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REPORTS ON SOME ACTIVITIES RELATIVE TO MALARIA IMMUNITY AND VACCINATION RESEARCH

Period Covered: June 4 - June 20, 1979

Reports Prepared by: Peter G. Contacos, M.D., Ph.D..

Conducted by: International Health Programs Staff
American Public Health Association
and
Office of Health, DS/HEA-M
Agency for International Development

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McGhaney

UNITED STATES GOVERNMENT

memorandum

DATE: August 9, 1979

REPLY TO ATTN OF: PPC/E/S, Barbara Pillsbury
DS/HEA, David Dunlop/Irv Taylor

SUBJECT: Evaluation and Primary Health Care

TO: See Attached Distribution

Your participation and serious questioning at the first working session on evaluation and primary health care was very encouraging and indicates what appears to be widespread concern with the issues discussed. As jointly agreed upon at that session, we will meet again on August 15 from 10 to 12 in room 1207-NS. (Participants from outside the building may call ext. 21660 or 21680 for entrance.)

Several agenda items were suggested at the first meeting for future sessions. The purpose of this second session will be:

1. to propose a set of appropriate, realistic criteria that could be suggested to the Agency for assessing the impact and effectiveness of primary health care programs. (It was suggested at the first session, for example, that an "impact-effectiveness" continuum be developed. It was also suggested that, alternatively, a hierarchy of evaluation levels might be established and appropriate criteria developed for each);

2. to identify projects that could be examined as illustrative case studies; and

3. in conjunction with the above, to review the results of the efforts several of us have been involved in during the past couple weeks in attempting to identify and describe for the Agency leadership projects and programs that have had beneficial impacts.

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THIS REPORT COVERS THE FOLLOWING ACTIVITIES:

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I. WHO MEETING OF THE SCIENTIFIC WORKING GROUP OF THE IMMUNOLOGY OF MALARIA

Panama

June 4-6, 1979

Visiting Team: Peter G. Contacos, M.D., APHA
Edgar Smith, AID

1. Background:

This was the 3rd meeting of the Scientific Working Group on the Immunology of Malaria for the special programme for research and planni in tropical diseases (UNDP/World Bank/World Health Organization). The specific title was "Meeting on the Immunodiagnostic Aspects of Malaria." The meetings were held both at the Ministry of Health of Panama and at the Gorgas Memorial Laboratory, June 4 through 6, 1979. There were about 35 participants.

2. Findings:

The meetings were inaugurated by the Minister of Health, Dr. Jorge Medrano, with comments by Dr. T. Lepes (WHO) and Rafael Miranda Franco (WHO/PAHO).

Working papers were presented and discussed on Monday and Tuesday morning. The three major categories of the working papers were:

(1) Antigenic Analysis, which included papers on blood stages, tissue stages, sporozoites, and the potential use of hybridoma techniques in antigenic analysis;

(2) Immunodiagnostic Tests, which included papers on technical aspects, standardization, measurement of stage specific antibodies, detection of malaria-immune complexes, and antigen-specific and non-specific cellular immune responses; and

(3) Epidemiologic Applicability of Immunodiagnostic Tests for baseline data, epidemiologic surveillance, epidemiologic evaluation under conditions of disappearing malaria and, most importantly, prospects for development of test systems reflecting protective immunity.

The most exciting working papers, in my opinion, were those concerned with the application of hybridoma technology to malaria.

Hybridoma technology allows the production of pure monoclonal antibodies directed against individual antigens present in crude (unfractionated) antigenic mixtures. The technology involves incubating myeloma cell lines with spleen cells (Lymphoid antibody forming cells) from animals immunized with specific antigens in the presence of polyethylene glycol which induces fusion. The mixtures are then tested for the production of malaria antibody. If found positive, the malaria-specific hybridoma is cloned. The antibodies produced by each clone can then be used to identify and/or isolate single antigenic components from complex mixtures of malaria antigens. This technology may indeed represent a breakthrough for malarial antigenic characterization and/or analysis.

Tuesday afternoon and all day Wednesday were devoted to working group sessions and discussions for preparation of the report. There were five groups:

(1) Antigenic Analysis
(2) Technical Aspects of Immunodiagnostic Tests - Test System Development, Sensitivity & Specificity
(3) Technical Aspects of Immunodiagnostic Tests - Reproducibility, Standardization of Tests & Reagents
(4) Applications of Immunodiagnostic Tests - As Epidemiologic Tools
(5) Applications of Immunodiagnostic Tests - As Research Tools

Each working group was asked to consider the inventory (State of the Art), lacunae, needs, suggested approaches, and recommendations. I was assigned to the 5th working group, and we considered it difficult to apply the above guidelines to our assignment. However, we produced a factual but somewhat philosophic discussion for our assignment this was well received by the Secretariat, which decided to use it as the introduction of the report rather than the originally intended 5th item of the report.

Attached is the very rough draft of the report of the working group on the Immunology of Malaria. It is hoped that the WHO Secretariat will drastically edit this version before final release.

3. Conclusions:

The third scientific working group on the Immunology of Malaria identified the following high priority areas as crucial to the further development of immunodiagnostic techniques and thus to the study of immune mechanisms, responses to immunization and the monitoring of the immune status of populations naturally exposed to malaria infection:

... The mass production of antigenic material, its purification & fractionation.

... Identification, as well as structual and functional characterization of species and stage specific antigens and related antibodies and their production.

... Development and standardization of tests especially for the assessment of protective immunity, both humoral and cellular, and for epidemiologic purposes.

... Widespread introduction of the use of immunodiagnostic techniques in field epidemiology.

Contacts: See attached list of participants.

II. SITE VISIT TO GORGAS MEMORIAL LABORATORY, PANAMA - RELATIVE TO PROPOSAL: IN VITRO CULTIVATION OF MALARIA PARASITES

June 7, 1979

Visiting Team: Peter G. Contacos, M.D., APHA
Edgar Smith, AID

1. Background:

This project was originally proposed in 1978, but for a multiplicity of reasons was not formally submitted by the Gorgas Memorial Institute of Tropical and Preventive Medicine, Inc. to USAID until recently. Therefore, it was only recently approved.

Unfortunately, in the meantime, Dr. Timothy T. Palmer, the principal investigator, has been reassigned to the Naval Medical Research Institute in Bethesda, Maryland. He is to be replaced by Dr. Monte Bawden, who will strive to carry out the objectives of the paper.

The Gorgas Memorial Laboratory has been invloved with malaria research for many years, especially during the last decade when Dr. Marting D. Young was Director of the laboratory. Dr. Richard Rossan, who worked actively with Dr. Young, is still involved with Malaria research at the Laboratory. In addition, Dr. Lopez-Antunana of PAHO is working with Malarial Sero-Epidemiologic studies and *in vitro* drug sensitivity studies at the Gorgas Laboratory.

2. Findings:

Dr. Palmer, because of his impending departure (on or about 23 June), has discontinued the few cultures of Falciparum Malaria he had established using the Trager-Jensen Petri Dish method.

Review of the research proposal reveals it to be scientifically sound, feasible and certainly of value to the overall objectives of the USAID Malaria vaccine program. The description of the chronologic staging for the various aspects of the proposal (See C-4) is noteworthy.

The objectives of the project are 1) to isolate and cultivate geographically isolated strains of *Plasmodium Falciparum* and *P. vivax* and 2) to produce, *in vitro*, Gametocytes of *P. Falciparum* that are infective to mosquitoes. Parenthetically, Dr. Palmer's primary interests were to study the factors that would stimulate fertile gametocyte production *in vitro*, certainly important to any sporozoite-based vaccine research programs.

The facilities and resources of the Gorgas Memorial Laboratory are more than adequate. Space and equipment are available. The

Gorgas Laboratory maintains an excellent and (indeed) large primate colony (almost exclusively Aotus)under the immediate supervision of a veterinarian, as well as a well-equipped insectary. Staff members of the Gorgas are available for consultation and/or collaboration.

Technical help has not yet been obtained,probably because the project has been temporarily suspended.

3. Conclusions:

(1) The project can make a valuable contribution to the overall objectives of the USAID Malaria vaccine research program and relates well to the other projects of the network. It can establish geographically distinct strains of Falciparum Malaria from this hemisphere in in vitro cultivation. In turn, these strains can be provided as needed to other projects of the network as well as to other groups concerned with Malaria Immunology/vaccine research.

(2) The Gorgas Memorial Laboratory is an ideal facility for the study of the factors that stimulate Gametocyte production in vitro can be studied. The reasons are multiple: (1) naturally occurring vectors are available for colonization in the laboratory's insectary facilities; (2) an entomologist-insect geneticist is currently in charge of the Vector Biology Department and insectaries and is available for consultation and collaboration.

(3) There should be no further delays in getting this project going. One of the first steps towards this end is to hire a laboratory technician and to immediately have this individual trained in the Trager-Jensen in vitro culture methods which have been repeatedly shown to be the most reliable and/or productive culture methods.

Contacts: At the Gorgas Memorial Laboratory:

Dr. Timothy Trow Palmer
Dr. Richard Rossan
Col. Bryce Walton (Retired)
Dr. Lopez-Antunana
Dr. J. A. Najera (PAHO)

III. MEETING OF THE INTER-AGENCY COORDINATION COMMITTEE ON MALARIA IMMUNITY AND VACCINATION RESEARCH

June 18, 1979

Informal Observer: Peter G. Contacos, M.D.

Background:

The Inter-Agency Coordination Committee on Malaria Immunity and Vaccination Research was established in October, 1978 "to expedite the development of a malaria vaccine." The specific objectives of the committee are to 1) develop coordination and stimulate cooperation and collaboration; 2) avoid unnecessary duplication of effort; 3) promote desirable replication and confirmation of results; 4) avoid duplication of funding; 5) eliminate unnecessary expenditures; 6) review research priorities, and 7) prepare an annual compilation of research projects which were funded by each of the participating agencies.

Membership of the Committee includes: USAID, U.S. Army Medical Research and Development (WRAIR), U.S. Naval Medical Research Institute (NMRI), National Institute of Allergy and Infectious Diseases (NIAID), Center for Disease Control (CDC). World Health Organization and Pan American Health Organization (WHO and PAHO) representatives attend as fully participating informal observers.

Results:

This was the 3rd meeting of the Inter-Agency Coordination Committee on Malaria Immunity and Vaccination Research. Agenda items discussed:

1. Coordination of Project Funding:

The problems concerning funding of malaria immunity and vaccination research were discussed. The possibility and/or desirability for coordinating requests for research funding between agencies and organizations involved in such funding was discussed. It was felt that such coordination would preclude some of the problems which occur when malaria immunity and vaccination research projects suddenly lose grant support for a short period. This loss of funding does not allow for the necessary continuity of the overall objectives of malaria immunity and vaccination research. This year, at least one such case has occurred. This might result in in the loss of highly trained personnel and specialized facilities. We hope that this will not happen.

2. The possible reorganization of USAID was reviewed.

3. The formalization of "Letters of invitation" to various U.S. Government Agencies with interest in the area of malaria immunity and vaccination research. This was done directly between the agency representatives and Dr. James Erickson.

Conclusions:

The next meeting was scheduled for December 15, 1979.

Note: The afternoon was spent with Edgar Smith discussing future scheduling of my consulting contract.

IV. SITE VISIT TO WARNER-LAMBERT/PARKE-DAVIS PHARMACEUTICAL RESEARCH DIVISION, DETROIT, MICHIGAN

June 19-20, 1979

Visiting Team: Peter G. Contacos, M.D., APHA
James Erickson, Ph.D., AID

The first day was spent with Charles A. Miller, Director, Research Planning and Contracts; and Harold E. Machamer, Ph.D., Director, Biological Research and Development. The second day was spent with the investigators working on the two research contracts; Robert T. Brackett, Ph.D. Director, Clinical Immunology and Director of both projects, George C. Cole, Ph.D., Richard L. Jacobs, Ph.D., and Theodore Green, Ph.D.

Our discussions the first day were concerned with the subject of long term planning for attaining goals and/or objectives of research in general. Charles Miller showed and explained to us PERT and/or FLOW charting which the company has used to meet specific objectives in producing drugs for eventual marketing. We devoted the afternoon to applying similar methods in making rough-draft outline that could apply to the eventual objective of producing a malaria vaccine. He offered to have the Parke-Davis staff working on the two projects, informally prepare such charts insofar as it related to their work.

1. Review of Contract AID/ta-C-1258: The *In Vitro* Cultivation of Malaria Parasites in Cell Culture:

Background:

One of the main problems for the development of malaria vaccine is a source of antigen suitable for mass production. The recently reported Trager-Jensen methods for *in vitro* cultivation are certainly a significant advance toward mass production of malaria antigen. However, a vaccine prepared from cultures containing human blood elements presents serious concerns regarding safety. There is a formidable risk of contamination of the final vaccine product not only with adventitious viruses but also antigenic materials. Ultrapurification (fractionation/filtration) might remove such contaminants from the antigen. However, a tissue culture cell with a known and accepted record of freedom from contamination with human viruses would certainly offer a promising alternative as a malaria vaccine substrate for *in vitro* cultivation of parasites.

The only published evidence for active penetration and development of blood stages of mammalian malaria parasites was reported by Dr. C Speer. He presented evidence of the penetration and development of rodent malaria in cultures of mouse testicular tumor leydig cells and in mouse or hamster bone marrow stem cells.

The objective of this contract is to develop cell culture technics for the *in vitro* cultivation of the erythrocytic stages of the human malaria parasite, *Plasmodium Falciparum*.

Results:

Drs. Cole and Jacobs have repeatedly tried to confirm Dr. Speer's work on culturing rodent malaria in leydig cells without success, and have abandoned this culture system as unproductive. The research team then attempted to grow stages of rodent malaria (*P. Berghei*) in 15 tissue culture cell types, some of which were primary cell cultures. The results of these attempts were uniformly negative.

When the Trager-Jensen method for growing *P. Falciparum in vitro* became available, the team substituted this human malaria species for the rodent species. A dual culture (co-culture) system was devised which basically combined their tissue culture method with the Trager-Jensen method. The co-culture system is a dynamic complex system wherein merozoites are continuously being released from infected red blood cells while the tissue culture cells continue to multiply and the percent parasitemia in the red blood cells increases. Twenty-one tissue cell lines were evaluated. In none were falciparum malaria parasites observed to penetrate and develop to maturity. However, parasite association with culture cells was observed to a very low degree in several cell lines, foremost of them was the vero cell line.

The team's approach was then directed toward treating the tissue cells in an attempt to modify them in order to promote association of parasite with tissue cell with eventual interiorization of the parasites. This parasite-tissue cell association has been principally observed with the merozoite stage (the invasive "penetrating" blood stage). Without tissue cell modification, 1% of the vero cells in co-culture were found associated with falciparum merozoites or early ring stages. After treating vero cells with ovomucoid, a protein protease inhibitor, the team found that this association could be increased to as high as 30% of the tissue cells.

It is difficult by light microscopy to conclusively determine if any of the associated parasites have actually invaded the tissue cells or whether the association is external (adhesion). The team entered into a collaborative agreement with Dr. Thomas Seed, an electron microscopist, to help clarify this point. We were shown electron photomicrographs of vero cells with associated falciparum

merozoites. The merozoites in these early studies appear to be externally associated with the vero cells by electron scanning. Sections showed no internal parasites but strongly indicated an association (interaction) between the surface of some merozoites and the vero cell membrane. This might suggest that one of the problems is the merozoite's inability to penetrate in the short period of time it has to do such. We know that the ability of merozoites to penetrate red blood cells is indeed very short-lived.

The research team proposes to continue their efforts to alter the cellular functions of the vero cell to further promote interiorization and allow such to develop to maturity. This will be accomplished by 1) determining the site of association of parasite and tissue cell; 2) optimizing interiorization of parasites; and 3) stablizing the vero cell-associated parasite to enhance growth and maturation. Some of these are already in progress.

2. Review of Contract AID/ta-C-1440: Malaria Immunity and Vaccination.

Background:

The objective of this project is to develop a safe and effective merozoite vaccine against Plasmodium Falciparum.

The project is divided into 4 phases: (1) increasing the yield of merozoites by modifying to be in vitro culture methods to improve parasite (antigen) yield; (2) development of methods for harvesting and purifying merozoites; (3) preparation and immunologic testing of experimental malarial vaccines preferably in the Aotus monkey and emphasizing freedom of contamination with agents and/or antigenic substances (extraneous; and (4) production of an experimental vaccine for testing in Aotus monkey and eventually in man.

Results:

Dr. Green presented some of the more recent findings of the project:

Parasite Culture: Parasites are being grown in culture using three methods; the Trager-Jensen candle jar method, the flow-flask culture system, and the Siddiqui stationary culture flask method. Dr. Green feels that the above methods have now been perfected for near optimal performance. At O_2:CO_2 gas ratio of 2.5, PH 7.0 to 7.2 and 6% hematocrit for culture medium, they can routinely attain a 60% parasitemia. He also reported that by microtiter culturing, they have obtained seven clones of falciparum malaria by using a critical microdilution method; this could be valuable for later studies on frequency of antigenic variation of human malaria parasites.

Production of Antimalarial Antibodies in Rabbits and The In Vitro Parasite Inhibition Test: Rabbits were immunized with merozoites with or without adjuvants. Sera from these immunized rabbits gave fluorescent titers as high as 1:2560. However, in the in vitro inhibition test, these antisera at a concentration of 10 to 50% of the medium did not inhibit reinfection of red blood cells; i.e. they failed to demonstrate "protective antibody". All rabbit antisera possessed antibody against human erythrocytes (expected); these could be removed by adsorption. The most interesting result reported to us by Dr. Green, in my opinion, was that they were able to demonstrate in vitro parasite inhibition (protective antibody) using a serum sample from an Aotus monkey which spontaneously developed a "sterile immunity" to a falciparum infection, some nine months before the serum sample was obtained. I saw the results of the test and there can be no question that the serum did inhibit reinfection of red in culture. The in vitro inhibition test developed by this group for use in human malaria is indeed impressive and in my opinion offers a valuable research tool for application to malaria vaccine development. Parenthetically, I might mention that they have been able to substitute rabbit serum for human when performing this test.

Antigenic Characterization and Purification: The eventual goal is to identify and purify the protective antigens and to use them as the immunogen in the development of a malaria vaccine. A variety of antigenic preparations were studied for "fingerprinting" identification of parasite-derived antigens. Antigens were prepared from (1) soluble antigens from culture supernatants, (2) particulate antigens from culture supernatants, SDS buffer solubilized, (3) antigens from cultures treated with sorbitol, and (4) antigens from cultures lysed by free-thawing. All were fractionated by precipitating with varying saturations of ammonium sulfate. The resulting fractions were subjected to polyacrylamide gel electrophoresis. As a result, two candidate antigen preparations have been identified for intensive study.

One antigen fraction is derived from sorbitol lysed cultures and is characterized by a low molecular weight, fast moving band with a characteristic cup-shaped migrating pattern. The other antigen preparation is derived from SDS buffer solubilized merozoites and is characterized by antigens which move more slowly than the sorbitol derived antigens and which are found in the upper portion of the gel separation migrating as typical protein bands. It is interesting that both of these parasite-associated proteins are precipitated from solution at 50% ammonium sulfate saturation. Antibodies produced to these antigen preparations were found to give positive fluorescent antibody test against parasites. All of the preparations mentioned above were found to contain some degree of red cell contamination (expected).

Conclusions:

The positive results to date on the cultivation of malaria parasites in cell culture have been on the lean side. However, the observed association of parasites with chemically modified cells certainly serves to justify the studies proposed in the contract extension; namely, to (1) determine the site of association, (2) optimize interiorization of parasites, and (3) stabilize the vero cell - associated parasite and enhance the growth and maturation. If significant interiorization of the parasites can be attained by whatever method, then the studies to stabilize associated parasites will assume even more importance if development, indeed maturation, of parasites is to obtain. In my opinion, it is highly unlikely that parasite maturation will occur without a source of hemoglobin or a substitute for it.

The research being carried out in the Malaria Immunity and Vaccination Project is indeed important to the overall objectives of the USAID malaria vaccine program. I was particularly impressed with their progress to date with antigen collection, fractionation and characterization. It might be advantageous to also prepare the antigen using other proven methodologies such as isoelectrofocusing (flatbed, qualitative and/or preparative), etc. Antigenic fractionation and characterization has one of the highest priorities in research and development of an effective and safe vaccine. Emphasis should be directed along these lines of research. The *in vitro* parasite inhibition test developed by this research group appears to be an excellent method for evaluating candidate malaria vaccines; namely, their ability to stimulate the production of antibodies which will protect against infection.

ATTACHMENT I

DRAFT REPORT ON WHO WORKING GROUP

ON

IMMUNOLOGY OF MALARIA

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CONTROLS

INTRODUCTION

The immunologic research program of the SWG-IMMAL has as its goals the development of malaria vaccines and the improvement of diagnostic tests for epidemiologic and clinical evaluation and for use in assessing control efforts (disappearing malaria). Meeting these goals will require a major expansion of knowledge regarding the immune response of man to malaria infection and/or immunization. The research requirements can be broadly stated as: (1) A requirement for biochemical definition and characterization of the antigenic components of malaria parasites (2) A description of the humoral response to individual antigenic components in response to infection or immunization. A complete description should include the magnitude, duration, antibody class, and subclass, and should consider variables such as duration of immunological memory and responses to reinfection, reimmunization, ~~genetics,~~ etc. (3) A description of cell mediated immune response with respect to specific antigens (4) Effects of malaria on response to other antigens.

At the present time the first requirement i.e. biochemical characterization of antigenic components is the principal limiting factor in the research program. Major advances will depend in larger measure on progress in this area.

Many sophisticated techniques are presently available for detection and quantification of malaria antibodies and antigens. Technical details of these tests have been discussed below. The various techniques have a wide range of intrinsic precision and reliability and many under optimal circumstances can achieve ex-

treme sensitivity. The limiting factors in the application of these tests to problems of malaria immunity relate primarily to the ~~degree~~ level of purity of antigens and definitive knowledge of the composition of test antigens or the specificity of antisera or antibodies used in the tests.

Selection of test systems for use in studies of malaria immunology is principally a function of the research question to be answered. Given the wide assay of technical alternatives now available which have adequate intrinsic sensitivity, reliability and precision, the choice test systems and configuration is best determined by the experimental design and the biochemical characteristics of the test antigen.

The availability of characterized antigen and antibodies of adequate purity and stability are considered a prerequisite to investigations designed to define:

1. Those antigens which can be used to measure protective immunity in animals under experimental conditions and in human populations resident in endemic areas.

2. Those antibodies which can detect antigens in human sera which comprise evidence of concurrent or recent malarial infections.

3. The sequence of serological changes induced by vaccination with antigens selected specifically for the induction of protective immunity in animal models and ultimately in humans.

4. The occurrence of antigenic change in parasites maintained in continuous cultivation systems.

5. The occurrence and sequence of antigenic variation of plasmodia.

6. The identification of antigens specific to the various plasmodial species and stages

7. The antigens and antibodies associated with immunopathological processes induced by malaria.

ANTIGENIC ANALYSIS

Source of antigen

There are three sources of malarial antigen available (1) *in vitro* cultivated forms of *Plasmodium*, (2) parasite stages and soluble antigen isolated from humans and animal models and the mosquito vector, (3) cell free translation systems of mRNA derived from malaria parasites and DNA cloning techniques. A recent review by the NMRI/USAID/WHO Workshop on the Immunology of Malaria and by the second meeting of the Scientific Working Group for the Immunology of Malaria, described the detailed technical aspects of the preparation of each of these systems.

At present only one stage of the malaria parasite can be grown in continuous culture (ie the erythrocytic stage) and this is possible with only a few species of the parasite. There is an urgent need to scale up the capacity of the *P. falciparum* culture system as well as to develop the *in vitro* culture of the other human forms and animal forms of malaria most useful as experimental models. It is stressed that although at present the exoerythrocytic and mosquito stages of malaria can be handled briefly *in vitro*, prolonged cultivation of large amounts of parasite material is still required for these stages.

The source of antigens should be defined with respect to malarial species, and, as far as possible, genetic variation of a given malarial species should be monitored by the use of the available genetic markers such as isozymes. This will facilitate comparison of the antigens identified by different laboratories. With

Best Available Document

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human sources of antigen, the geographical location and clinical status of the host should be defined. The establishment of reference banks of parasite material is of major importance. P. knowlesi in the rhesus monkey is at present the only model system which exhibits serologically defined antigenic variation and can readily be used to study this phenomenon. It should also be possible to clone the erythrocytic stages of P. falciparum in vitro culture. Cloned forms of the parasite would greatly facilitate studies on antigenic variation. It is essential that antigenic activity is retained during transport of material from source of origin to the laboratory for antigenic analysis. Furthermore, for studies with living parasites it would be important to preserve the viability of the heterogenous natural population during transport. Although methods for cryopreservation of malaria parasites are available, they do not allow the preservation of all parasite forms. For definitive experiments with human malaria it will therefore be required to improve cryopreservation techniques and perform some experiments in the field.

Purification of specific life-cycle stages of malaria parasites

In order to identify and fractionate malarial antigens, purified stages of the parasite are required. For some studies it will also be necessary to prepare parasites free from contaminating host material. Methods for the purification of intact and viable ring forms, trophozoites, schizonts, sporozoites and gametocytes should be standardized and adopted to large-scale preparation. As the important antigens may be labile during experimental manipula-

tion their identification may be critically dependent on the retention of parasite viability.

Separation of host membranes and parasites

It is important to be able to distinguish parasite membrane antigens and host red cell membrane components, and also to identify ~~internal~~ intraerythrocytic parasite antigens. Surface labelling of infected red cells has been used to identify surface membrane molecules and to monitor the purity of red blood cell membrane and parasite components during the preparation of membranes from schizont-infected red blood cells. Assays of erythrocyte membrane enzymes may also be useful in monitoring the efficiency and purity of sub-cellular fractionation.

Several approaches are available for releasing the parasite from the red blood cell, some of which are suitable for subsequent antigenic analysis. Physical methods including N_2 cavitation, flow-through sonication, French Pressure cell and the Stansted Cell Disruptor will allow parasite purification with minimum loss of antigenic activity. Immune lysis may also be useful for releasing parasites from infected cells. Techniques ~~need~~ exist to be developed for the fractionation of the external red blood cell membrane, the parasitophorous vauolar membrane and parasite surface membranes.

The parasite stages at which malarial antigens are expressed on the erythrocyte surface should be examined. The nature of these antigens, i.e. host modified red blood cell components or parasite material inserted into the red blood cell membrane, should be established, possibly by the use of metabolic labelling. It will

also be necessary to improve the methods of purification of sporozoite stages from mosquito-derived contaminants.

Chemical and Immunochemical analysis of antigens

Chemical and immunochemical analysis can be carried out on internal and membrane components. Membrane antigens may be an important group of antigens relevant to protective immunity, and their characterization without loss of antigenic activity will require solubilization of the material to be analyzed. Different ionic and non ionic detergents could be used for solubilization and compared in their efficiency. For analytical purposes, metabolic labelling of the parasite, ~~particularly of blood forms~~, and external labelling of membrane proteins of the parasites could help distinguish parasite and host material.

Different analytical methods are available to characterize the antigens isolated.

1. Physical and biochemical methods, for example: gel filtrations, SDS-polyacrylamide-gel ~~analysis~~ electrophoresis, isoelectric focussing, the recently developed two dimensional gel analysis techniques which allow mapping of proteins on the basis of their isoelectric point and molecular weight.

2. Immunochemical methods: Defined sera, such as those described above can be used for antigen identification by

a) affinity chromatography,

b) double immunoprecipitation followed by SDS gel analysis or two-dimensional gel analysis and

c) crossed immunoelectrophoretic techniques.

The analytic techniques for protein separation will allow the mapping of proteins characteristics of particular stages of the parasite and subcellular fraction for example. Crossed immunoelectrophoretic techniques will provide a means of comparing complex antigen mixtures and antibody specificities in polyvalent sera.

Functional characterization of antigens

Antigens obtained by methods described above would be analysed for functional and immunological activity in the following ways:

a) vaccination and challenge

b) production of antibody *in vivo* against a specific antigen and subsequent testing of the antibody for antiparasitic activity. Antiparasitic activity could be tested *in vitro* by methods such as inhibition of merozoite invasion, inhibition of gamete fertilization, and passive transfer and testing for protection *in vivo*. ~~We~~ It is recommended that new *in vitro* tests should be developed to assay ~~antibody which may be relevant for~~ protective immunity.

Isolated antigens from antigenic variants of *P. knowlesi* and different isolates of human malaria parasites should be compared in order to identify possible variant specific molecules.

Purified antigens may also be used in diagnosis e.g. in tests to identify antibodies specific for parasite species and stage of parasite and in tests for diagnosis of recent or ongoing infections. ~~See recommendations by Group 3.~~

Hybridomas

This meeting has seen the introduction of hybridoma technology into parasitology. This technique allows the isolation and

immortalization of an antibody-forming-cell secreting a specific antibody of interest by transforming it into a tumour cell. Such transformation is achieved by fusing immunized lymphoid cells containing the antibody-forming-cell with an established myeloma cell line. At this meeting, several groups have reported production of hybrid cell lines secreting monoclonal antibodies specific for various types of parasite antigens in human, rodent and avian malarias. We make two recommendations for future involvement of hybridoma technology: (1) to improve immunization ~~programs and~~ and screening procedures in order to obtain biologically useful hybridoma antibodies; (2) to establish a system for distributing hybridoma antibodies amongst the appropriate research laboratories to ensure their maximum utilization.

The potential uses of monoclonal antibodies in malarial research are manifold: (1) they allow isolation of pure antigens from crude unfractionated antigenic mixtures; (2) they can be used for generation of anti-idiotypic antibodies which may prove useful substitutes for parasite antigens difficult to obtain in adequate quantities; (3) they can be used as exquisitely specific probes for monitoring antigenic variation, an application of monoclonal antibodies which has already been demonstrated in the case of influenza virus; (4) they should aid malaria typing programs. One particular advantage of the hybridoma technology relates to reagent standardization, as any hybridoma antibody of interest will be available in large amounts and could be readily distributed worldwide.

Standardization of test systems

Methods

A wide range of methods is available for the identification and assay of microbial antigens and specific antibodies. They may be classified into simple two component tests, usually carried out in gels, and more complex systems involving three or more components.

Amongst two component systems are immuno-double diffusion (IDD) and single-radial-difussion (SRD) tests. IDD tests with specific immune sera are valuable tools for examination of the identity of antigens or antibodies, they are not however sensitive or precise methods for assay. SRD techniques employing gels incorporating specific immune sera may be used for the precise assay of antigens. In these techniques it may be necessary to treat the antigen to be assayed with suitable detergents (eg sarkosyl or mulgofen) to release antigen molecules able to diffuse. The use of SRD tests in which the zone of reaction is demonstrated by protein staining allows concentrations of antigen as low as 1 to 10 µg/ml to be detected. The sensitivity of SRD test may be considerably enhanced by the use of radioactively ~~Lobleed~~ labelled antibody or antigen and demonstrating reaction zones by autoradiography. SRD tests may also be used for the accurate assay of antibody. In this case appropriate antigens at defined concentrations are incorporated in gels and sera for assay are added to wells in the gel. With stable antigens transportable immunoplates may be prepared which can be shipped from a central laboratory to areas where studies are to be made. When used in conjunction with standard calibrated reagents (sera or antigens) SRD methods provide reliable and precise assays with

coefficients of variability which are generally less than 10%.

Many multi-component systems for assay of antibodies or antigen are available. These include microbial infectivity assays and infectivity or growth inhibition by antibody. The latter tests may be of particular value in indicating antibody levels consistent with protection in the host. Hemagglutination and hemoagglutination inhibition tests have been widely used with agents (eg viruses) which exhibit hemagglutination. Complement fixation tests have been widely used in the demonstration of diagnostic antibody responses or the recognition of antigens. For viruses, single-radial haemolysis test in gels, which depend on the ability of specific antibody to virus surface antigens to cause the complement mediated lysis of erythrocytes coated with viral antigen, have been used widely for the assay of specific antiviral antibody. These tests have been of particular value for extensive seroepidemiological studies of viruses (eg influenza and rubella) since they do not require serial dilution of test sera, haemolysis zone size being a direct measure of antibody concentration. Antigens which do not naturally adhere to erythrocytes may be attached by suitable coupling agents ($CrCl_3$, tannic acid).

Other useful complex systems include the many variations of radio-immunoassay and ELISA test which are available for the assay of antibody and antigens. These tests are often highly sensitive but require careful control for specificity.

In general multi-component systems have a much higher degree of variability in response and in specificity than two component

tests and the use of appropriate controls for specifity and sensitivity is emphasized. However, when calibrated standard reagents are incorporated in the tests and the characteristics of the dose response are fully understood some systems have been used reliably for antibody or antigen assays.

Standards

In order to establish a sound basis for quantification of antibody and antigen, such as might be used to establish control of vaccination programs against malaria, standard reference antisera and antigens of accurately known concentration would be useful. These should be established as international WHO references by appropriate collaborative studies.

1. Antibodies. It is desirable to have highly specific reference antisera which could be used for diagnosis, seroepidemiology and control of vaccination. These would include monoclonal antibodies and might be of four types:

a. Malaria group-specific

b. Malaria species-specific

c. Malaria variant-specific

d. Malaria stage-specific

2. Antigens. At this stage the technology in characterization of malaria antigens is in a primitive state as compared to that of virus antigens. The present unsatisfactory position will only be resolved when antigens can be provided on a reasonable scale and contemporary immunochemical methods applied to them.

Logistics

Workshops. There are already indications that the terminology of malaria parasite antigens is becoming confused. For example, different numbering systems for P. knowlesi antigens are used. By analogy with other fields (eg human HL-A), workshops should be held at which the investigators would meet, exchange reagents and agree on nomenclature. Working reagents would be kept in a reference laboratory and made available in small amounts.

Serum banks. The availability of defined antigens would be complemented by having antisera reacting with them. Reference sera, especially monoclonal antibodies of different specificity, should be kept in a bank and made available to interested investigators.

Training courses. When malaria parasite antigens and antibodies become available and immunological techniques are more widely used in research and in control of malaria, training courses in method, and reagents would be useful. These would follow the courses in parasite cultivation which have been held in different parts of the world.

Collaborative assays. In clinical immunology, collaborative assays have been performed in which serum samples are distributed to different laboratories for measurements by several techniques using various classes of immunoglobulins, immune complexes ~~and so forth~~. Similar collaborative assays of malaria antigens, antibodies and immune-complexes would be useful.

Central provision of antigens. Several laboratories have a need for antigens prepared from different stages of the life cycle

of malaria parasites. This would include both human and a limited number of lower animal parasites. At present, efforts to produce antigens are being duplicated in several laboratories. Difficulties of antigen production are a major factor limiting the entry of persons with complementary skills (eg biochemistry or cell biology) into the field, as well as the extension of research from one host-parasite system to others. It would be useful if production of relatively large amounts of antigen by contract could be arranged. The distribution of antigens would be controlled ~~by the IMM/MAL~~ under the auspices of the Steering Committee. of the SWG-IMMAL.

ANTIGENS ~~Serology~~ TECHNICAL ASPECTS OF TEST SYSTEMS

The antigen preparations used in immunodiagnostic test should ideally contain those antigens which stimulate immune responses in natural infections in man. ~~In~~ This discussion ~~we will~~ is restricted ~~our~~ to a consideration ~~of~~ the detection of humoral responses. For this purpose ~~we will deal with~~ antibody responses against blood stages and sporozoites is discussed.

Sources of antigenic material from human malaria parasites

1. Blood stages

There has been a severe shortage of material suitable for serological tests. The availables sources of each human malarial parasite are listed below.

P. falciparum

In the past infected erythrocytes were obtained from peripheral blood or better still from placentae of naturally infected people in endemic areas. Peripheral blood contains few or no mature schizonts and so for use in serological tests short term culture of this blood ~~is~~ has been essential to produce the necessary mature stages of the parasite. In endemic areas this source of antigen is probably still adequate for indirect immunofluorescence, but not for those tests which require a soluble antigen. Infected placentae were a good source of schizonts but the introduction of malaria prophylaxis during pregnancy has virtually eliminated this material.

The discovery that Aotus monkeys will support high P. falciparum parasitaemia provided an alternative. However, these

animals are now scarse and expensive, factors which severely restrict their use for malarial antigen production. Fortunately the recent advent of continuous cultivation *in vitro* of *P. falciparum* provides ~~gives us~~ a potentially unlimited supply of antigen. To date technical aspects of cultivation and its cost have somewhat limited the amount of antigenic material produced especially in those endemic test areas where the tests are needed.

P. malariae, *P. ovale* and *P. vivax* infected erythrocytes can occasionally be obtained in endemic areas, from naturally infected people. However, the very low density of parasites has restricted the use of this material for antigen, for indirect immunofluorescence.

P. malariae and *P. vivax* (but not *P. ovale*) have been adopted to *Aotus* monkeys, and can provide antigen for indirect immunofluorescence. The density of parasitaemia is lower than *P. falciparum* but can still be used, especially in the case of *P. vivax*, to provide soluble antigen. The limited availability of these monkeys again means that this is a very restricted source of antigen. This is even more serious as the parasite cannot yet be grown in continuous *in vitro* culture.

Sporozoites

Tests for antibodies to sporozoites could well become a valuable tools for monitoring transmission. Outside endemic areas there are virtually no practical means of providing this material (because of unavailability of fertile gametocytes).

Even in endemic areas it is impracticable to produce infected

mosquitoes in mass.

Technical aspects of immunoassay

Antibodies

High quality antisera or antibodies are of fundamental importance for detection and purification of specific malaria associated antigens. At the present time certain antisera from humans and monkeys can be used for detection of malaria antigens. These antisera are extremely useful but are highly variable in terms of antibody specificity, titre and degree of contamination with undesirable antibodies and in addition, are of limited availability. Sera from animals such as rabbits and sheep present similar, but more severe types of problems. In any case, continued production of large amounts of antisera using conventional immunization procedures requires substantial amounts of antigen which, as discussed in the previous section, is difficult to obtain. In order to obtain highly specific antibodies in amounts suitable for international distribution as standardized immunodiagnostic reagents, it is recommended that cell fusion technology be used.

Test systems

1. Inventory and limitations of presently used methods.

Indirect immunofluorescence on ~~dried~~ entire parasites is used extensively for detection of anti-malarial antibodies. The limitations of present technique are (1) the requirements for special equipment, reagents and skilled personnel (2) the lack of automation and time-comsumption for large scale studies. In addition the analysis of the immune response to distinct antigens would not

be easily achieved.

2. Needs

For epidemiological studies, there is a need for immunoassays allowing for a better definition of the immune response to antigens relevant to recent infection. Therefore a particular effort should be made to develop test systems in which defined antigens such as surface antigens would be used rather than dead parasites or whole parasite extracts for the detection of antibodies or for analysis of cell-mediated immunity. Similarly there is a need for tests to be developed to detect parasite antigens in very low amounts, as an index of active infection or very recent infection. Since there is a frequent coexistence of antigen release and of corresponding antibodies, immune complexes may be formed. Their detection should be considered in relation to some immunopathological manifestations of malaria.

The use of these tests will obviously require their feasibility to be tested in tropical conditions.

3. Approaches to fulfill the needs

A large variety of new serological methods have been developed in the last decade for use in microbiology, clinical immunology or endocrinology. Many of these methods which can reach a high degree of sensitivity would be applicable to immunodiagnosis of malaria, provided the proper antigens or antibodies would be available.

3.1 Assays for antibodies

Among the presently available immunoassays for antibodies the following methods seem applicable to malaria as a comple-

ment to the regular immunofluorescence method.

a) solid phase or fluid phase radioimmunoassays (RIA)

b) Enzyme linked immunoassays (ELISA)

c) Erythrocyte aggregation: (solid phase aggregation tests, passive hemagglutination)

d) Various immunodiffusion assays

e) Solid phase fluorometry with fluorescent markers (SAFA)

f) Change in water condensation pattern on thin layer plates (TIA)

Other methods may also be considered for particular studies, such as counter immunoelectrophoresis or immunofluorescence on living parasites.

3.2 Assays for antigens

The detection of soluble or particulate parasite antigens can be done on blood or on serum samples. The methods available are usually the inhibition of the antibody binding assays listed under 3.1. However, adoption of these tests for the direct measurement of antigen may also be achieved. The practicability of this approach has been clearly demonstrated in other infectious diseases such as in Hepatitis B.

3.3 Assays for immune complexes

A number of methods have been recently devised to detect immune complexes through the measurement of the biological reactivity of these complexes with complement, cellular receptors (Fc or C3) or anti-globulines (see review WHO Technical Report

Series). In addition, antigen specific methods can be easily developed to detect the proportion of one given antigen appearing free or bound to antibody molecules. Obviously the latter approach requires monospecific antibodies. The obvious advantage of direct measurement of antigen-antibody interactions (homogenous immunoassays) must be emphasized.

Tests for Cell-Mediated Immunity

Proliferation of peripheral blood cells. Mononuclear cells prepared by Ficoll-Paque or Percoll methods are incubated with soluble or particulate antigen preparations, and increased DNA synthesis measured. Complete titrations of all parameters, including antigen concentration, cell density, kinetics and nutritional requirements eg. serum, must be performed.

Production of mediators

Tests for MIF production by human lymphocytes are difficult to perform reproducibly. Possibly one of the simplest mediators to assay and most informative is interferon. Again complete titrations of all parameters must be undertaken.

Generation of Cytotoxic cells. In other systems, exposure of peripheral blood cells from immune donors to specific antigens results in production of interferon and consequent activation of non-specific cytotoxic cell activity. This can be studied using human effector cells from peripheral blood and human target cells (eg K562 erythroblastic cell line). The possible generation of cytolytic T lymphocytes in malaria has not been extensively tested. From what is known from other systems, secondary *in vitro* stimula-

tion is often necessary for generation of effective killing activity. One difficulty in this system is that T lymphocytes frequently recognize microbial or other cell surface antigens associated with histocompatibility antigens. Human and monkey erythrocytes lack the major histocompatibility antigens and consequently may be inappropriate targets for T cell-mediated cytolytic activity.

Other effector cells. Possible alternative effector systems include antibody dependant cell mediated cytotoxicity and activated macrophages which should be tested after primary and secondary stimulations.

Present status of immunodiagnostic tests as epidemiological tools.

Almost 20 years ago it was shown that the antibodies produced in response to infection with malaria could be detected. In subsequent years many studies were done in different parts of the world to validate different test procedures such as the indirect fluorescent antibody (IFA), indirect haemagglutination test, complement fixation and gel diffusion tests; of them, the most useful for epidemiological studies was found to be the IFA test, in terms of sensitivity, specifity and reproducibility, although, as pointed out elsewhere, it is by no means the ideal test. This test has, therefore, been the one most widely used for a variety of studies which include the following: 1) as a supplement to standard malariometric techniques of splenometric and parasitological surveys to give more accurate information on the endemicity of malaria. Such studies have been widely applied and have been done in tropical America, Africa, Asia, the Pacific, and in Mediterranean countries, in different population groups exposed to different amounts of malaria. It is particularly at the lower levels of transmission that serology has given useful additional information, particularly on the period prevalence of malaria 2) serological surveys have been used to confirm the success of malaria eradication programmes notably in Tunisia, Mauritius and Greece 3) by contrast, they have been used, as an adjunct to parasitology, to detect the return of malaria into countries in which eradication was thought to have been achieved, for example in Grenada. In Tunisia serology was useful in detecting residual foci of P. vivax malaria which could have been missed by using only standard blood film examination.

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4) serology has been used to measure the effect of chemotherapy on immune status. This is a field in which its use could be much extended. 5) it has been used as a surveillance tool for detecting possible carriers of malaria in immigrant groups as in Costa Rica, 6) in certain clinical conditions serology has been useful for the retrospective diagnosis of malaria.

Antigen

A dependable antigen source is an essential prerequisit for malaria immunodiagnostic work. This material should preferably be homologous with the *Plasmodium* species.

For sero-epidemiological studies, at the present moment, the IFA test appears to have the widest application and the antigen could be obtained from three sources:

a) *P. falciparum*: from the continuous *in vitro* culture

b) *P. vivax*: from infected monkeys

c) *P. vivax* and *P. malariae*: from heavily parasitized human beings in endemic areas following the "in vitro" maturation of trophozoites.

Aotus to which human malarias have been best adapted, are difficult to maintain in captivity. Many major laboratories were unable to replenish their colonies in view of the present export restrictions. Therefore part of the production of antigens in these primates should be carried out in countries where the monkeys area indigenous.

Models

For the interpretation of results there exist epidemiological

models which try to draw conclusions about the amount or the variations of transmission from the different variables that can be measured by serological surveys.

So far sero-epidemiological models have used percentage of positivity and age specific positivity as a basis for descriptions of endemicity or past history of malaria transmission.

Appart from applicability to individual diagnosis or follow-up in longitudinal studies, variations in titers have not been found to be useful as a main epidemiological parameter.

Descriptive models include:

a) malaria serological profiles, i.e. age specific distribution of sero-positivity, providing general picture of malaria endemicity.

b) estimation of period average infection rates such as the models developed by Carpentier, Draper and Voller, Doensburg and Van Drutten.

c) a new one which is being developed in an attempt of interpolating continuous functions between estimated infection rates at central points of age groups.

Future needs

Although there are a number of useful serological tests available to detect circulating antibodies, no single technique is available that would differentiate a past infection from a present infection, an acute infection from a chronic infection or a primary infection from a relapse recondescence. Positive serological tests indicate the experience of an individual with malaria. ~~The presence~~

of IgM, however, may indicate relatively recent malaria experience.

Serological cross reactions are common between different human species and therefore, individual differential diagnosis of various species is difficult.

In the individual there is no direct correlation between severity of malaria and antibody levels nor between peripheral parasitemia and antibody titre levels. Furthermore antibody levels do not reflect the degree of clinical protection in an individual subject.

Malaria serological techniques are often demanding in equipment and material, time consuming and laborious; only a few laboratories do malaria serological tests routinely.

An almost universal problem is the supply of adequate quantities of antigen of the required quality. The value of various asexual blood satages as antigens is not clearly established, although it is known that mature schizonts give the relatively strongest reactions in IFA tests. It is hoped that continuous in vitro culture of malaria parasites will become a suitable source of antigen.

Other reagents like conjugates are expensive and difficult to obtain in large quantities. Lots obtained at different times from the same source or those obtained from different sources vary in their quality and each lot needs calibration.

A major constraint are variations in quality and characteristics of the antigens.

Considerations for field implementation

Despite the fact that some serological techniques have been developed nearly twenty years ago, and the validity of some, like IFAT, demonstrated for epidemiological purposes, there are ~~less~~

only a limited number of

~~than 15~~ laboratories around the world utilizing these techniques for epidemiological purposes. Admittedly, techniques available have certain weaknesses and are laborious; moreover it is almost impossible to standardize all the biological reagents. Nevertheless the use of serological techniques for epidemiological purposes is recommended.

The recent resurgence of malaria in the world has prompted numerous countries to reorientate their malaria control/eradication programmes. In view of the present epidemiological situation it has been postulated that malaria affected countries should undertake practically simultaneously the following four types of activities:

a) control of epidemics and prevention of further spreading

b) development of longtime malaria control/eradication programmes

c) training

d) research

In view of technical problems encountered and ecological or intervention-related changes leading to ~~changes~~ modifications in the epidemiological potential of malaria in many affected countries, national programmes must be reoriented towards an epidemiological approach in the selection of control methodology. This approach essentially requires thorough epidemiological investigation and analysis. ~~In countries where malaria control/eradication programmes have been carried out for nearly 20 years, the immune status of the population has been altered; this is an additional reason for using serology in epidemiological studies.~~ based on the use of appropriate techniques for the collection of essential data, including the use of immunodiagnostic methods wherever required.

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In order to realize the reorientation of the malaria programmes, research should be included as an integral part of every national control/eradication servies programme. This needs to be secured through specialized training and in order to promote it, young scientists interested in this field should be supported through apprentieship training as to enable them to acquire the technique. These activities could be carried out either in well established laboratories or on the spot in endemic countries through consultants. The second step would be to secure the necessary equipment and the services of visiting consultants who would assist the newly trained in gaining the necessary experience. At the same time a few laboratories could be appointed as collaborative laboratories, ready to help with advice, reference material exchange of research workers until the newly established laboratories have become totally independant.

The analysis of data obtained would require consultation with epidemiologists and matehmaticians, who should be part of the epidemiological teams. This should facilitate the appropriate analysis and interpretation of data obtained.

The preparation of a detailed plan for the implementation of sero-epidemiological studies on malaria will require additional consultation, which WHO should take up in consultation with interested scientists. At the same time it is necessary to give privide more information on the results of past sero-epidemiological studies, particularly to malariologists responsable for national malaria control/eradication programmes, and to include the subject of malaria sero-epidemiology in the curricula of training

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courses for malariologists and epidemiologists.

Inasmuch as seroepidemiological studies are urgently required for malaria affected countries carrying out malaria control/eradication programmes, such studies will also have to be undertaken in countries where no organized programmes exist for the time being. This applies particularly to areas in which major sections of the population are being subjected to chemoprophylaxis or other forms of mass drug administration.

RECOMMENDATIONS

The following areas were identified as crucial to the further development of immunodiagnostic techniques and thus to the ~~further development of~~ study of immune mechanisms, responses to immunization and the monitoring of the immune states of populations naturally exposed to malaria infection:

- The mass production of antigenic material, its purification and fractionation.
- Identification, as well as structural and functional characterization of ~~pure~~ species and stage specific antigens and related antibodies and their production.
- Development ~~of~~ and standardization of tests especially for the assessment of protective immunity, both humoral and cellular, and for epidemiological purposes.
- Wide spread introduction of the use of immunodiagnostic techniques in field epidemiology.

These areas of high priority are reflected in the following detailed recommendations.

Antigenic Analysis

1. ~~Increase~~ The production of antigen ~~by~~ should be increased by

   a) mass production of parasite material from *in vitro* cultures.

   b) production of parasites from *in vivo* animal models including the development of alternative primate models for human malarial parasites.

   c) production of antigens using recombinant DNA technology.

2. Antigenic analysis of cultured parasites compared to those isolated from parasites of animal models.

3. Characterization of parasites ~~variants~~ using genetic enzyme markers and cloning techniques.

4. Establishment of reference banks of antigens.

5. Improvement and standardization of cryopreservation technicques.

6. Standardization of techniques for purification of different stages of the parasite, for antigen identification and for separation of parasites from host materials.

7. Developments of techniques for fractionation of membrane antigens e.g. red cell membrane, parasite membrane and internal membranes (parasitophorous vacuole).

8. Determination of time course of appearance of new antigens on red cell membrane.

9. ~~Antigen~~ Characterization and purification of antigens by

   a) Optimization of techniques to solubilize membrane antigens.

   b) Physicochemical methods

   c) Immunochemical methods

10. Development of assays for the biological activity of antigens

a) By immunization and testing antibodies for anti-parasitic activities *in vivo* and *in vitro.*

b) Development of new *in vitro* tests reflecting protective immunity.

11. The further development in application of hybridoma technology to produce highly specific antibodies.

a) For the ~~production~~ isolation of antigens for the use in immunization studies

b) For the production of purified antigens of diagnostic value.

12. Establishment of an antibody bank with the appropriate distribution systems.

DEVELOPMENT OF TEST SYSTEMS

1. Analysis by two-dimentional gel analysis of total protein gene product.

2. Development of test systems using defined antigens, for example surface antigens, instead of dead parasites and parasites extracts.

3. Application of other methods e.g. counter current immuno-electrophoresis for immunofluorescence in parasites.

4. Development of assays for immune complexes by measurement of biological reactivities with complement, Fc recept~~ar~~ors, and anti-globulines or by specific antigen antibody binding assays.

5. Simple serological techniques for used in epidemiological assessment and monitoring.

6. Development of assays for therapeutic of malaria

STANDARDIZATION OF TEST SYSTEMS

1. Production and standardization of both reference antigens and antisera.

2. Organization of workshops to review technical aspects and the development of standard nomenclature.

3. The development of collaborative assays of malaria antigens, antibodies and the immune complexes.

4. Production of antigen and the contract with central laboratories, the distribution of which is under the auspices of the Steering Committee of the SWG-IMMAL.

EPIDEMIOLOGICAL APPLICATION OF TESTS

1. The development of tests for:

   a) To measure protective immunity.

   b) To distinguish recent infections from past infection.

   c) To determine current, low grade infections.

   d) To distinguish a primary infection from a relapse.

   e) to measure antigenic memory

IMPLEMENTATION FOR FIELD USE

1. Training and support for the establishment of serological laboratories in endemic areas, connected or within malaria control/eradication programmes, for carrying out seroepidemiological studies in a wide variety of conditions.

2. Training should be continued by the maintenance of linkages of the newly established laboratories with well recognized ones for sharing expertise and needs and for maintaining quality control.

3. Support for seroepidemiological studies aimed at a better interpretation of serological surveys.

4. Support for epidemiological studies needed for the validation of serological tests which may offer important advantages in field applicability.

ATTACHMENT II

SPECIAL PROGRAMME FOR RESEARCH AND
TRAINING IN TROPICAL DISEASES

THIRD SCIENTIFIC WORKING GROUP ON THE
IMMUNOLOGY OF MALARIA

MEETING ON THE IMMUNODIAGNOSTIC ASPECTS OF MALARIA

Gorgas Memorial Hospital, Panama, 4-6 June 1979

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