV) vaccine is needed in both developed and developing countries. The goal of such a vaccine should be the prevention of severe RV diarrhea during the first 2 years of life, when RV disease is most serious. Since animal studies indicate that intestinal immunity plays a major role in resistance to RV disease, current efforts in RV vaccine research

are aimed at developing a live attenuated vaccine for oral administration, effective against all 4 serotypes.

Approaches to the development of a RV vaccine range from conventional cell culture cultivation of human or animal RV strains, to molecular biologic techniques. The most promising and most extensively evaluated method, is the "Jennerian" approach in which a related RV from a different host is used as the immunizing agent. Efficacy trials of a bovine RV strain (NCDV-Lincoln [RIT 4237]) by Vesikari et al. in children one year of age or less demonstrated a protection rate of over 80% against clinically significant RV diarrhea. We are pursuing another animal RV strain - the rhesus RV MMU18006 - as a vaccine candidate. This strain is similar, if not identical to human RV type 3, and grows efficiently in a semicontinuous diploid cell strain (DBS FRhL-2) from fetal rhesus monkey lung. It is undergoing efficacy trials in collaborative studies in the U.S., Latin America and Europe involving approximately 1,000 children <12 months of age. The target population for ongoing or future studies is infants <5 months of age. The delicate balance of achieving a vaccine dose which is attenuated enough to be nonreactogenic but antigenic enough to induce protection is a challenging task especially in neonates who characteristically possess high levels of maternal RV antibodies.

If the "Jennerian" approach is not effective in protecting against all 4 human RV serotypes, then the rhesus RV (or bovine RV) strain can be used as a donor of attenuating genes that can be transferred to form a hybrid or reassortant RV that possesses only the major neutralization protein (VP7) of a human RV belonging to serotype 1,2,3, or 4 and the remaining genes from the animal rotavirus donor. Such single gene substitution reassortants have been prepared for each human RV serotype with the rhesus and/or bovine (UK) RV strains. Since VP3 (another outer capsid protein) has been shown to induce high levels of neutralizing antibody also, and such antibody can protect against disease experimentally, it might be possible to use a reassortant which possesses a broadly reactive VP3, and the VP7 of a human RV as a vaccine candidate. In addition, RV strains which were isolated from neonates with asymptomatic infections may be promising vaccine candidates since they appear to be naturally occurring attenuated strains and they represent each of the 4 serotypes. In another approach, reassortants comprised of 10 genes from a neonatal strain and a single gene (VP7) from different RV serotypes may yield attenuated strains and broad protection. Finally, cold adaptation of human or animal rotaviruses may yield attenuated strains for use as vaccine candidates.

SHIGELLA VACCINES

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Shigella infections occur throughout the world, but are of special importance in developing nations. In these countries, bacillary dysentery is the second or third leading cause of acute diarrhea in young children. The prevelence of infections, especially those which are symptomatic, is low in breast-fed babies, but increases marked after weaning. The incidence in two to three year old children in same regions is 200,000 cases per 100,000 per year.

Attempts to prevent the disease by the parenteral administration of conventionally killed vaccines have not been successful. The fact that in endemic areas, older children and adults tend to be resistant to clinical disease suggests that attenuated oral vaccines might be effective in protection. Experimental studies in monkeys have demonstrated that living oral vaccine strains which can penetrate the intentional mucosa are more effective than vaccines which do not have this property. Both experimental and field studies have indicated that that protection is associated with the shigella somatic antigen. Safe oral vaccines have been constructed by transferring genetic material which encode for the expression of somatic antigens from shigella to carrier strains. The carrier strains

include the *Salmonella typhi* Ty21a strain which was effective in protecting against typhoid fever in Egypt. A second antigen-carrier strain has been constructed from *E. coli* K-12. Both carrier strains are thought to penetrate the intestional mucosa. Two lots of a the Ty-21a strain which expresses the *S. sonnei* somatic antigen have protected volunteers against serious disease. A third production lot failed to confer protection. Two different protection tests, in which the *E. coli* carrier expressing the *S. flexneri* 2a somatic antigen was employed as a vaccine, protected monkeys against challenge with virulent *S. flexneri* 2a.

SALMONELLA TYPHI VACCINES

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From the early 1950s to the early 1970s, the World Health Organization sponsored a series of field trials of efficacy of the heat-phenolized and acetone-inactivated killed whole cell parenteral *Salmonella typhi* vaccines in several countries, including Yugoslavia, Poland, the Soviet Union, Guyana, and Tonga. Both vaccines provided moderate to excellent efficacy for up to seven years, although the acetone-killed vaccine was somewhat superior. Despite their efficacy, these vaccines are rarely used as public health tools because they cause notable adverse reactions, including fever, malaise, and severe local reactions, in approximately 20-25% of recipients, commonly resulting in absenteeism for 1-2 days. Thus the need persists for a typhoid vaccine that is effective without causing significant adverse reactions. Several attractive alternative vaccines to prevent typhoid fever have reached the stage of clinical testing and definitive field trials of efficacy. These include attenuated mutants used as live oral vaccines and the purified capsular Vi polysaccharide of *S. typhi* employed as a parenteral vaccine.

In the early 1970s, studies in volunteers showed that attenuated strains of *S. typhi*, including a streptomycin-dependent and a gal E mutant, when used as live oral vaccines could provide

remarkable protection without eliciting adverse reactions. The gal E mutant, Ty21a, has since been extensively evaluated in field trials. In a trial in Alexandria, Egypt, approximately 16,000 6 and 7 year old schoolchildren ingested three 10^9 organism doses (within one week) of a liquid formulation of Ty21a following neutralization of stomach acid with 1.0 gm. $NaHCO_3$; a similar number of children ingested placebo. The liquid formulation in this trial provided 96% protection for a period of at least 3 years.

In an attempt to identify a more practical formulation of vaccine and the possibility of immunizing with fewer doses, a randomized, double-blind, placebo-controlled trial was carried out in Area Norte, Santiago, Chile, where approximately 90,000 schoolchildren ages 6-19 yrs. were randomized to receive 2 doses of vaccine in enteric-coated capsules (that do not require pre-treatment with $NaHCO_3$), 2 doses of placebo, or a dose of each. Two doses of vaccine provided moderate protection during the first 2 years of surveillance (50% in year 1 and 72% in year 2), while 1 dose was only 16%-40% protective. In the third and fourth years of surveillance, the efficacy of 1 dose disappeared and the protective effect of 2 doses fell to insignificant levels.

A second field trial in Santiago (in Area Occidente) compared the relative efficacy of 3 doses of vaccine in enteric-coated capsules versus in gelatin capsules with $NaHCO_3$. In this trial two different immunization schedules were also compared (3 doses given within 8 days versus 21 days between doses). Three doses of enteric-coated vaccine gave significant protection (60%) that has endured for at least 3 years, with no difference evident between the two immunization schedules. The efficacy of the gelatin capsule/$NaHCO_3$ formulation (31% efficacy) was significantly inferior ($p=0.0031$).

A third field trial in Santiago involving 248,544 schoolchildren has compared the efficacy of 2, 3, and 4 doses of vaccine in enteric-coated capsules. Four doses were significantly superior to 2 or 3 doses ($p<0.002$).

Field trials scheduled for late 1986 in Chile and Indonesia will compare the relative efficacy of three doses of Ty21a vaccine given in enteric-coated capsule or liquid formulations.

Clinical studies have begun with an Aro-, Pur- mutant strain of S. typhi prepared by B. Stocker et al that has precise deletions of genes in metabolic pathways making it auxotrophic for substrates not available in human tissues. In these preliminary

studies, the vaccine (strain 541 Ty) was well-tolerated and stimulated notable cellular immune responses.

Two purified parenteral preparations of Vi polysaccharide were tested for reactogenicity and immunogenicity in volunteers, one having 5% and the other 0.2% contamination with LPS. The more purified preparation was well-tolerated and caused Vi seroconversions in 85% of vaccinees. Two field trials of efficacy of this purified Vi vaccine are in progress.

In recent years, promising new candidate vaccines have appeared to prevent typhoid fever that do not elicit adverse reactions and thus are attractive alternatives to the reactogenic whole cell parenteral vaccines. One of these vaccines has reached the point of being ready for use in public health programs.

VIBRIO CHOLERAE VACCINES

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The first attempts to develop a cholera vaccine came within one year of the identification of *Vibrio cholerae* as the causative agent of cholera in 1883. During this century of research, a variety of immunizing agents have been developed, each suffering from one or another short-coming. While a number of vaccine strategies are currently under study, two separate approaches to the development of a cholera vaccine appear particularly promising. These approaches are: 1) killed whole vibrios plus purified B subunit combination vaccine and 2) a genetically engineered non-enterotoxinogenic attenuated *V. cholerae* strain. Each vaccine is administered orally to maximize stimulation of mucosal immunity and each vaccine contains multiple vibrio antigens.

The killed combination vaccine is composed of multiple killed strains of *V. cholerae* plus purified B subunit of cholera enterotoxin. Antibodies directed against the B subunit will neutralize the action of the holotoxin which consists of the B or binding subunit and the A or active subunit which is responsible for the ADP-ribosylation characteristic of this toxin. This vaccine has proven to be

completely safe in volunteers and provides significant protection to challenge, although the protection is not equivalent to that conferred by the disease itself. Further evaluation of this vaccine is currently underway in a field trial in Bangladesh.

Our research at the Center for Vaccine Development has focused on the development and testing of a genetically engineered attenuated V. cholerae strain. We have cloned and sequenced genes encoding cholera enterotoxin and have constructed mutations in vitro using restriction endonucleases. These mutations were then introduced into the chromosome of virulent V. cholerae strains which had been well-characterized in volunteers. The resulting attenuated strains produced either the B subunit only or neither the A nor B subunits. The first two strains to be constructed were an El Tor Inaba strain (JBK70) which was A^-B^- and a Classical Ogawa strain (CVD101) which was A^-B^+. Both strains produced excellent immune responses in volunteers including both antibacterial and antitoxic (CVD101 only) responses. Volunteers immunized with a single dose of JBK70 were significantly protected against challenge with a 89% vaccine efficacy, equivalent to the protection seen with the fully virulent parent strain N16961. However, these vaccine strains were not entirely satisfactory because mild to moderate diarrhea was seen in 40 - 50% of volunteers upon initial immunization.

We have recently been evaluating a vaccine strain with minimal or no reactogenicity in volunteers. This strain, CVD103, is a derivative of the highly toxinogenic Classical Inaba strain 569B. Of 26 volunteers who have received CVD103 to date, only two experienced any diarrhea. In one volunteer, diarrhea consisted of two loose stools totaling 378 ml, while in the other, four loose stools over four days totaled 911 ml. This strain produces significant protection in volunteers against challenge. In the first efficacy study, nine volunteers were immunized with a single oral dose of 2×10^8 CVD103 and subsequently challenged with toxinogenic V. cholerae 569B. Diarrhea was seen in only one of nine vaccinees (stool volume of 0.5 liter) versus seven of eight unimmunized controls ($p=0.007$). The mean diarrheal stool volume in the controls was 1.4 liters with a range of 0.5 to 3.9 liters. These results are very encouraging and further volunteer studies are ongoing to fully evaluate this vaccine candidate.

Development of a Synthetic Malaria Vaccine

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Vaccination of rodents, monkeys and humans with attenuated, irradiated sporozoites leads, in many instances, to complete protection against malaria. Protective immunity which is species- and stage-specific [generally not strain-specific], is at least in part antibody-mediated. Incubation of sporozoites with the serum of vaccinated, protected animals and humans, neutralizes their infectivity both in vivo and in vitro, and results in the appearance of prominent deposits of antibody on the whole surface of the parasite, the "circumsporozoite' reaction.

Although protective immunity induced by irradiated sporozoites is not long-lasting, it can be boosted and maintained by the recurrent bites of infected mosquitoes. This may also occur in humans living in malaria endemic areas. The aquisition of resistance to malaria with increasing age is associated with a higher percentage of positive reactions and higher titers of antisporozoite antibodies.

The protective antigens [CS proteins] of sporozoites have been identified in several malaria species by means of monoclonal

antibodies. Passive transfer of as little as 10 μg of moAb to mice induces complete protection against sporozoite challenge. These antibodies also prevent the attachment and interiorization of sporozoites into liver cells in vitro.

The CS proteins display unusual immunological properties. They have an immunodominant epitope, which occurs more than once per molecule. The strong immunogenicity of this epitope is highlighted by the finding that antibodies from humans living in malarious areas or from a volunteer vaccinated and protected against malaria are mostly directed against the repetitive epitope.

The genes encoding the CS proteins of various Plasmodia were recently cloned and sequenced, including those of the two human malaria parasites, P. falciparum and P. vivax. The central area of these proteins is formed by tandem repeats of short amino acid sequences, which contain the immunodominant epitope, and which vary among different Plasmodial species. In the case of these two human malaria parasites, MoAb directed against the respective CS proteins recognize the repeats, in a large number of isolates from different areas of the world.

In view of the impossibility of obtaining sufficient amounts of CS protein from sporozoites, engineered vaccines are being developed. To date, two candidate vaccines, based on the NANP repeats of P.falciparum are being assayed. One of these is a conjugate of a carrier protein [tetanus toxoid] with the synthetic peptide $(NANP)_3$, the epitope recognized by the majority of antisporozoite antibodies in the serum from humans living in endemic areas. Rabbits and mice injected with $NANP_3$-conjugate make high titers of antipeptide antibodies. Most of these also recognize the native CS protein on the sporozoite's surface and neutralize parasite infectivity. The other candidate vaccine, made by recombinant DNA technology, consists of a fusion protein produced in Escherichia coli and contains 32 NANP repeats. Synthetic peptides corresponding to dimers of the nonapeptide repeat of the CS protein of P vivax are being assayed for antigenicity.

We have recently found that lymphokines, in particular gamma interferon, inhibit greatly the development of exoerythrocytic stages of Plasmodia, both in vivo and in vitro. The targets of this antimalarial activity of gamma interferon are not the sporozoites but the next stage of development, i.e., the liver stages. It is conceivable that the inoculation of viable sporozoites, during challenge, will trigger the release of gamma interferon by sensitized T cells, and thus contribute towards the protection of immunized animals and perhaps also man.

CURRENT STATUS OF HAEMOPHILUS INFLUENZAE TYPE B VACCINE: POTENTIAL FOR USE IN THE DEVELOPING WORLD.

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Haemophilus influenzae type b (Hib) is the most common cause of bacterial meningitis in children <5 years of age in the United States. The organism also is an important cause of other diseases such as pneumonia. Currently, an Hib polysaccharide (PS) vaccine is recommended in the U.S. for children 2-4 years of age. This age group accounts for approximately 20% of all cases of disease. Unfortunately, this vaccine is ineffective in younger children, the group at greatest risk. Hib PS vaccine also may be ineffective in preventing disease in certain subgroups of the population who are genetically at increased risk of disease and who showed impaired antibody responses to immunization. Currently, several new Hib PS-protein conjugate vaccines are under evaluation. In contrast to the plain PS vaccine, conjugate vaccines are immunogenic in infants as young as 2 months of age, and elicit boostable increases in Hib PS antibody on reinjection of vaccine. Thus, conjugate vaccines may prevent disease in children in the age group in the U.S. at greatest risk of disease. Several studies indicate that Hib is an important cause of invasive bacterial infections in children from developing countries. However, more information is needed on the epidemiology of Haemophilus diseases in these populations, including the age-incidence of disease and whether serotypes other than b are also important. Also needed is information on the immunogenicity of Hib conjugate vaccines in the specific populations. In developing countries, it is possible that genetic factors, malnutrition or coexisting, chronic infections may affect the immune responses of children immunized with these vaccines.

RESPIRATORY SYNCYTIAL VACCINE

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Respiratory syncytial (RS) virus is the major etiologic agent of severe lower respiratory tract disease during early life and the need for effective immunoprophylaxis has been clearly established in studies performed throughout the world. The challenge of vaccination against RS virus disease is formidable because the virus has its major impact during the second through sixth month of life. Prevention of such illness by decreasing the spread of virus in the community through vaccination of adults and older children is unlikely to succeed because reinfection by RS virus occurs with appreciable frequency. Since natural infection with the virus does not provide effective resistance to reinfection, it is not likely that immunization would succeed in achieving this result. However, infection does decrease the severity of illness during subsequent re-infection. Thus, we are left with the difficult task of immunizing the target host for severe RS virus disease, the young infant. This means that RS virus vaccine must be administered shortly after birth if it is to be effective in preventing the major share of severe RS disease. Unfortunately, this is a difficult period in which to attempt immunization because the infant's immunologic processes are not fully responsive to antigenic stimulation. Also, antibody in serum which is passively acquired from the

mother acts to suppress the infant's immunologic response.

Previous attempts to develop a safe and effective RS virus vaccine have all met with failure. A formalin-inactivated virus vaccine tested 20 years ago failed to protect against RS infection or disease. Instead, disease was enhanced during subsequent infection by RS virus. The basis for this potentiation of disease by formalin-inactivated virus was recently elucidated and laboratory systems are now available to test current and future vaccines for evidence of disease potentiation.

Safe and immunogenic temperature sensitive (ts) mutants of RS virus were developed but could not be used as a live vaccine because of over-attenuation or genetic instability which allowed the mutants to lose their ts phenotype. Immunization by parenteral inoculation of live RS virus has also been proposed, but a field trial of this approach indicated it to be ineffective.

Recently, several encouraging developments suggest that effective immunization against RS virus can be achieved. The two surface glycoproteins of RS virus, i.e., F and G, have been shown to be the major protective antigens. Complete copies of the genes for these antigens have been cloned and sequenced. Vaccinia recombinant viruses were then prepared by recombining F or G cDNA into the TK gene region of vaccinia virus. Each of the RS-vaccinia recombinants expressed the appropriate RS gene product during infection in vitro. Also, infection of cotton rats induced a specific, high level antibody response to the appropriate RS glycoprotein. Significantly, both recombinants induced a high level of neutralizing antibodies to RS virus in cotton rats. Prior infection with the RS F-vaccinia recombinant provided complete resistance in the lungs to subsequent infection by RS virus. The RS G-vaccinia recombinant also induced significant resistance in the lungs but protection was less than that observed with the RS F-vaccinia recombinant.

Pneumococcal Polysaccharide Vaccine

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Recognition of the importance of acute respiratory infections as causes of morbidity and mortality in infancy and early childhood has focused attention in the past decade on their diagnosis, treatment and prevention. Although a significant proportion of deaths from respiratory infection appears to result from bacterial pneumonia, definitive data regarding the causes of such infections are limited because of the difficulty in making a precise bacteriologic diagnosis without invading the lower respiratory tract. Available data suggest that the pneumococcus and Haemophilus influenzae are the most important causes of bacterial pneumonia in early life.

Although some studies of the treatment of acute respiratory infection, including pneumonia, in infancy and early childhood with antimicrobial drugs in the absence of clinical microbiologic studies have shown a reduction in mortality, it appears from what is known of the epidemiology of drug resistance in the pneumococcus that such a strategy would not be effective over a prolonged period. Resistance of pneumococci to penicillin and to other drugs tends to emerge in populations in which such drugs are widely used, and children are among

those receiving these agents most frequently. For this reason, strains of pneumococcal types most often responsible for infection in early life, types 6A, 6B, 14, 19F, 19A and 23F, are among those found most often to manifest drug resistance. For this and other reasons, prophylactic vaccination, if a safe and effective vaccine were available, would be a preferable means of controlling pneumococcal infection.

Both 14- and 23-valent formulations of polyvalent pneumococcal vaccine contain capsular polysaccharides of pneumococci in groups 6, 14, 19 and 23. Studies of the responses of infants and young children to the purified polysaccharides of these strains show them to be poorly immunogenic, a finding confirmed by their failure to prevent infection with these same types at this age. In addition, the antibodies to these and to other pneumococcal types induced by purified polysaccharide antigens is predominantly of the IgM class, and protection afforded is transitory.

The finding in experimental animals that administration of capsular polysaccharides of bacteria chemically linked to proteins, including bacterial toxoids, alters immunologic responsiveness to the carbohydrate moiety with the resulting development of antibodies of both the IgG and IgM classes and of "immunologic memory" has led to the administration of similar conjugates to man with analogous results. If safe and immunogenic conjugates of the capsular antigens of those bacterial strains responsible for the preponderance of pneumococcal infection can be developed and at an acceptable cost, it should be possible to reduce significantly the morbidity and mortality of infections caused by them.

IMPEDIMENTS TO THE DEVELOPMENT OF ADDITIONAL VACCINES; VACCINES AGAINST IMPORTANT DISEASES WHICH WILL NOT BE AVAILABLE IN THE NEXT DECADE

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There are over 200 infectious diseases or syndromes caused by multiple species and/or serotypes of bacteria, viruses and parasites. Slightly more than one vaccine for the prevention of a few of these diseases has been developed for each of the decades since Jenner inaugurated the era of immunization 190 years ago. The most effective ones are for nine diseases of children; thirteen others of varying quality and efficacy are used in various high-risk groups in different countries. Now, the availability of new biotechnologies such as genetic engineering for the design of attenuated organisms or the expression or synthesis of purified antigens has led to the prediction that new or improved vaccines can be developed for 28 diseases within the next decade, a 25-fold acceleration in research and development.

Consideration of the reasons why such optimism cannot be extended to the availability of vaccines for many other infectious diseases is facilitated by reviewing the steps in vaccine development from identification of the etiologic agent, through elucidation of the mechanisms of recovery and immunity and identification of the protective antigen, to construction of an attenuated or inactivated vaccine. Impediments to vaccine development may exist, or arise, at any point in this pathway, e.g., multiplicity of serotypes, inability to cultivate the pathogen, multi-stage

life cycles with multiple antigens, infection in infancy, unpredictability of epidemics, long incubation period, inadequate knowledge of pathogenesis and immunity, reversion to virulence, fear of gene splicing, need for an adjuvant, lack of profitability, liability. Examples of diseases for which vaccines are not likely to be available in the next decade, by organ system, are: Ocular: conjunctivitis, trachoma, onchocerciasis; Respiratory: common cold, pneumonia due to Klebsiella, Legionella, and mycoplasma; Gastrointestinal: non-typhoid salmonellosis, amebiasis and giardiasis; Hepatic: Non-A, non-B hepatitis, schistosomiasis; Genitourinary: syphilis, chancroid, chlamydial urethritis and pelvic inflammatory disease; Nervous: most viral encephalitis, trypansomiasis; Skin: yaws and leishmaniasis; Systemic: filariasis and Epstein-Barr virus infections. Although more than a decade may be required, vaccines for some of these diseases are likely to become available eventually. Meanwhile, efforts must continue to improve and extend environmental and vector control and to design more effective antivirals and better antiparasitic drugs. Vaccines will never be able to do it all.

Improving the Heat Stability of Vaccines - Problems, Needs, and Approaches

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The hope for temperature stabilization of Biologics suffers from (a) the desire to take the product beyond the confines of even physiologically normal temperature and (b) the assumption of a database that does not exist. Biologicals, whether live, killed or subunits of organisms are composed of organic polymers that are inherently unstable and that must retain their primary and subtle secondary structures as well as their appropriate interrelationships if their desired activities are to be retained. The rate for loss of chemical integrity and activity is largely temperature dependent. The principles currently applied to preservation of existing biologics are based largely on empiricism backed by historic precedents that, in practice, are no more than rules of thumb.

Recognizing the technical problems involved, it is perhaps the more realistic to ask what can be done now to optimize and improve the current situation, even though, modest, within the limits of present knowledge. There are 4 principal elements involved. First, there must be a vastly increased scientific database. Second, there must be an economic incentive for the producer. Third, there must be an improved means to protect the rights of the inventor. Fourth, someone must be willing to pay the cost. There are no meaningful incentives for the producer.

There are exceptions and the example of how a vaccine of greatly improved heat stability was accomplished without economic reward will be reviewed.

It is critical at this time to weigh the costs for improving vaccine stability against the costs for establishing an effective cold chain. The pros and cons and inevitable conclusion will be presented.

VACCINE DELIVERY MANAGEMENT

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The vaccine cold chain describes the distribution system which ensures that vaccine arrives at the right place, the right time and in a potent state. This voyage from the factory to the village typically takes between 12 to 18 months.

The development of heat stable vaccine will not, unfortunately, eliminate the need for a cold chain. The "cold" part would not be needed but a management chain of effective supplies will be needed.

This paper covers two topics: first, some of the progress that has been made in the past 7 years in the technology and the management of the cold chain and second, a look ahead a few years to some of the developments we would like to take place.

1. Past progress

Effective equipment is no longer a major constraint. There are over 100 items of equipment that have been tested for use in tropical conditions. This equipment includes refrigerators, freezers, solar refrigerators, sterilizers, syringes, voltage stabilizers, spare parts and almost any item of equipment that will be needed in an immunization programme. There is, however still some work to be done in this area: we need more ice in the rural areas and we need burners for kerosene refrigerators that are tolerant of polluted or low grade fuels.

EPI training materials are no longer a constraint. Materials existe for management of the EPI and the cold chain at all levels from the senior manager to the volunteer vaccinator. The training materials also cover equipment maintenance and equipment repair.

Many thousands of people have been trained using these materials and of course there are still many thousands to train.

Management and supervision, however, are still serious problems. Some progress has been made. Time-temperature monitors are in use in almost all tropical countries of the world. Many innovative management systems have been developed by the ministries of health to overcome the special problems of the cold chain. More are needed.

2. Future challenges

In spite of the advances there are still some important challenges for the cold chain of the future.

The first is an over-riding one that affects all the others: national self sufficiency. This is not so much a challenge of technology or management as it is a challenge to a change of attitude. The goal is to achieve cold chains (and immunization programmes) that are, as far as possible, independent of outside support.

With this attitude towards self sufficiency understood as a background, eight ideas are listed below that are needed for the cold chain to progress. These are listed under the headings of the three major suppliers of ideas for the EPI.

The countries.

o When possible, make greater use of the skills of the private sector. For example, for the maintenance of equipment or the management of fuel supplies.

o Management of transport must be improved. This is an area where much work has been done but little has been achieved.

The donors.

o The whole life costs of equipment must be considered when selecting equipment for the EPI. Each dollar spent by an aid agency on cold chain equipment results in three dollars of foreign currency being spent by the ministry of health to support that piece of equipment.

o A greater challenge lies in developing this equipment in the countries themselves. Buy equipment in the country whenever possible.

o With the countries, develop innovative ways to solve fuel shortages.

The manufacturers.

o Price the vaccine in a way that reflects the number of doses contained in a vial.

o Extend the vaccine expiry dates to three to four years.

o More heat stable vaccines - four to six weeks at 45°C.

If any one of these ideas could be implemented over the coming three to four years we would see an important step forward for the cold chain and for the EPI.

SOME OPERATIONAL ISSUES IN IMMUNIZATION PROGRAMS

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The biological and technical aspects of vaccine research and production have progressed substantially in recent years. However, effective immunization of a target population requires the transfer of this knowledge to the field and its proper utilization, sometimes under very difficult conditions. In an effort to improve the level of immunization coverage achieved in rural India, a study has been made of the operation of the vaccine distribution system in three districts of one State of south-central India. The distribution study has covered the entire range of program elements: supply of vaccines to the district, transport within the district, supply to the final consumption and utilization points (Primary Health Centers and sub-centers), vaccine storage, monitoring of the program, and population coverage achieved. Population coverage achieved is highly variable, and disappointingly low. Analysis of data collected on each of these system elements has identified a number of operational problems, and has pointed to possible solutions. Among the problems identified are irregularities in order quantities and order intervals, which result in stock-outs as well as periods of excessive inventory, along with high workload

SOME OPERATIONAL ISSUES IN IMMUNIZATION PROGRAMS

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variation; correction of these require improved inventory management. Transport time, too, is highly variable; transport distance and time can be improved through route optimization. Systematic refrigerator maintenance as well as improved routing and better inventory control can decrease the incidence of cold chain failure. A simulation model of the immunization system has been developed and used to estimate system performance under alternative feasible system arrangements and under different field conditions. Results show that there is vast scope for increasing the efficiency of the immunization program, thus ensuring that the successful efforts in research and technology result in benefits for target populations.

IMMUNIZATION PROGRAMS AND HTLV-III/LAV

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The emergence of HTLV-III/LAV as a significant cause of infection and disease in the world today has been amply documented. The presence of this is virus, which is capable of infecting cells of the immune system and which can remain in a latent form for many years, has important implications for immunization programs which must be considered as these programs are planned and implemented. First, the infection by HTLV-III/LAV results in immunosuppression. As a consequence, infected invididuals may not respond to vaccines with a protective response. Second, stimulation of the immune system may be a co-factor for the development of AIDS since stimulated lymphocytes are needed for the replication of HTLV-III/LAV. Thus the progression of disease may be enhanced by immunization. Third, in areas which are endemic for the virus and since this virus can be transmitted through exposure with blood and blood products, immunization programs must take steps to minimize the possibility of accidental transmission of infection during the vaccination procedure itself. Finally, immunization programs must consider the higher probability of vaccinating an immunosuppressed person and the consequences this immunization will have for the vaccinee.

Global Control of Vaccine Preventable Diseases - An assessment of Progress

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Immunization is one of the most cost-effective of all health services. It remains tragically under-utilized. In the developing world, excluding China, less than 40% of infants receive a third dose of DPT or polio vaccines. Coverage with measles vaccine is only about half that for DPT and polio, in part because it is only now being introduced in some programmes. Over three million children still die annually from measles, neonatal tetanus and pertussis, while over a quarter of a million children are crippled by poliomyelitis.

If under the current EPI strategy less than 40% of infants in developing countries have so far received a third dose of DPT or polio vaccine, some 60% have probably received the first dose, as drop-out rates remain of the order of 30% in many areas. This situation represents a major public health gain in the space of the past 10 years. Yet such a gain is clearly not enough.

Acceleration of existing efforts constitutes the overriding priority for EPI at present, using approaches that must differ according to the requirements of individual programmes. Recommendations include:

- promoting collaboration between the public and private sectors,
- adopting a mix of complementary strategies for programme acceleration which can be sustained,
- providing immunization at every contact point, immunizing even children suffering from minor illness or malnutrition,
- reducing drop-out rates between first and last immunizations,
- improving immunization services to the disadvantaged in urban areas, and
- increasing the priority for the control of measles, poliomyelitis and neonatal tetanus.

In evaluating immunizations programmes, the priority should be placed on monitoring immunization coverage and disease incidence levels. Routine reports are essential for this purpose, although they may be usefully supplemented by surveys. Other evaluation instruments include outbreak investigations, vaccine quality control, cold chain monitoring, serological surveys, costing studies and programme reviews.

The problems revealed by an evaluation of immunization programmes can be taken as being generic to the health services as a whole until proven otherwise. In remedying these problems, approaches which improve the health services as a whole should be sought.

ROLE OF NON-GOVERNMENTAL AGENCIES IN VACCINE DELIVERY

THE ZAIRE EXPERIENCE

MAMBU-MA-DISU

Ministry of Health

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Zaire, a large central African country with a population of more than 30,000,000 has had much experience with non-governmental agencies in its health sector. For many years missionary groups have played an important role in the development of the country's health infrastructure. With financial backing from their church groups, they established health facilities and conducted "mobile" health activities in much of rural Zaire. Missionaries also established efficient communications and supply networks. Because they could refrigerate vaccines, they were among the first groups to deliver vaccines in rural areas. After participating in the very successful smallpox eradication program, missionary health personnel concentrated on the establishment of rural zones as part of the government plan to implement its primary health care strategy through fixed centers. Early EPI efforts were aimed at vaccine delivery in major urban areas where government facilities generally had vaccine delivery capabilities. Expansion of services to rural areas was only made possible through the efforts of non-governmental agencies. Since 1932 when this expansion was initiated, the national EPI has played a vital role in the development of primary health care services by providing national and peripheral training programs.

POLITICAL, ECONOMIC, & ADMINISTRATIVE RESOURCES AVAILABLE FOR THE CONTROL OF VACCINE-PREVENTABLE DISEASES

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The harnessing of broad social mobilization with immunization appears to be the only feasible way of controlling the vaccine-preventable diseases and achieving universal immunization by 1990. Field experience in the developing countries since 1974 indicates that rapid headway against such diseases as measles and tetanus will not be made through conventional EPI operations or through health ministries working on their own. Sizeable immunization gains have been made in the past three years, however, by countries that have marshalled broad national forces to assist the health sector. Among these countries are Columbia, Turkey, Nigeria, and Burkina Faso. Syria is now preparing to follow suit.

The conventional health sector approach to immunization is handicapped for two main reasons. First, it generally fails to secure the active personal commitment of the country's political leadership. Second, it fails to look outside the health sector for the economic and administrative resources needed for acceleration. Since the percentage of the national budget given to health in the average developing country is almost certain not to increase in the difficult economic circumstances of the foreseeable

future, and since the child populations of most countries will continue to increase rapidly, the chances of a conventional health sector solution are virtually nil.

This leads to a choice. One either accepts continued low coverage and gives up on the goal of UCI by 1990, or one turns towards the huge supplementary resources that exist outside the health system.

How can such resources be brought to bear to accelerate immunization? The countries mentioned earlier can each furnish many examples.

The most crucial outside resource -- the personal involvement of the top political leaders -- has been effectively utilized in all the countries mentioned. Colombian President Belisario Betancour involved himself directly in the tactics and promotion of his country's national immunization days, and President Kenan Evren personally vaccinated children to launch each round of Turkey's campaign. Equally strong personal leadership has been shown by presidents Babangida of Nigeria and Sankara of Burkina Faso. The commitment of all these leaders communicated itself immediately to their main functional ministries -- health, interior, and education -- which then passed urgent instructions to staff in the provinces, whose work would be closely monitored by provincial governors.

It was up to health and the other functional ministries to guarantee the "supply" site of these national immunization efforts -- to provide the vaccines, vaccination, vaccination stations, and organization. To the media, especially national television, fell the job of creating demand. Turkish television contributed $5 million of free prime time programming. Nigerian television also energetically took up the immunization theme. The radio network Caracol and the newspaper El Tiempo became synonymous with vaccination in Colombia. Each of these media set out to deliver a single message: get your child vaccinated.

That message needed reinforcement on the ground -- almost on a house-to-house basis. A coalition of imams, primary teachers, and muhtars (village heads)

carried this out alongside health workers in Turkey, guaranteeing that parents would turn out with their children. In Nigeria, Catholic priests, imams, and traditional chiefs addressed themselves to the task, with the energetic backing of state governors. Priests and policemen were among the main reinforcers in Colombia. Soldiers, village officials, and even truck drivers were involved on the ground in Burkina Faso's Vaccination Commando. Imams, teachers, and muhtars are expected to have major roles in Syria. Because each of these partners in health speaks from an angle that impinges differently on the awareness of the community, they can have a formidable multiplier effect.

Much support for immunization can also be provided by non-governmental organizations and individuals. Rotary International and individual Rotary clubs provided substantial funding and in-kind contributions in Nigeria and Turkey. Turkish companies provided five million child health passports and after-vaccine aspirins for all children. For each of the countries mentioned, celebrity support for immunization has come from actors, comedians, and star athletes.

The immunization successes in each of these countries demonstrate that broad national mobilization is indispensable for the control of the vaccine-preventable diseases. Further, it appears that political leaders, the media, teachers, religious and traditional leaders, service clubs, celebrities, and individual donors comprise a low-cost, elastic, permanently renewable resource base that stands ready to aid health ministries in maintaining immunization coverage.

PROSPECTS FOR UNIVERSAL IMMUNIZATION: STRATEGIES FOR ACHIEVEMENT

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Of the 15 million children who die each year under the age of 5, 97% are from developing countries. Many of these children die of diseases which are not lethal in the developing world. Three and one-half million die from childhood diseases for which there are effective vaccines and 4 million die from diarrheal diseases.

In spite of the immensity of the problem, progress is being made. Vaccine usage has trebled in the last 3 years. Immunization levels (third dose DTP) have increased from 10% to over 33%, and studies show that 60% of children are being reached with at least one dose of some immunization. Over the last 2-1/2 decades, infant mortality has gone down, life expectancy at birth is up, and birth rates are slowly decreasing.

The existing technology for global immunization is good, and even better technology will be available. The major barriers to global program now are social, political and managerial, rather than technical. Studies and evaluations in these field are needed to obviate and/or eliminate these barriers.

The political will on the part of developing countries is being generated to make immunizations universally available. However, continuing assistance from the developed world will be required over many years, probably in the range of 20%-30% of the cost of the total effort. Also, it is apparent from experience in the United States and other developed countries that maintenance efforts must be given highest priority.

Immunization programs are not only cost effective in their own right, they can serve as effective models for primary health care and the total public health effort in general. This is being demonstrated by the PAHO campaign to eliminate polio and the measles control program in the United States.