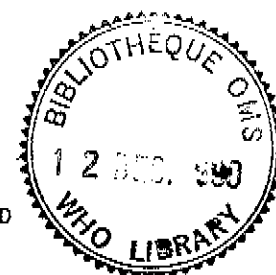




UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR
 RESEARCH AND TRAINING IN TROPICAL DISEASES

Geneva, 26-30 September 1988



SCIENTIFIC WORKING GROUPS ON IMMUNOLOGY OF MALARIA AND
 ON APPLIED FIELD RESEARCH IN MALARIA

IMMUNOLOGICAL ASPECTS OF MALARIA EPIDEMIOLOGY

CONTENTS

	<u>Page</u>
INTRODUCTION	2
1. WORKING GROUP 1. DIVERSITY OF HUMAN MALARIA PARASITES AND OF THE IMMUNE RESPONSE TO THEM	2
1.1 General Recommendations	3
1.2 Stage-specific Recommendations	4
2. WORKING GROUP 2. THE DEVELOPMENT OF THE IMMUNE RESPONSE TO MALARIA PARASITES AND ITS CORRELATION WITH PROTECTION	7
2.1 Indicators of the Immune Response whose Possible Association with Protection should be Investigated	7
2.2 Clinical Indicators of Immunity to Malaria	9
2.3 Study Design for Investigation of the Relationship Between Immunological Measurements and Protection Against Malaria	9
3. WORKING GROUP 3. NATURAL PATTERNS OF INOCULATION, THEIR GENERATION AND THEIR CONSEQUENCES	12
3.1 The Infectious Reservoir of Human Malaria	12
3.2 Sporogonic Development in Relation to Inoculum Size	13
3.3 Time/Space/Intensity Distribution of Entomological and Parasitological Inoculations	13
3.4 The Relationship Between Sporozoite Load and Clinical Outcome of Infection	14
3.5 Mixed Infections	14
4. WORKING GROUP 4. USE OF IMMUNOLOGICAL TESTS FOR EPIDEMIOLOGICAL CHARACTERIZATION AND CLASSIFICATION OF MALARIA SITUATIONS	16
5. LIST OF WORKING PAPERS	17
6. PARTICIPANTS	18
ANNEX SELECTED RECENT REFERENCES	22

This report contains the collective views of an International group of experts convened by the UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR RESEARCH AND TRAINING IN TROPICAL DISEASES (TDR). It does not necessarily reflect the views of TDR/WHO. In the interests of rapid communication it has been submitted to only minimal editorial revision. Moreover, any geographical designations used in the report do not imply the expression of any opinion whatsoever on the part of TDR or WHO concerning the legal status of any country, territory, city or area or of its authorities concerning the delimitation of its frontiers or boundaries.

Ce rapport exprime les vues collectives d'un groupe international d'experts réuni par le PROGRAMME SPECIAL PNUD/BANQUE MONDIALE/OMS DE RECHERCHE ET DE FORMATION CONCERNANT LES MALADIES TROPICALES (TDR). Il ne représente pas nécessairement les vues du TDR/OMS et, en vue d'une diffusion accélérée, il n'a pas été l'objet d'une mise en forme particulièrement soignée. En outre, les noms géographiques utilisés dans le présent rapport n'impliquent, de la part du TDR ou de l'OMS, aucune prise de position quant au statut juridique de tel ou tel pays, territoire, ville ou zone, ou de ses autorités, ni quant au tracé de ses frontières.

INTRODUCTION

In 1985, the first joint meeting of the Scientific Working Groups on the Immunology of Malaria (IMMAL) and on Applied Field Research in Malaria (FIELDMAL) considered the basic principles of malaria vaccine trials and the phases in which they would be carried out.¹ Since then important advances have been made in malaria vaccine research. A number of vaccine candidate molecules have been identified and several of the corresponding genes cloned and sequenced. There has been progress in the analysis of immune mechanisms in natural and induced immunity to malaria and in the methodology for such studies.^{2,3,4}

Further development, including the selection of immunogens and the evaluation of vaccines, raises a number of questions which are at the interface of malaria immunology and epidemiology. These questions particularly concern: the possible uses of malaria vaccines; the diversity of human malaria parasites and of the immune response to them; the development of the immune response to malaria parasites and its correlation with protection; the natural patterns of inoculation, their generation and their consequences; and the assessment of clinical malaria and protection, including uses of sero-epidemiology.

The second joint meeting of the IMMAL and FIELDMAL Scientific Working Groups (Geneva, 26-30 September 1988) was therefore convened to review research on immunological aspects of malaria epidemiology, and to provide guidance for the identification of further research required in this area.

This report is largely devoted to the recommendations prepared by four working groups during the meeting; it also includes a list of working papers presented at the meeting and a list of the participants.

1. WORKING GROUP 1. DIVERSITY OF HUMAN MALARIA PARASITES AND OF THE IMMUNE RESPONSE TO THEM

The antigens and epitopes of human malaria parasites are partly conserved and partly diverse, both within and between geographic areas; there may in addition be related diversity of man's naturally acquired immune response, of virulence, and of responsiveness to drugs. Working Group I was convened to discuss the nature of this diversity, and how its importance might be assessed. The group made a series of general recommendations, and specific recommendations concerning the different life-cycle stages of the parasites.

-
- 1/ Principles of malaria vaccine trials: Report of a joint meeting of the Scientific Working Groups on Immunology of Malaria and on Applied Field Research in Malaria (1985) Document TDR/IMMAL-FIELDMAL/VAC/85.3
 - 2/ Sporozoite vaccine development and research towards development of asexual blood-stage vaccines. Report of the Ninth Meeting of the Scientific Working Group on the Immunology of Malaria (1986) Document TDR/IMMAL/SWG(9)86.3.
 - 3/ Exoerythrocytic and asexual blood-stage antigens of human malaria parasites: Report of the Tenth Meeting of the Scientific Working Group on the Immunology of Malaria (1988) Document TDR/IMMAL/SWG(10)/88.3.
 - 4/ Malaria Diagnosis: Memorandum from a WHO Meeting. Bulletin of the World Health Organization, 66(5): 575-594 (1988).

1.1 General Recommendations

1. Further studies were recommended to increase our knowledge of diversity within and between malaria species. (i) These should employ existing immunological reagents to study antigenic diversity but also could involve studies of markers such as drug resistance and isoenzymes; (ii) New panels of immunological reagents are required for P. ovale, P. malariae and P. vivax. These reagents should be produced using total parasite preparations, and also using antigens known to be of importance from previous studies on P. falciparum and P. vivax; (iii) Existing DNA probes and cloned genes should be used to identify homologues in other species.

2. Studies should be supported which investigate the genetic mechanisms involved in the generation of diversity in malaria parasites.

3. Studies should examine whether new diversity arises as a result of the selection pressure imposed by active or passive immunization. This should involve detailed analyses of breakthrough parasitaemias in human and monkey vaccine trials. Important information may also be gained from malaria parasites infecting non-primate laboratory animals.

4. Further studies are required to extend our knowledge of the location of dominant T- and B-cell epitopes in candidate vaccine molecules, particularly with respect to whether these are in conserved or variable regions.

5. Although there is much information on primary structure of P. falciparum antigens, little is known about their conformation and studies addressing this question, which would include X-ray crystallographic analyses, should be supported.

6. Some diversity may reflect the selection pressure of immune responses. However, some parasite diversity may also reflect genetically determined diversity of host structures, perhaps of importance in biology of the parasite and this concept should be investigated.

7. Studies were recommended to examine the genetic element in immune responses to defined antigens or epitopes. These studies should include longitudinal studies so that non-responder/responder status can be assessed at more than one point in time.

8. Field studies should be complemented by laboratory studies in which MHC restriction of immune responses to defined antigens is investigated, preferably using human T-cells.

9. The effect of non-MHC polymorphisms, particularly those of the erythrocyte should be investigated for effects on the development of immune responses.

10. Studies on diversity of parasites should be undertaken at multiple levels e.g. infected single mosquitoes, infected individuals, within and between households, communities and different geographical areas. These studies should include areas that vary in endemicity, including single areas where transmission and/or morbidity varies over time, as well as areas where malaria control projects are instituted.

11. Multiple cross-sectional studies should be carried out to determine whether the frequency of parasite phenotypes or alleles varies over space and time. These should employ a wide range of polymorphic markers so that the rate of reassortment of independent alleles can be assessed.

12. Longitudinal cohort studies should be carried out to determine the relationship between parasite diversity and the development of (strain-specific) acquired immunity and/or specific immune responses.

13. Studies should be designed in a variety of epidemiological situations to determine whether diversity in parasite markers and host genotypes can discriminate among infections with differing clinical outcomes including HMS (Hyperreactive Malarial Splenomegaly).

14. In vitro studies should examine the relationship between parasite diversity and properties such as cytoadherence or reduction of cytokine production which may relate to the outcome of the host-parasite interaction.

15. A variety of methods and reagents are available to examine the extent of parasite diversity. These include monoclonal and polyclonal antibodies, nucleic acid probes, the polymerase chain reaction (PCR) and dot blot hybridization. The PCR has specific application in establishing sequence differences in alternate alleles and is especially useful with very low parasitaemias. It also avoids the necessity of culturing parasites. The PCR can also be used to determine the genotype when this is not known.

Monoclonal antibodies (MAbs) and other immunological reagents are useful for determining whether infections are mixed and for directly determining antigenic phenotype.

Pulse-field gel electrophoresis (PFGE) and DNA fingerprinting may also be applied in the analysis of diversity of field isolates. PFGE coupled with hybridization of specific probes may be used in analysis of mixed infections.

16. Methods for measuring antibody responses (such as micro-ELISA, immunoblots, IFA, etc.), which in many cases show good sensitivity and specificity, are not standardized between different laboratories and need to be made comparable. Availability of standardized antigens and standard reference sera of known titre (possibly through WHO) would greatly facilitate comparison of studies.

There is an urgent need for improved and standardized T-cell assays that can be applied in epidemiological studies and which will provide easily interpretable information concerning cellular function (effector and helper). It is strongly recommended that antigens employed in these studies should have been assessed for reactivity with human T-cell clones that react against native parasite proteins.

17. Existing specific antigen, antibody, and nucleic acid probes should be made widely available for these studies. WHO should institute procedures for making these more available to investigators (dissemination and/or production).

18. The group recommended formation of an annotated data bank including nucleotide and amino acid sequence information. This should include information concerning availability of reagents. The ready availability of sequence information will enable a number of important biological questions to be examined.

1.2 Stage-specific Recommendations

Relevant to all stages is a requirement for studies on the regulation of parasite gene expression particularly as it relates to the expression of stage-specific antigens. In addition, further studies are required in relation to the different life-cycle stages of the malaria parasite.

Sporozoites

(a) Circumsporozoite (CS) proteins

Analysis of the non-repeat flanking regions, including their putative T-cell epitopes should be extended. This should involve sequence analyses on further isolates. It is important that T-cell sites of CS proteins be assessed in studies using native CS protein and sporozoites for priming and challenge as well as with synthetic peptides. In addition CS protein variants should be assessed with respect to other aspects of sporozoite biology (e.g. transmissibility from mosquito to man and invasiveness of hepatocytes). Heterogeneity of CS proteins introduced by post-translational modifications should be examined.

The effect of immune pressure operating in the mammalian host and the mosquito vector should be examined for effects on the emergence of new CS protein variants.

(b) Non-CS antigens of sporozoites

We recommend research into non-CS protein antigens of sporozoites that may play a role in sporozoite immunity (involving, for example, intermolecular help between different sporozoite antigens).

Exoerythrocytic (EE) stages

The extent to which EE antigens cross-react with blood stages and sporozoites should be further investigated. Comparisons should be made of antigens in EE and blood stage merozoites. Liver-stage specific antigens, (LSA-1 and any others) should be further characterized and examined for diversity among different isolates. It is a priority to examine diversity in EE stage antigens as this has relevance not only to vaccine potential but possibly also because detection of such antigens, or antibodies to them, could be of diagnostic value. Possible diversity among relapse populations in P. vivax should be examined.

Asexual stage antigens

(a) The precursor of the major merozoite surface antigens (Pfl95, Pv200). This is an important candidate vaccine molecule and further information is required about its structural diversity. It is a priority to determine which are the dominant T- and B-cell epitopes in both experimental and natural priming and whether these correspond to conserved, dimorphic or polymorphic regions of the molecule.

Studies of the function of this molecule are recommended to determine whether the dimorphic form has functional significance at the level of receptor heterogeneity. In addition, the function of processed products of this antigen which are released in soluble form should be examined. The dimorphism of Pfl95 as it relates to processing and post-translational modification should be investigated. Existing antibody and oligonucleotide probes for Pfl95 should be used to examine parasite diversity in epidemiological studies.

For Pv200, it is a priority to undertake further sequencing and to generate additional monoclonal antibodies and oligonucleotide probes for assessing diversity within this species. Some regions of Pv200 show homology with Pfl95. It is important to determine the significance of this conservation. The homologue in P. ovale and P. malariae should be examined as they will provide opportunities for the assessment of diversity in these species.

(b) Other merozoite surface antigens

The extent of diversity in MSA-2 and other merozoite surface antigens requires further definition.

(c) Other merozoite vaccine candidates

Other vaccine candidates including several rhoptry antigens have been identified. There is little evidence for diversity in any of these molecules but this needs to be further explored.

(d) Infected red cell surface antigen

An antigen on the surface of mature-parasite-infected erythrocytes exhibits extensive antigenic diversity. It is important to investigate the genetic basis of this diversity, and in particular, to determine whether antigenic variation (phenotypic switching of clonal parasite populations) is responsible for this phenomenon. Further studies to assess whether a component of strain-specific immunity is directed towards this antigen should be carried out.

(e) Other polymorphic asexual stage antigens

Some other asexual stage antigens not necessarily considered as vaccine candidates exhibit extensive polymorphisms. Oligonucleotides and antibody probes for the different forms of these antigens should be developed and put to use in epidemiological studies. These include:

S-antigens, Pf 11-1, MESA/Pf EMP2, CRA/exp-1.

Homologues of these P. falciparum antigens should be identified in other species of Plasmodium infecting humans, to allow similar epidemiological studies. Antigens such as FIRA, RESA and the heat-shock proteins (Pf hsp-1) are apparently non-polymorphic immunogens in P. falciparum which could be used as constant markers in epidemiological studies. (The lack of expression of certain genes, e.g. RESA and HRPs, seen in parasites adapted to in vitro culture is not thought to occur in the field but this cannot yet be excluded).

Sexual stage antigens

A large number of P. falciparum isolates have been examined for diversity of target antigens of transmission modulating immunity. The results suggest that epitopes of these antigens of P. falciparum are generally conserved. These studies should be extended with larger panels of antibodies against a wider range of sexual stage antigens. Cloning and sequencing of the genes for these antigens is still a priority.

More extensive diversity has been observed in the sexual stage antigens of P. vivax than in P. falciparum. There is a need to establish how relevant this diversity is to transmission-modulating (blocking and enhancing) immunity in natural infections.

Studies are required to identify T- and B-cell epitopes in the various sexual stage antigens that are potentially involved in the development of transmission-blocking immunity.

Further work is required to identify antigens involved in transmission modulating immunity in P. falciparum which, like P. vivax GAM-1, are shared between blood stages, and to define the extent of the diversity.

2. WORKING GROUP 2. DEVELOPMENT OF THE IMMUNE RESPONSE TO MALARIA PARASITES AND ITS CORRELATION WITH PROTECTION

Working Group 2 considered the development of immune responses to malaria parasites, and possible correlates with protection, including clinical indicators of immunity to malaria and possible study designs to investigate the relationship between immunological measurements and protection.

2.1 Indicators of the Immune Response whose Possible Association with Protection should be Investigated

The following assays were recommended as potentially helpful tests.

SPOROZOITES

(a) Antibody

- i) IFA - whole sporozoites: (a) dried (b) wet/surface
- ii) ELISA - against repeat and non-repeat epitopes
- iii) ISI - measure of invasion: current assays give low levels of infection and improvement of the assay needed for assessing penetration and to measure development
- iv) CSP precipitation test.

(b) Cell-mediated immunity

1. Lymphocyte proliferation test to peptides
2. Production of cytokines IL-1, IFN- γ , TNF, etc.
3. Tests with partially purified cell preparations

All CMI tests must employ appropriate controls

LIVER STAGES

(a) Antibody

1. ELISA - against liver stage specific peptides including one which has been sequenced
2. IFA - against infected primate liver cells

(b) Cell-mediated immunity

No assay has yet been standardized

ASEXUAL STAGES

(a) Functional antibody tests

- i) Opsonization of merozoites: best assessed visually; probably measures same antibodies as iii).
- ii) Opsonization of schizonts: limited experience of usefulness.

- iii) Antibody-dependent cell-mediated cytotoxicity (ADCC): this measures inhibition of parasite multiplication in the presence of antibodies and cells; uses either purified monocytes or unfractionated mononuclear cells from infected or normal individuals; best assessed visually, but radiolabelled hypoxanthine incorporation can be used; not strain specific; can measure both antibody and CMI depending on donor cells used.
 - iv) Growth inhibition assay and inhibition of invasion by serum: tests can be used to detect antibodies which inhibit merozoite dispersion, which inhibit invasion of RBCs by merozoites or which inhibit growth of parasites within RBCs, depending on how the assays are designed. The tests are partially isolate specific; these will be influenced by non-immunological factors such as nutritional status of the test serum, or the presence of drugs.
 - v) Inhibition of cytoadherence: either melanoma cells or endothelial cells can be used (the best target is not yet identified - there may be differences between melanoma and endothelial cells, and endothelial cells from different sites may have different binding properties).
 - vi) Reversal of cytoadherence: limited experience.
 - vii) Agglutination of fresh parasitized RBCs: isolate and possibly variant specific.
 - viii) IFA of fresh parasitized RBCs: needs a double sandwich technique; probably measures same antibodies as agglutination; problem with high backgrounds.
- (b) Antibody assays measuring exposure
- i) IFA using fixed parasitized RBCs: well characterized
 - ii) ELISA against unpurified antigens: many different assays in use; need for standard antigens and control sera
 - iii) IHA: not widely used
 - iv) Western blot to whole lysates or recombinant proteins
- (c) Antibody tests for vaccine candidate antigens and other defined antigens
- i) ELISA would usually be used
 - ii) Dot blot and Western blot
- (d) Cell-mediated immunity tests
- i) Tests with: unpurified antigens and/or specific peptides
 - ii) Determination of proliferative response and cytokine production
 - iii) Determination of frequency of T-cell clones

General Comments

1. Isotype and antibody affinity: for some assays it would be appropriate to measure the isotype of the antibodies being tested and in some cases antibody affinity.

2. Standardization

- is particularly difficult for biological assays, as these depend on live parasites and frequently mammalian cells, both of which are very variable.
- comparison between results in different laboratories would be easier if cell lines used in these assays could be standardized.
- it may not be appropriate to use a standard parasite isolate because of strain variation between regions.
- for simpler assays comparison of results obtained in different laboratories would be facilitated by making standardized control positive and negative sera.
- synthetic and recombinant peptides should be assessed as control antigens for serological tests.
- standardization of cytokine assays and the methods for sample collection for these tests is required.

3. Further Development Needed

The group recommended that assays which should be developed further as possible tests of protective immunity should include:

- Skin tests
- Assays to test invasion of liver cells and growth in liver cells
- Cellular cytotoxicity assays, and other tests of CMI to liver stage antigens.
- Cytoadherence assay which uses an endothelial cell line
- Micromethod assays, including assays for CMI

2.2 Clinical Indicators of Immunity to Malaria

The group discussed various approaches to clinical measurements of malaria immunity in man including measurement of mortality, morbidity, infection and consequences of infection.

The group agreed that despite the many difficulties involved, case definitions for death from malaria, severe and mild clinical malaria and malaria infection were essential for epidemiological studies. The definitions presented in Dr Brown's paper (see list of working papers) were discussed and were agreed with some minor modifications. The same definitions would not necessarily be applicable in all circumstances but whenever parallel studies are being undertaken attempts at standardization should be made.

2.3 Study Design for Investigation of the Relationship between Immunological Measurements and Protection against malaria

a) Longitudinal studies

The group agreed that longitudinal cohort studies were the best method available for investigating the relationship between immunological parameters and protection against malaria.

Cohorts to be studied

It was recommended that cohort studies should be undertaken in different geographical areas with different levels of transmission. (The strength attached to the demonstration of a relationship between one immune response and protection against malaria would be greatly enhanced if it was found in areas with different levels of transmission). The cohort must contain subjects who are likely to have varying levels of protection.

Groups that might be studied include:

- infants and children in malaria endemic areas
- adults in malaria endemic areas
- individuals who migrate to malaria endemic areas
- women before and during their first pregnancy
- previously immune individuals who have lost some protective immunity as a result of residence in a non-endemic area

Characterization of the cohort:

It is essential that the level of transmission in the study population should be clearly defined.

It is desirable that subjects in the cohort should be characterized for any likely confounding factors such as: Hb-genotype, thalassaemia, possibly HLA type, HIV-infection and nutritional status.

Measurements to be made:

- i) Episodes of malaria parasitaemia with symptoms
- ii) Incidence of malaria infection
- iii) Whenever possible parasites causing new infections in cohort subjects should be characterized

It is important that measurements are made frequently enough to detect nearly all clinical episodes and new infections. The frequency needed will vary with area.

In most cases cohort studies will not be large enough to allow information to be obtained on the relationship between immunological measurements and severe and complicated malaria, which is usually a rare event.

An epidemic of malaria, in which a higher attack rate is seen over a short period, is one situation in which it might be possible to study severe and complicated malaria.

Confounding factors

Possible confounding factors in cohort studies are:

1. Variability in exposure to infective mosquitoes. If possible some assessment of individual exposure of study subjects to infection should be made. The possibility of undertaking a challenge experiment in semi-immune individuals with a defined sporozoite inoculum, (maybe as part of a vaccine trial), was discussed.
2. Treatment. Radical cure should be undertaken before longitudinal observations are started. It is very difficult to control treatment, but the problem may be partially overcome if the investigators take over clinical care of the study subjects.

b) Cross-sectional studies

The group considered that cross-sectional surveys in which comparisons are made between different groups within a community have a role to play in identifying immunological factors worth further investigation as possible indicators of protective immunity. However interpretation of the significance of different patterns of association between age-dependent features of malaria and of age-dependent immunological changes is difficult.

It was suggested that cross-sectional studies directed at the measurement of a single immunological parameter would rarely be cost-effective.

c) Case control studies

Case control studies have an important role to play in identifying non-immunological risk factors for severe versus mild malaria.

Study of immunological risk factors is difficult if subjects are entered into the study on presentation with clinical malaria because of the perturbing effect of malaria infection on immune responsiveness. However case control studies might be used to answer specific questions.

d) Passive transfer studies

It was recommended that whenever serum or serum products were used for disease treatment or prevention, the opportunity should be taken to investigate the possible role of individual constituents in giving protection against malaria.

e) Other possible studies

Other possible studies that might give information on the relationship between immunological measurements and protection against malaria that were discussed were:

- i) Study of young women before and during their first pregnancy.
- ii) Previously immune individuals who have lost some protective immunity as a result of residency in a non-endemic area.
- iii) Comparison of immunological findings in patients with high levels of parasitaemia who despite treatment progress to cerebral malaria and those who do not.

General Comments

The group considered the question of detection of immunological markers, which could indicate susceptibility to immunopathological complications of malaria such as quartan malaria nephrosis, hyperreactive malarial splenomegaly and Burkitt's lymphoma, but the group could not make any positive suggestions.

The discussions of the group were directed primarily at studies of Plasmodium falciparum infection as few assays for measuring immune responsiveness to Plasmodium vivax, Plasmodium ovale and Plasmodium malariae are yet available.

Recommendations

1. A detailed review should be made of the reports describing early studies which involved induced infection with Plasmodium falciparum in human subjects.

2. Consideration should be given to the acceptability of leaving untreated asymptomatic parasitaemic individuals resident in endemic areas detected during epidemiological studies. The consensus of the group was that this is justifiable in the context of epidemiological studies in areas of high and stable malaria transmission provided that parasitaemia is only modest, regular follow-up is undertaken, that treatment is continuously available and implemented in accordance with local practice.

In areas of lower endemicity, perhaps in the context of vaccine trials, the level of parasitaemia at which treatment should be instituted must be carefully assessed in the light of local conditions and clinical practice.

3. Studies of possible indirect indicators of morbidity from malaria as opposed to malaria infection should be undertaken. Possible indicators that could be investigated are haptoglobin, acute phase proteins, complement components and indicators of macrophage activation.

3. WORKING GROUP 3. NATURAL PATTERNS OF INOCULATION, THEIR GENERATION AND THEIR CONSEQUENCES

Certain qualitative and quantitative aspects of human malaria transmission under natural conditions are inadequately understood. The practical implications of this area of research fall into four main categories:

- (1) Implications for malaria transmission and specifically the effects of transmission-blocking immunity. Do variations in the infectivity of gametocytes and numbers of oocysts and salivary gland sporozoites affect the number of sporozoites inoculated?
- (2) Implications for the epidemiology of clinical malaria as distinct from effects on transmission dynamics. Here the basic questions are, do variations in the dose (number/frequency) of sporozoites inoculated into individuals affect the clinical severity of the ensuing infection? and do the effects of ant sporozoite immunity depend upon the size and frequency of sporozoite inoculum?
- (3) How are sporozoite inocula distributed in time and space in micro-environments?
- (4) Implications of the occurrence within mosquito and human populations of mixtures of different species of malaria or of different genotypic forms of one species.

3.1 The Infectious Reservoir of Human Malaria

The infectious reservoir in the human population should be defined under different conditions of endemicity.

- (a) What is the distribution of gametocytes in the human population?
- (b) Who is infectious to mosquitoes? Such studies should pay attention to the age of the host, stage of infection and to changes in the composition of the human population. It is important that "intrinsic" infectivity of gametocytes be distinguished from "actual" infectivity. This will entail the collection of serum and gametocytes from infected individuals. Infectivity should be detected by direct feeding of mosquitoes and also by membrane feeding in normal (non-immune) and in the patient's serum.

3.2 Sporogonic Development in Relation to Inoculum Size

Information is required in the following areas:

(a) Quantification of oocyst and sporozoite numbers in naturally infected mosquitoes. Particular attention should be paid to ensuring that low numbers of sporozoites are not missed. Immunological methods currently available are the only means for distinguishing species of sporozoite. However they cannot be entirely relied upon for accurate measurement of sporozoite loads. These would have to be confirmed by dissection.

(b) Relationship between oocyst numbers and sporozoites successfully entering the salivary glands. This relationship is likely to be affected by the density of parasites at each stage and will have to be defined under laboratory conditions.

(c) The most critical area is the number of sporozoites actually injected during feeding. More work is required to develop methods to measure this in wild-caught vectors in a manner which will closely simulate natural feeding conditions. Ideally accurate measurements of sporozoite numbers inoculated should be obtained. However even semi-quantitative methods that distinguish between small (e.g. 10 sporozoites) and large (e.g. 1000 sporozoites) would be valuable.

(d) Factors affecting the development of sporozoite stages. These would include the effects of host immunity and antimalarial drugs on sporogonic development, not only on stages in the mosquito midgut but also on subsequent sporogonic development in the haemocoel and in the salivary glands.

(e) Factors affecting vector competence. This would include genetic changes in vector populations detected by karyotypic monitoring and the effects of the vector immune system.

3.3 Time/Space/Intensity Distribution of Entomological and Parasitological Inoculations

There has been a tendency in both theoretical and practical approaches to malaria transmission to regard subjects living in a single environment as having random exposure to sporozoites. It is now becoming clear that this is unlikely to be the case; thus studies in a number of areas with varying transmission characteristics have indicated that there is a non-random distribution both of mosquitos and infected humans within relatively small microenvironments. This may be important in determining patterns of disease and has implications for control strategies and study design.

There is a need for detailed investigations of sporozoite inoculation distribution within microenvironments (i.e. between individuals within a house and among small groups of houses) with emphasis on determining changes over time. These studies are a necessary prerequisite for studies to determine the possible clinical/parasitological relevance of variations in sporozoite distribution (see below).

It is expected that such studies may define different patterns of sporozoite exposure, for instance some subjects may be exposed to relatively constant challenges, some to intermittent challenges with varying doses and some to predictable patterns where frequency and dose of inoculations build up at certain times of the year. These patterns, for instance "trickle" inoculation versus intermittent inoculations may be important in determining the host immune response to infection. This aspect is best examined in laboratory based studies and it is important that such studies be undertaken in appropriate models (i.e. models in which even low dose inoculations lead to uniformly fatal infections are not appropriate).

Studies in several areas have indicated that an individual's level of antisporezoite antibodies may provide a useful indicator of recent exposure to sporozoites. This needs to be confirmed, such studies should take into account the relative transience of the antisporezoite response and attempt to define the age ranges and transmission conditions in which this is a potentially useful tool. It is important that whenever possible such studies should use comparable methodologies.

3.4 The Relationship Between Sporozoite Load and Clinical Outcome of Infection

As indicated above individuals living in apparently similar environments may be exposed to different doses and patterns of sporozoite inoculation. Clearly sporozoite inoculation is a prerequisite to establish malaria infection but it may be that dose is also a factor determining the severity of clinical disease. It would be helpful to investigate this possibility in appropriate animal models or ideally in a human experimental situation. It is accepted that the accurate determination of absolute sporozoite loads received in the field is not at present feasible but it may nonetheless be possible to examine the question using relatively simple measurements of relative sporozoite load. Such studies will be dependant on initial descriptive studies recommended above and a number of different study designs may be appropriate in areas of varying endemicity. It is an essential requirement of such studies that field methodologies be developed for the identification and classification of clinical disease due to malaria.

A range of factors operating in the human host may govern the chance of a particular inoculation resulting in infection. These include both specific and non-specific immune status, nutritional status and genetic background. Most of these are best investigated under standardized laboratory conditions. One particular question which deserves a joint laboratory and field based approach is the question of whether superinfection is prevented or modified by pre-existing hepatic infection, concurrent blood stage infection or even infection with other organisms.

3.5 Mixed Infections

The term 'mixed infection' encompasses two possible arrangements, firstly the presence in vector or host of more than one malarial species at the same time; secondly, the presence in vector or host of multiple genotypic representatives of one malarial species.

(a) Infections with more than one malarial species

In many areas where P. falciparum occurs, the epidemiology of infection with other malarial species is not adequately documented. This may reflect both the perception of P. falciparum as a more serious problem and the fact that other species are less prevalent and reach lower parasite densities, so that they may be overlooked if P. falciparum parasites are present in a blood film.

Several lines of evidence from epidemiological and clinical studies in endemic areas and from both experimental infections in man and animal models suggest that, when present at the same time, one species of malarial parasite may modify the characteristics of infection by another species of parasite. Two situations where this may be relevant to human malariae were considered. Firstly it is possible that in areas where P. falciparum and P. malariae occur together, P. malariae infections may to some extent be suppressed. The question arises as to whether the specific reduction of P. falciparum alone, for instance in a vaccination campaign, would lead to changes in the natural history of P. malariae infections -- the most important possible consequence

being an increased prevalence of associated renal disease with P. malariae. It would be helpful to pay more attention to interactions of different malarial species in epidemiological studies even when the main focus of interest is on P. falciparum. Advantage should be taken, as developments permit, of the availability of species-specific diagnostic probes which may overcome some of the problems of determining true prevalences. Points of transition, either temporal or geographical, offer particular opportunities for studying the effect on one parasite species of changes in prevalence of another species.

A second potentially important phenomenon is the apparent modification of the severity of P. falciparum infection by preceding infection P. ovale, reported during the treatment of neurosyphilitic patients. The relevance of these observations has not been studied in endemic areas. The availability of monoclonal antibodies both to P. ovale CS protein and to blood stages offers a means of examining this in epidemiological studies focused on clinical P. falciparum malaria.

(b) Mixtures of genetically distinct parasites of the same species

There is a rapidly expanding awareness of the degree of genetic diversity within malarial species, particularly in P. falciparum and P. vivax. This has important implications both for our understanding of the epidemiology of malaria and in particular for our thinking on vaccination. It is essential that studies be undertaken to examine the degree of genetic diversity both in human and mosquito populations and in individual human and mosquito infections. Because opportunities for genetic recombination may vary widely depending on transmission characteristics, these studies should be carried out in areas covering the complete range of endemicities. The availability of monoclonal antibodies, DNA probes and technologies based on the polymerase chain reaction (PCR) will facilitate these studies and steps should be taken to make reagents and facilities as widely accessible as possible. An area of particular importance is the pattern of large seasonal peaks of clinical disease against a relatively constant background of asymptomatic infection, which is characteristic of many endemic areas. The question of whether this is due to the transmission of different genetic forms or whether it is related to changes in sporozoite inoculum size should be tackled using approaches outlined above.

General Recommendations

1. It is necessary that epidemiological studies are done under a range of endemic conditions. There is especially a lack of information from areas of low endemicity.
2. Entomological facilities are essential for comprehensive epidemiological studies. Facilities for maintaining vectors and for conducting infectivity studies should be established where these studies are to be carried out.
3. Mechanisms should be formulated by TDR to facilitate the linkage between laboratories in developed countries where technologies are available and those in disease endemic countries so as to make available information, reagents and technology for these studies in endemic regions. For example monoclonal antibodies, and oligonucleotide probes to be used in the polymerase chain reaction for typing parasites.
4. Development of new techniques that could facilitate studies suggested above, e.g. sexual and sporogonic stage (ookinete, oocyst) specific diagnostic reagents.
5. TDR should offer continued support to selected institutions in endemic areas which are capable of carrying out comprehensive epidemiological studies.

4. WORKING GROUP 4. USE OF IMMUNOLOGICAL TESTS FOR EPIDEMIOLOGICAL CHARACTERIZATION AND CLASSIFICATION OF MALARIA SITUATIONS

The group attempted to identify immunological tests that would serve as epidemiological measures of the various stages of the malaria life cycle and be applicable to various malaria situations. These are listed in terms of the priority assigned to them.

1. Detection of CS antigen in mosquitoes as a measure of species-specific endemicity. For falciparum malaria, standardization has been accomplished. Availability of reagents needs to be centralized. For vivax malaria the basic methodology still requires standardization. For malariae and ovale malaria methodology requires development.

2. Detection of CS antigen in mosquito tissues is still controversial as a measure of sporozoite inoculation rate. The group recommended that high priority be given to the resolution of this problem.

3. Detection of P. falciparum CS serum antibodies allows measurement of exposure to sporozoites and assessment of levels and patterns of transmission in endemic populations. Availability of reagents needs to be centralized. The selection of a single capture antigen is recommended.

4. Detection of CS serum antibodies to other malaria species requires standardization and selection of appropriate capture antigens. The group recommended a high priority for this task.

5. Emphasis should be given to the identification of EE-specific antigens as an epidemiological tool and as a possible measure of malaria incidence.

6. ELISA methodology should be developed to replace the conventional IFAT used as a measure of asexual blood stage immune response. Priority has to be given to identification of appropriate defined antigen(s).

7. Further studies should be carried out to identify sexual stage antigens that would be useful in epidemiological studies in addition to those related to vaccine development.

General Considerations

As was the case with antibodies to sporozoites, epidemiological studies on other defined stage-specific antigens and antibodies against them could reveal further useful malariological indicators.

The group recommended further research to identify serological markers of the severity of malaria disease and specific complications of malaria, e.g. cerebral malaria.

Alternative methodologies should be developed for the identification of parasite stages and species such as oligonucleotide probes, and research should be encouraged on the identification of markers for parasite virulence and for relapse and recrudescence of infection.

The group recommended that validation and standardization of methodology be done in established institutions in various geographical locations. This implies a role for TDR in collaborative networking between individual investigators, and the TDR assume a positive role in promoting the availability of standardized reagents to responsible investigators and institutions.

5. LIST OF WORKING PAPERS

- Possible uses of malaria vaccines. Some implications for research, by
L. Molineaux
- Antigenic diversity of sporozoite and liver stages and diversity of immune
response, by L. Schofield
- Epidemiology of anti-sporozoite immunity in Thailand, by H.K. Webster,
C. Wongsrichanalai, A.E. Brown & J.B. Gingrich
- Differential antibody responses to P. falciparum and P. vivax CS proteins in a
human population, by T.R. Burkot, P.M. Graves, R.A. Wirtz, B. Brabin,
D. Battistutta, J.A. Cattani, T. Maijeli & M.P. Alpers
- Association between HLA type and antibody response to malaria sporozoite and
gamete epitopes is not evident in immune Papua New Guineans, by
P.M. Graves, K. Bhatia, T.R. Burkot, R. Carter, M. Prasad & R.A. Wirtz
- Biological functions of the CS proteins as detected by P. gallinaceum stage-
specific anti-sporozoite mab's, by A.U. Krettli, A.D. Ramirez,
E.M.M. Rocha & M.A.G. Fontana
- The antigens and epitopes of the asexual blood-stages of human malarias, their
diversity and the diversity of the human immune response to them, by
R. Anders, N. Barzaga, J. Smythe, L.M. Thomas, R.L. Coppel, V.M.J. Robson,
P.T. Shi & G.V. Brown
- The development of the human immune response against the major surface protein
(gp 190) of P. falciparum, by H.M. Muller, K. Frieh, A. von Brunn,
F. Esposito, S. Lombaroli, L. Theilmann, A. Crisanti & H. Bujard
- Antigenic diversity of the Pfl95 molecule in the field populations of P.
falciparum, by J. McBride
- The antigens and epitopes of the sexual stages of Plasmodium vivax, their
diversity and the diversity of man's immune response to them.
Epidemiological aspects, by K.N. Mendis, R. Carter, J.S.M. Peiris,
A.P.K. Zoysa & P.H. David
- The antigens and epitopes of the sexual stages of Plasmodium falciparum, their
diversity and the diversity of man's immune response to them. Epidemio-
logical aspects, by J.H.E.T. Meuwissen, T. Ponnudurai & P.J. Beckers
- Correlation between immune response to P. falciparum and protection, and their
development in human populations, by B.M. Greenwood
- Identification of a potentially protective T-cell epitope on the Plasmodium
falciparum CS protein, by S. Hoffman, C.N. Oster, C. Mason, J.C. Beier,
J.A. Sherwood, W.R. Ballow, M. Mugambi & J.D. Chulay
- Correlation between immune response to P. vivax and protection, and their
development in human populations, by D.F. Clyde
- Malaria transmission potential of wild afrotropical Anopheles: Sporozoite
loads and in vitro sporozoite transmission, by J.C. Beier, F.K. Onyango,
M. Ramadhan, D.K. Koech & C.R. Roberts
- Effect of antibodies or chloroquine administered to malaria infected mosquitos
and its possible implications for transmission, by V.E. do Rosario

- Sporozoite dose and the outcome of malaria infections, by K. Marsh
- Natural inoculations as potential boosters of artificial immunity, by R. Carter & M. Good
- Immunotechniques for malaria epidemiology. The use of immunological tests for the epidemiological characterization and classification of malaria situations, by F. Esposito, A. Habluetzel, S. Lombardi & S. Goriup
- Epidemiological aspects of immunopathology in malaria, by G.E. Grau & P.H. Lambert
- The epidemiology of mixed infection in relation to the possible uses of malaria vaccines for malaria control. Some implications for research, by L. Molineaux
- Interactions between HIV infection/AIDS and Plasmodium falciparum malaria, by P. Nguyen-Dinh
- The diagnosis and grading of clinical malaria, by I. McGregor
- Assessment of immunity (protection) against malaria, by G.V. Brown
- Comparative in vivo/in vitro analysis of immunity to P. falciparum blood stages: study design in 8 Thai patients treated with African IgG and preliminary results, by Tan Chongsupajaisiddhi & P. Druihe

6. PARTICIPANTS

- ALZATE, Dr A. Faculty of Health, Universidad del Valle, Apartado Aero 2188, Cali, Colombia
- ANDERS, Dr R.F. Walter & Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia
- BEIER, J. Walter Reed Army Medical Center, Walter Reed Army Institute for Research, Washington, D.C. 20307, USA
- BJORKMAN, Dr A., Department of Parasitology, Statens Bakteriologiska Laboratorium, 105 21 Stockholm, Sweden
- BROWN, Dr G.V. Walter & Eliza Hall Institute of Medical Research, Post Office, Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia
- BURKOT, Dr T., Queensland Institute of Medical Research, Bramston Terrace, Herston, Brisbane, Queensland 4006, Australia
- CAMPBELL, Dr C.C. Dept. of Health & Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA 30333, USA
- CARTER, Dr R. Department of Animal Genetics, University of Edinburgh, West Mains Road, Edinburgh EH9 3JN, Scotland
- CHONGSUPHAJASIDDHI, Dr T. Dept. of Tropical Pediatrics, Hospital for Tropical Disease, Faculty of Tropical Medicine, Mahidol University, 420/6 Rajvithi Road, Bangkok 10400, Thailand
- CHULAY, Dr J., Department of Immunology, Walter Reed Army Medical Center, Walter Reed Army Institute of Research, Washington, D.C. 20307, USA

- CLYDE, Dr D.F. University of Maryland, 10 South Pine Street, Baltimore,
MD 21201, USA
- CRISANTI, Dr A., IMF 282 ZMBH, 6900 Heidelberg, Federal Republic of Germany
- DAVID, Dr P.H., Unité d'Immunoparasitologie, Institut Pasteur, 25, rue du
Dr Roux, 75015 Paris, France
- DEL GIUDICE, Dr G., Département de Pathologie, Université de Genève, 1, rue
Michel-Servet, 1211 Genève 4, Switzerland
- DIGGS, Dr C.L., Agency for International Development, Washington, D.C. 20523
USA
- DRUILHE, Dr P., Institut Pasteur, 25, rue du Dr Roux, 75015 Paris, France
- ESPOSITO, Dr F., Dipartimento di Biologia Cellulare, Università di Camerino,
2, Via Camerini, 62032 Camerino, Italy
- FORSYTH, Dr K. Walter & Eliza Hall Institute of Medical Research, Post Office,
Royal Melbourne Hospital, Melbourne, Victoria 3050, Australia
- FRUH, Dr K., ZMBH, Zentrum für Molekular Biologie, Universität Heidelberg,
Postfach 106249, 6900 Heidelberg 1, Federal Republic of Germany
- GRAU, Dr G. WHO Immunology Research & Training Unit, University of Geneva,
Dept. of Pathology, Centre Medical Universitaire, 1 rue Michel-Servet,
1211 Genève 4, Switzerland
- GRAVES, Dr P., Queensland Institute of Medical Research, Bramston Terrace,
Herston, Brisbane, Queensland 4006, Australia
- GREENWOOD, Dr B. MRC Laboratories (Medical Research Council), P.O. Box 273,
Fajara, Banjul, Gambia
- HOFFMAN, Dr S.L., Naval Medical Research Institute, Naval Medical Command,
Bethesda, MD 20814, USA
- HOGH, Dr B. Department of Immunology, University of Stockholm,
106 91 Stockholm, Sweden
- HOLDER, Dr A.A. National Institute for Medical Research, Medical Research
Council, The Ridgeway, Mill Hill, London NW7 1AA, UK
- JERSEN, Dr S., Malaria Research Laboratory, Department of Trepanematoses,
State Serum Institute, 80 Amager Boulevard, 23 Copenhagen S, Denmark
- KAISER, Dr R.L. Center for Infectious Diseases, Public Health Service,
Centers for Disease Control, Atlanta, GA 30333, USA
- KRETTLI, Dr A.U., Malaria Research Laboratory, Centro de Pesquisas "René
Rachou" FIOCRUZ, C.P. 1743, 30 000 Belo Horizonte, Brazil
- MACPHERSON, Dr C., Swiss Tropical Institute Field Laboratory, P.O. Box 53,
Ifakara, United Republic Tanzania
- MARSH, Dr K. University of Oxford, Nuffield Dept. of Clinical Medicine, John
Radcliffe Hospital, Headington, Oxford OX3 9DU, UK
- MCBRIDE, Dr J. Department of Zoology, University of Edinburgh, West Mains
Road, Edinburgh EH9 3JT, Scotland

- MCGREGOR, Sir Ian. Liverpool School of Tropical Medicine, Pembroke Place
Liverpool L3 5QA, UK
- MENDIS, Dr K.N. University of Colombo, Faculty of Medicine, Kynsey Road,
Colombo 8, Sri Lanka
- MEUWISSEN, Dr J.H.E.T. Institute of Medical Parasitology, University of
Nijmegen, Geert Grooteplein Zuid 24, P.O. Box 9101, 6500 HB Nijmegen,
Netherlands
- MILLER, Dr C., Agency for International Development, Washington, D.C. 20523,
USA
- MILLER, Dr. K., Agency for International Development, Washington, D.C. 20523,
USA
- NGUYEN-DINH, Dr P., Parasitic Diseases Division, Centers for Infectious
Diseases, Centers for Diseases Control, 1600 Clifton Road, N.E. Atlanta,
GA 30333, USA
- PERRIN, Dr L.H., Centre de Transfusion sanguine, Hôpital cantonal
universitaire, 24, rue Micheli-du-Crest, 1211 Genève 4, Switzerland
- PETERSON, Dr E., Karolinska Institute, Roslagstulls Hospital, 114 89 Stockholm,
Sweden
- PHILIPPS, Dr R.S. University of Glasgow, Department of Zoology, Glasgow,
G61 1QH, Scotland,
- PINICHPONGSE, Dr S. Department of Communicable Disease Control, Ministry of
Public Health, Devavesm Palace, Bangkok 2, Thailand
- RELF, Dr W., Queensland Institute of Medical Research, Bramston Terrace,
Herston, Brisbane 4006, Australia
- RIECKMANN, Dr K.H. Army Malaria Research Unit, MILPO, Ingleburn NSW 2174,
Australia
- ROBERTS, Dr C.R., US Army Medical Research Unit, Kenya, Walter Reed Project,
Kenya Medical Research Institute, P.O. Box 54840, Nairobi, Kenya
- ROSARIO, Dr V.E., Biomedical Research Institute, 1211 Parklawn Drive,
Rockville, MA 20852, USA
- ROUGEMONT, Dr A., Unité de Santé communautaire et Médecine tropicale, Hôpital
Cantonal, 1211 Genève 4, Switzerland
- SCHOFIELD, Dr L. NYU Medical Center, Dept. of Medical & Molecular
Parasitology, 550 First Avenue, New York 10016, USA
- SMITH, Dr P.G. London School of Hygiene & Tropical Medicine, Keppel Street,
London WC1E 7HT, UK
- SOUZA, Dr O.E., University of Panama, Panama, Panama
- STEKETEE, Dr R., Parasite Disease Division, Centers for Disease Control,
Atlanta, GA 30333, USA
- TOURE, Dr Y. Centre National de la Recherche Scientifique et Technologique,
B.P. 3052, Bamako, Mali

WEBSTER, Dr H.K. United States Army Medical Comp., Armed Forces Research
Institute of Medical Sciences (AFRIMS), Rajvathi Road, Bangkok 10400,
Thailand

WERNSDORFER, Dr W. Anilingasse 2 (App. 2/15), 1060 Wien, Austria

WINTER, Dr P.E., American Institute of Biological Sciences, Malaria Immunity
and Vaccination Research, 1800 N. Kent Street, Suite 930, Arlington
22209, USA

WHO Secretariat

BEALES, Dr P.F., Programming and Training, Malaria Action Programme

CATTANI, Dr J., Special Programme for Research and Training in Tropical
Diseases

DOBERSTYN, Dr B., Research and Technical Intelligence, Malaria Action Programme

GODAL, Dr T., Special Programme for Research and Training in Tropical Diseases

GORIUP, Dr S., Epidemiological Methodology and Evaluation, Malaria Action
Programme

KOUZNETSOV, Dr R., Programming and Training, Malaria Action Programme

LAMBERT, Dr P.-H., Microbiology and Immunology Support Services, Division of
Communicable Diseases

MARTINEZ, Dr L., Research and Technical Intelligence, Malaria Action Programme

MOLINEAUX, Dr L. Epidemiological Methodology and Evaluation, Malaria Action
Programme

MORROW, Dr R., Special Programme for Research and Training in Tropical Diseases

MUIR, Dr D.A., Malaria Action Programme

NAJERA, Dr J.A., Malaria Action Programme

TRIGG, Dr P., Research and Technical Intelligence, Malaria Action Programme

VLASSOFF, Dr C., Special Programme for Research and Training in Tropical
Diseases

ANNEX

SELECTED RECENT REFERENCES

- ANDERS, R.F. et al. (1987) Structure and function of candidate vaccine antigens in Plasmodium falciparum. Biochem. Soc. Symp. 53, 103-114
- BAKER, E.Z. et al. (1987) Detection and Quantification of Plasmodium falciparum and P. vivax infections in Thai-Kampuchean Anopheles (Diptera: Culicidae) by enzyme-linked immunosorbent assay. J. Med. Entomol. 24, 536-541
- BALLOU, W.R. et al. (1987) Safety and efficacy of a recombinant DNA Plasmodium falciparum sporozoite vaccine. Lancet, (i) 1277-1281
- BROWN, A.E. et al. (1988) IgM antibody responses to the circumsporozoite protein in naturally acquired falciparum malaria. J. Clin. Immunol. 8, 1-7
- BURANAKITJAROEN, P. and NEWBOLD, C.I. (1987) Antigenic cross-reactivity between p195 and a distinct protein of 100 kDa in Plasmodium falciparum. Mol. Biochem. Parasitol. 22, 65-77
- BURKOT, T.R. et al. (1987) The efficiency of sporozoite transmission in the human malarías, Plasmodium falciparum and P. vivax. Bull. World Health Organization 65, 375-380
- CAMPBELL, G.H. et al. (1987) Detection of antibodies in human sera to the repeating epitope of the circumsporozoite protein of Plasmodium falciparum using the synthetic peptide (NANP)₃ in an enzyme-linked immunosorbent assay (ELISA). Am. J. Trop. Med. Hyg. 37, 17-21
- CARTER, R. et al. (1988) Restricted or absent immune responses in human populations to Plasmodium falciparum in gamete antigens which are targets of malaria transmission-blocking antibodies. J. Exp. Med. 169, 135-147
- CONTRERAS, C.E. et al. (1988) RESA-IFA assay in Plasmodium falciparum malaria, observations on relationship between serum antibody titers, immunity and antigenic diversity. J. Parasitol. 74, 129-134
- DE ZOYSA, A.P.K. et al. (1988) Modulation of human malaria transmission by anti-gamete transmission blocking immunity. Trans. R. Soc. Trop. Med. Hyg. 82, 548-553
- DEL GIUDICE, G. et al. (1987) Detection of human antibodies against Plasmodium falciparum sporozoites using synthetic peptides. J. Clin. Microbiol. 25, 91-96
- DEL GIUDICE, G. et al. (1987) Antibodies to the repetitive epitope of Plasmodium falciparum circumsporozoite protein in a rural Tanzanian community: a longitudinal study of 132 children. Am. J. Trop. Med. Hyg. 36, 203-212
- DELORON, P. et al. (1987) Antibodies to the Pfl55 antigen of Plasmodium falciparum, measurement by cell-ELISA and correlation with expected immune protection. Am. J. Trop. Med. Hyg. 37, 22-26
- EGAN, J.E. et al. (1987) Efficacy of murine malaria sporozoite vaccines: implications for human vaccine development. Science 236, 453-456

- GOOD, M.F. et al. (1987) Human T clones reactive to the sexual stages of Plasmodium falciparum malaria. High Frequency of Gamete-reactive T-cells in peripheral blood from nonexposed donors. J. Immunol. 138, 306-311
- GOOD, M.F. et al. (1988) Human T-cell recognition of the circumsporozoite protein of Plasmodium falciparum: immunodominant T-cell domains map to the polymorphic regions of the molecule. Proc. Nat. Acad. Sci., USA 85, 1199-1203
- GRAVES, P.M. et al. (1988) Antibodies to Plasmodium falciparum gamete surface antigens in Papua New Guinea sera. Parasite Immunol. 10, 209-218
- GREENWOOD, B.M. et al. (1987) Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. Trans. R. Soc. Trop. Med. Hyg. 81, 478-486
- HERRINGTON, D.A. et al. (1987) Safety and immunogenicity in man of a synthetic peptide malaria vaccine against Plasmodium falciparum sporozoites. Nature 328, 257-259
- HOFFMAN, S.L. et al. (1987) Naturally acquired antibodies to sporozoites do not prevent malaria: vaccine development implications. Science 237, 639-642
- KEMP, D.J. et al. (1987) Repetitive proteins and genes of malaria. Ann. Rev. Microbiol. 41, 181-208
- KUMAR, N. (1987) Target antigens of malaria transmission blocking immunity exist as a stable membrane bound complex. Parasite Immunol. 9, 321-335
- LEE, M. et al. (1988) Interaction of Malaysian sera with Plasmodium vivax sporozoite antigen. Am. J. Trop. Med. Hyg. 39, 535-539
- MARSH, K. et al. (1988) Anti-sporozoite antibodies and immunity to malaria in a rural Gambian population. Trans. R. Soc. Trop. Med. Hyg. 82, 532-537
- MENDIS, K.N. et al. (1987) Malaria transmission-blocking immunity induced by natural infections of Plasmodium vivax in humans. Infect. Immunity 55, 369-372
- MENDIS, K.N. et al. (1988) Diversity of P. vivax induced antigens on the surface of infected human erythrocytes. Am. J. Trop. Med. Hyg. 38, 42-46
- MIETTINEN-BAUMANN, A. et al. (1988) A 46 000 Da Plasmodium falciparum merozoite surface antigen not related to the 185 000-195 000 Da schizont precursor molecule: isolation and characterization. Parasitol. Res. 74, 317-323
- NICHOLS, M.E. et al. (1987) A new human Duffy blood group specificity defined by a murine monoclonal antibody. Immunogenetics and association with susceptibility to Plasmodium vivax. J. Exp. Med. 166, 776-785
- PANG, L.W. et al. (1988) Circumsporozoite antibodies and falciparum malaria incidence in children living in a malaria endemic area. Bull. World Health Organization 66, 359-364
- PEIRIS, J.S.M. et al. (1988) Monoclonal and polyclonal antibodies both block and enhance transmission of human Plasmodium vivax malaria. Am. J. Trop. Med. Hyg. 39, 26-32

- PERLMANN, H.K. et al. (1987) Absence of antigenic diversity in Pf155, a major parasite antigen in membranes of erythrocytes infected with Plasmodium falciparum. J. Clin. Microbiol. 25, 2347-2354
- PETERSON, M.G. et al. (1988) A third form of the precursor to the major merozoite surface antigen of P. falciparum. Mol. Cell. Biol. 8, 2664-2667
- PONNUDURAI, T. et al. (1987) Transmission blockade of Plasmodium falciparum: its variability with gametocyte numbers and concentration of antibody. Trans. R. Soc. Trop. Med. Hyg. 81, 491-493
- QUAKYI, I.A. et al. (1987) The 230kDa gamete surface protein of Plasmodium falciparum is also a target for transmission-blocking antibodies. J. Immunol. 139, 4213-4217
- RANAWAKA, M.B.R. et al. (1988) Boosting of transmission-blocking immunity during natural P. vivax infections in man depends upon frequent re-infection. Infect. Immunity 56, 1820-1824
- RILEY, E.M. et al. (1988) Cellular immune responses to Plasmodium falciparum antigens in Gambian children during and after an acute attack of falciparum malaria. Clin. Exper. Immunol. 73, 17-22
- ROMERO, P. et al. (1987) Antigenic analysis of the repeat domain of the circumsporozoite protein of Plasmodium vivax. J. Immunol. 139, 1679-1682
- SADOFF, J.C. et al. (1988) Oral Salmonella typhimurium vaccine expressing circumsporozoite protein protects against malaria. Science 240, 336-338
- SAINT, R.B. et al. (1987) Changes in repeat number, sequence, and reading frame in S-antigen genes of Plasmodium falciparum. Mol. Cell. Biol. 7, 2968-2973
- SAUL, A.J. et al. (1988) Delineation of epitopes on a Plasmodium falciparum merozoite surface antigen using inhibitory monoclonal antibodies. In: Technological Advances in Vaccine Development. Alan R. Liss, Inc. New York
- SCHOFIELD, L. et al. (1987) Gamma-interferon, CD8+ T-cells and antibodies required for immunity to malaria sporozoites. Nature 330, 664-666
- SMITH, D.B. and JOHNSON, K.S. (1988) Single-step purification of polypeptides expressed in Escherichia coli as fusions with glutathione S-transferase. Gene 67, 31-40
- SMYTHE, J.A. et al. (1988) Identification of two integral membrane proteins of Plasmodium falciparum. Proc. Natl. Acad. Sci. USA 85, 5195-5199
- STAHL, H.D. et al. (1987) Structure of the FIRA-gene of Plasmodium falciparum. Mol. Biol. Med. 4, 199-211
- SULZER, A.J. et al. (1988) Antibodies to the Ring-infected Erythrocyte Surface Antigen Plasmodium falciparum elicited by infection with Plasmodium malariae. Infect. Immunity 56, 729-733
- TANABE, K., et al. (1987) Allelic dimorphism in a surface antigen gene of the malaria parasite Plasmodium falciparum. J. Mol. Biol. 195, 273-287

- UDAGAMA, P.V. et al. (1987) Antigenic polymorphism in Plasmodium vivax malaria: comparison of 50 parasite isolates with a panel of 30 monoclonal antibodies. Infect. Immunity 55, 2604-2611
- VAUGHAN, J.A. et al. (1988) Plasmodium falciparum: Ingested anti-sporozoite antibodies affect sporogony in Anopheles stephensi mosquitoes. Exp. Parasitol. 66, 171-182
- WEBSTER, H.K. et al. (1988) Characterization of antibodies to sporozoites in Plasmodium falciparum malaria and correlation with protection. J. Clin. Microbiol. 26, 923-927
- WEISS, W.R. et al. (1988) CD8+ T-cells (cytotoxic/suppressors) are required for protection in mice immunized with malaria sporozoites. Proc. Nat. Acad. Sci. USA 85, 573-576
- WIRTZ, R.A. et al. (1987) Field evaluation of enzyme-linked immunosorbent assays for Plasmodium falciparum and Plasmodium vivax sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. J. Med. Entomol. 24, 433-437

* * *