

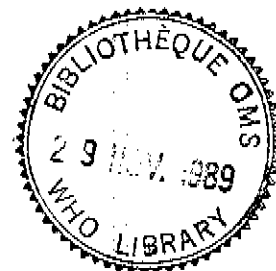


UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR  
 RESEARCH AND TRAINING IN TROPICAL DISEASES

Geneva, November 1989

GUIDELINES FOR THE EVALUATION OF PLASMODIUM FALCIPARUM  
 ASEXUAL BLOOD-STAGE VACCINES IN POPULATIONS  
 EXPOSED TO NATURAL INFECTION

Prepared jointly by the WHO Malaria Action Programme  
 and the UNDP/World Bank/WHO Special Programme for  
 Research and Training in Tropical Diseases



CONTENTS

	Page
INTRODUCTION . . . . .	2
A. GENERAL BACKGROUND . . . . .	3
1. Development of <u>Plasmodium falciparum</u> asexual blood-stage vaccines . . . . .	3
2. The possible parasitological and clinical consequences of immunization with <u>P. falciparum</u> asexual blood-stage vaccines . . . . .	3
3. Asexual blood-stage vaccines and malaria control . . . . .	5
4. Factors likely to affect the outcome of <u>P. falciparum</u> asexual blood-stage vaccine trials . . . . .	6
B. FIELD TRIALS IN MALARIA-ENDEMIC COUNTRIES . . . . .	8
1. Objectives . . . . .	8
2. Information required from early clinical trials (Phase I and Phase II) . . . . .	9
3. Criteria for the selection of areas/populations for field trials . . . . .	10
4. Background information required to design a trial . . . . .	11
5. Trial design . . . . .	13
6. Implementation . . . . .	17
7. Ethical considerations . . . . .	20
C. SELECTED REFERENCES . . . . .	21
ANNEX I - METHODS OF MEASUREMENT . . . . .	26
ANNEX II - CANDIDATE ANTIGENS FOR INCLUSION IN VACCINES . . . . .	30
ANNEX III - RESEARCH TOPICS RELEVANT TO VACCINE TRIALS . . . . .	33

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

Recent developments in immunology and molecular biology indicate that vaccines against malaria are on the horizon. There are three main approaches to malaria vaccine development, based on different stages in the life cycle of malaria parasites. The vaccine targets currently envisaged are the pre-erythrocytic stages (sporozoite and liver forms), the asexual blood-stages, and the sexual and sporogonic forms which develop in the mosquito vector. Vaccines based on these life cycle stages can be expected to elicit different forms of protective immunity, and a number of specific considerations will arise in the planning of the clinical and epidemiological evaluation of each type. The general planning of malaria vaccine trials and the phases in which they will be developed are considered in the report of a meeting held in WHO, Geneva, in February 1985<sup>(1)</sup>. Another WHO document considers the epidemiological evaluation of Plasmodium falciparum sporozoite vaccines in malaria-endemic areas<sup>(2)</sup>. The present document considers the evaluation of P. falciparum asexual blood-stage vaccines in populations exposed to natural challenge.

Field trials of the kind discussed in this document should be carried out only after early clinical studies (Phase I and Phase II) are completed in volunteers in both non-endemic and endemic areas. These early trials should establish the basic safety, immunogenicity and efficacy of the vaccine (against some end points of interest), and the appropriate dosage.

This document is addressed to national health authorities, in particular to those of malaria-endemic countries interested in the potential use of P. falciparum asexual blood-stage vaccines for the control of malaria, and to research scientists interested in the development and field evaluation of such vaccines. The guidelines may help public health officials to make decisions about malaria vaccine trials to be conducted in their countries, including not only field (Phase III) trials, but also the required earlier clinical trials.

This document was produced by a group of consultants<sup>(3)</sup> and a working group composed of members of the WHO Malaria Action Programme (MAP) and the UNDP/WORLD BANK/WHO Special Programme for Research and Training in Tropical Diseases (TDR) in WHO Headquarters, in consultation with colleagues from the same and other divisions of WHO, and with the TDR Steering Committees on the Immunology of Malaria (IMMAL) and on Applied Field Research in Malaria (FIELDMAL).

In this report, the terms "immune" and "immunity" indicate resistance to infection, resulting from previous infection or vaccination; "immune response" is used to describe the host's humoral or cellular immune response which follows exposure to antigens, e.g. following infection or vaccination, but which does not necessarily reflect or correlate with a state of protection against adverse effects of infection. Immunity may be reflected by either resistance against the clinical effects of malaria infection or against the development of parasitaemia.

- 
- (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. Document TDR/IMMAL-FIELDMAL/VAC/85.
  - (2) Guidelines for the epidemiological evaluation of Plasmodium falciparum sporozoite vaccines. Document TDR/MAP/SVE/PF/86.5
  - (3) B.M. Greenwood, T.K. Ruchush and P.G. Smith

A. GENERAL BACKGROUND

1. Development of Plasmodium falciparum asexual blood-stage vaccines

Most of those living in areas where P. falciparum is endemic develop effective immunity to malaria only after suffering multiple infections, usually over a period of several years. The resulting may not completely suppress parasitaemia and adults who have been exposed to malaria throughout life frequently have asymptomatic parasitaemias. Experimental infection of humans has shown that a single infection with P. falciparum tends to modify the severity of subsequent infections and among children living in an endemic area the clinical effects of successive infections become progressively less severe. It is possible that an asexual blood-stage vaccine would have an effect similar to that produced by repeated natural exposure, dramatically reducing mortality and morbidity while not completely preventing parasitaemia. In such a situation subsequent infections might boost vaccine-induced immunity.

The feasibility of vaccinating against the asexual blood-stages of malaria has been demonstrated by immunizing animals with whole merozoites. Relatively large numbers of asexual blood-stage parasites of P. falciparum can be produced in vitro but it is not feasible presently to base a vaccine on such material because it is impossible to produce enough parasites sufficiently free of components of the human serum and erythrocytes in which they are grown. Therefore, current efforts are directed at developing a subunit vaccine.

The asexual blood-stages of P. falciparum are antigenically complex and infection results in immune responses directed against many different antigens. It is assumed that immune responses induced by the majority of these antigens are irrelevant to protection, or may even be undesirable, and that only a small number of the antigens induce protective immune responses. The first stage in the development of a vaccine against the asexual blood-stages of P. falciparum is the identification of the antigens that are capable of inducing protective immune responses. Although useful information has been obtained from immunization trials with antigens purified from infected erythrocytes, it is necessary to clone the genes for candidate vaccine antigens so that their vaccine efficacy can be assessed using recombinant proteins or synthetic peptides.

A large number of antigens of the asexual blood-stages of P. falciparum have now been described and the genes encoding many of them have been cloned. None of these antigens appears dominant as a target of protective immune responses. Rather, several antigens appear to be capable of inducing immune responses that limit the growth or development of P. falciparum and it is possible that maximum efficacy will be achieved only with a vaccine that combines several of these antigens.

Antigens currently considered to be candidates for inclusion in anti-P. falciparum asexual blood-stage vaccines are described in Annex I.

2. The possible parasitological and clinical consequences of immunization with P. falciparum asexual blood-stage vaccines

Evidence from animal models suggests that immunization with asexual blood-stage vaccines may not prevent the appearance of parasitaemia, but that such vaccines may reduce the density of asexual blood-stage infections and enhance the clearance of parasites from the blood, thus reducing the severity of clinical disease. The immunity produced by blood-stage vaccines may be similar to the naturally acquired immunity of human populations exposed to intense transmission of P. falciparum. An effective asexual blood-stage vaccine would be expected to reduce the number of gametocytes produced and hence reduce the infectivity of the host to the vector, as in naturally acquired immunity. However, there may be differences in this respect between natural and artificial immunization, as well as between different asexual blood-stage vaccines.

Potential effects of P. falciparum asexual blood-stage vaccines in vaccinated individuals are summarized in the following Table:

Variable affected	Probable effects	Other possible effects
Infection (parasitaemia)	Little or no change in incidence Decreased density of <u>P. falciparum</u> asexual blood-stages and, probably, gametocytes  Decreased duration	Increased prevalence and density of <u>P. malariae</u> , <u>P. vivax</u> , <u>P. ovale</u> (1)
Immune response	Increased response to <u>P. falciparum</u> asexual blood stages No change in response to sporozoites(2)	Decreased response to <u>P. falciparum</u> sexual stages (3)
Morbidity (disease)	Decreased incidence Decreased severity	Increased incidence of complications during infections (5)
Mortality(4)	Decreased mortality rate Decreased case fatality rate	

(1) Supposing that P. falciparum infection itself suppresses or masks infections with the other malaria parasites.

(2) Unless there is suppression of the response to sporozoites by the asexual blood-stages, and removal of that suppression by immunization.

(3) Assuming decreased production of P. falciparum gametocytes.

(4) Decrease in mortality caused directly or indirectly by malaria; in terms of vital statistics, there is likely to be a decrease both in malaria-specific mortality and in the mortality rate from all causes combined, and the absolute decrease in the latter may be greater than in the former.

(5) A malaria vaccine could theoretically induce a state of altered immunity which would result in an exaggerated and harmful immune response on natural exposure.

### 3. Asexual blood-stage vaccines and malaria control

A major purpose of field trials of a vaccine should be to determine its potential usefulness for disease control. A discussion of the potential uses of asexual blood-stage vaccines is important for planning their evaluation and their further development, as the most desirable characteristics of such vaccines may vary according to the epidemiological situation in the area in which they are used. Two broad categories of use for malaria vaccines can be distinguished: the protection of vaccinated individuals and the control of transmission. Sporozoites vaccines may fulfil both these roles, gamete vaccines only the second. Asexual blood-stage vaccines should induce clinical protection; whether they will also reduce transmission will depend upon their effect on the infectivity of vaccinated individuals, which is not known at present.

#### 3.1 The protection of vaccinated individuals

##### 3.1.1 Protection of residents of endemic areas

Permanent residents in endemic areas constitute by far the largest populations that would benefit from malaria vaccines and they are the major target group for eventual vaccine use. Vaccines may be of special importance among permanent residents, for newborn infants, with or without passive (maternal) immunity, and pregnant women. If an asexual blood-stage vaccine is to "shortcut" an otherwise slow and hazardous acquisition of immunity, it may have to be administered soon after birth. In some situations non-immune immigrants settling into endemic areas may constitute another especially important target group.

As the protection given by a vaccine may not be long lasting, periodic revaccination may be required for continuous protection. Alternatively, immunity might be maintained after vaccination by natural exposure to infection, depending on the effect of natural infections and on the intensity of infection in a vaccinated population.

##### 3.1.2 Protection of non-immune visitors to endemic areas

The role of asexual blood-stage vaccines in the protection of nonimmune visitors to endemic areas will depend on the degree of protection given by the vaccines, and on the relative merits of alternative methods of protection, such as chemoprophylactic drugs or sporozoite vaccines.

Non-immune visitors are a significant target group for vaccination. This could affect vaccine design, as the requirements of non-immune visitors may differ from those of permanent residents of endemic areas. For example, a vaccine giving a high degree of protection, but for a short duration, may be adequate for many visitors. Also the convenience of mass administration is less important for temporary visitors than for permanent residents. The addition of a sporozoite vaccine to an asexual blood-stage vaccine may be desirable for short-term visitors, but not necessarily for long-term residents (e.g. if natural boosters are important). The acceptable cost of a vaccine is likely to be higher for many visitors than for residents of endemic areas.

Certain classes of temporary visitors may be useful for the evaluation of malaria vaccines, including asexual blood-stage vaccines. Organized groups (e.g. military personnel) have specific advantages: they can be closely monitored; chemoprophylaxis may be withheld, since treatment can be made readily available; they may be non-immune; they may be exposed to a wide variety of epidemiological situations; and prolonged follow-up under close medical supervision may be possible.

### 3.2 Control of transmission

Little is known about the effects of suppressing asexual blood-stage infections on the production of infectious gametocytes, but it is likely that an effective asexual blood-stage vaccine will have some effect on gametocytes thereby reduce transmission.

### 3.3 Conclusion: priorities

In assessing the possible role of asexual blood-stage vaccines in malaria control, priority should be given to the evaluation of the impact of vaccination on the following:

- mortality, in communities where malaria is an important cause of death
- incidence and severity of clinical malaria
- incidence, density and duration of asexual parasitaemia
- incidence, density and duration of gametocytaemia, and infectivity for vectors

### 4. Factors likely to affect the outcome of P. falciparum asexual blood-stage vaccine trials

A number of factors may affect the outcome of vaccine trials, either because they interfere with the effectiveness of the vaccine or because they have an independent effect on exposure to malaria or on its manifestations, or on both. Variation of such factors may affect vaccine efficacy in different places, times and persons and may lead to variability in the response to vaccination between communities. Thus, it is important to review these factors, in order to decide which, if any, should be taken into account either in the planning of a vaccine trial (e.g. by stratification and/or randomization) or in analysis of its results (e.g. by stratification). Some of the potentially relevant factors that should be considered before a trial is started are listed below.

#### 4.1 Parasitological factors

##### 4.1.1 Antigenic diversity and variation

Antigenic diversity of asexual blood-stage antigens has been demonstrated both between and within geographic areas. The outcome of a vaccine trial might be influenced by the degree of similarity between the antigenic characteristics of the parasites from which the vaccine was prepared and the antigenic characteristics of the malaria parasites dominant in the study area. Thus, it is desirable to know something about the antigenic characteristics of the parasites prevalent in a proposed study area before a vaccine trial is planned, and to monitor whether the antigenic characteristics of the dominant parasites in the study community are changed as a result of vaccination.

##### 4.1.2 Drug sensitivity

Knowledge of the drug sensitivity of the malaria parasites in the study area will be important for decisions on which drugs are used to treat infections at the beginning of the trial and during its course.

##### 4.1.3 Mixed infections

P. falciparum infection is commonly accompanied by infection with one or more other species of malaria parasites. It is generally believed that, within the human host, P. falciparum parasitaemia suppresses or masks the parasiti-

taemias due to other malaria parasites and a successful P. falciparum blood stage vaccine might result in an increased incidence of clinical attacks of malaria caused by other species. Thus parasitaemia due to other species of malaria must be measured in a P. falciparum vaccine trial.

#### 4.2 Human host factors

##### 4.2.1 Immunological

- (a) Pre-existing acquired active immunity. The level of existing immune responsiveness resulting from previous natural exposure may influence the response to vaccination and prior investigation of the study community will be required.
- (b) Passive immunity in infants. It is possible that maternally-derived factors could suppress the immune response of infants to an asexual blood-stage vaccine.
- (c) Suppression of the immune response to vaccination might result from a concurrent blood-stage infection. Such an effect could be removed by treatment, possibly a few weeks before vaccination. Treatment would also facilitate the detection of new infections and hence the assessment of protection provided by vaccination.
- (d) Heterogeneity of immune responsiveness. If heterogeneity of immune responsiveness to the vaccine antigen is an important factor, this should be detected at the stage of vaccine development. An antigen that is not recognized by a significant fraction of the target population is not a good candidate as an immunizing agent.

##### 4.2.2 Other human host factors

- (a) Age and sex and pregnancy may influence both the response to vaccination and the risk of infection so that trials done in subjects of different groups may have different outcomes.
- (b) Several genetic factors affect host responses to P. falciparum, in particular Hb-S, thalassaemias, ovalocytosis and some types of G-6-PD deficiency and the prevalence of these factors in a community might influence the outcome of a vaccine trial.
- (c) Human behaviour may influence vaccine trials in several ways. Occupational, social or other activities may affect exposure to vectors, personal protective measures may reduce exposure to malaria, and treatment seeking and drug usage patterns may alter the apparent effect of a vaccine. These factors must be taken into account during the design of protocols for vaccine trials. Vaccine trials should not take place in communities where these factors are likely to change during the course of the trial.
- (d) Other diseases, such as HIV infection, may alter the response to malaria infection or to the vaccine and their presence may complicate evaluation of a malaria vaccine trial. Records should be kept of such diseases in trial participants.
- (e) Introduction of a new vaccine may influence the acceptability of other vaccines according to how well it is tolerated. It may also affect the immune responses to other vaccines and influence their side-effects. These potential interactions will need to be considered in evaluating malaria vaccine trials.

- (f) The nutritional status of a study community might influence both the immune response to vaccination and the pattern of malaria in that community. It is probably advisable, at least initially, not to conduct early vaccine trials in severely malnourished populations or individuals.

#### 4.3 Entomological factors

The entomological factors which could affect the outcome of malaria vaccine trials are the intensity and seasonality of natural challenge and booster immunization by sporozoite inoculations. A vaccine may prove to be more effective in an area of low challenge than in an area of high challenge, or vice-versa, so that it will be important to know the level of challenge to which vaccinated subjects have been exposed.

#### 4.4 Control measures

Malaria control measures, i.e. vector control, control of man-vector contact (bednets, etc.) and antimalarial drugs, may all affect the endpoints (infection and disease) of malaria vaccine trials. It is recommended that vaccine trials should be conducted in areas and populations in which no significant changes in malaria control measures are planned, except as part of the trial. It is also recommended that any measures, applied either on a communal or on an individual basis, be monitored. In particular, records should be kept of the use of antimalarial drugs, from whatever source.

### B. FIELD TRIALS IN MALARIA-ENDEMIC COUNTRIES

#### 1. Objectives

- 1.1 To measure the effect of a P. falciparum asexual blood-stage vaccine on:
- (i) mortality due to malaria;
  - (ii) incidence and severity of P. falciparum symptomatic malaria;
  - (iii) incidence and density of P. falciparum asexual blood-stage parasitaemia;
  - (iv) prevalence of P. falciparum gametocytaemia and infectivity to the vector;
  - (v) the immune response to blood-stage antigens, and its correlation with any effects on (i), (ii) and (iii).
- 1.2 To measure any side-effects attributable to vaccination.

The major endpoint of public health importance in the use of a malaria vaccine is the reduction of mortality. It is possible that a vaccine that reduces morbidity (the incidence of disease) would have little impact on mortality. It would be highly desirable, therefore, to measure mortality in early field trials of a candidate vaccine. The primary reason for conducting such studies early in the field evaluation of a vaccine is that it may be judged unacceptable to do so (in a double-blind comparison including a placebo) once a vaccine has been shown to reduce morbidity. However, an early trial of mortality will involve the immunization of a large number of children with a vaccine whose efficacy is unknown.

2. Information required from early clinical trials  
(Phase I and Phase II)(1)

Early clinical trials are required to produce the information needed for the planning of field trials, in particular information regarding: safety; acceptability, immunogenicity and efficacy (protection, including its duration) following artificial challenge, by age, sex, and past malaria experience; vaccine formulation, storage, dosage; and the booster effect of revaccination.

There are major differences between non-immune subjects in non-endemic areas and populations living in endemic areas that could influence the response to malaria vaccines and the evaluation of the results of vaccine trials (see section 4). Because of these differences, the results of clinical trials in non-endemic areas cannot provide an adequate basis on which to begin field trials in endemic areas and it will be necessary to repeat early clinical (Phase I) trials conducted in non-malarious areas in some, but not necessarily all, endemic areas. The need to repeat Phase I trials before proceeding with field trials will depend on the availability of information from other trials carried out in areas in which the epidemiology of malaria is similar. In addition, for ethical reasons, clinical trials in some special high-risk groups, e.g. pregnant women and infants, can only be carried out in malarious areas.

It is envisaged that the process of vaccine evaluation leading to large-scale field trials is likely to take place in the following sequence:

- (i) trials in non-immune adults in a non-malarious area to evaluate vaccine safety and immunogenicity (Phase I)
- (ii) trials in non-immune adults in a non-malarious area to measure protection following artificial challenge (Phase IIa)
- (iii) trials in partially-immune adults in an endemic area to evaluate vaccine safety and immunogenicity (Phase I)
- (iv) trials in small groups of partially-immune adults to measure protection following artificial or natural challenge (Phase IIb).
- (v) small trials of safety and immunogenicity in older children, young children and pregnant women.

Trials involving artificial challenge may not be necessary in all endemic areas in which field trials are envisaged. The decision on whether or not to conduct artificial challenge trials before embarking on a large scale field trial will depend, among other considerations, on whether the level of natural challenge is sufficiently high and predictable in the study area to allow the evaluation of protection against development of parasitaemia in a small number of subjects and on local technical capabilities and interest.

A special problem exists with respect to the evaluation of asexual blood-stage vaccines. Because of the potentially life-threatening nature of P. falciparum infections in non-immune subjects, it will be necessary to treat artificially induced infections very soon after parasitaemia becomes patent. Consequently, it may be impossible in Phase II trials in non-immune subjects to identify vaccines that reduce the level of parasitaemia and/or the severity of

---

(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. Document TDR/IMMAL-FIELDMAL/VAC/8.3

symptoms but do not provide complete protection against parasitaemia. Such a mode of action is likely with vaccines directed against the asexual blood-stages and it will be possible to show the efficacy of such vaccines only in field trials done in endemic areas.

The decision on whether or not to proceed with a field trial of a vaccine for which efficacy has not been demonstrable in Phase IIa trials will be a difficult one; it will be influenced by the results of other immunological studies, including trials in non-human primates. Demonstration of efficacy after artificial challenge in a Phase 2 trial is not an absolute requirement for a Phase 3 trial of an asexual blood-stage vaccine.

### 3. Criteria for the selection of areas/populations for field trials

The selection of areas/populations for field trials will be critical. In making this selection, and in planning the baseline studies for a trial, it will be important to take account of the fact that it will not be possible to carry out successive malaria vaccine trials in the same population. Such trials would be likely to produce insoluble problems for the interpretation of results obtained.

#### 3.1 Epidemiological criteria

A candidate vaccine should be evaluated in the range of epidemiological situations and geographic areas in which it is likely to be relevant to control. The following populations merit highest priority: residents of areas with intense seasonal transmission; residents of areas with intense perennial transmission; and non-immune immigrants of different age groups (previously unexposed or with limited exposure) settling in areas with intense seasonal or perennial transmission. Judicious selection of the timing of vaccination in relation to the transmission season could be useful. The inclusion of both areas with seasonal and with perennial transmission is justified because those two kinds of malaria transmission are likely to induce different patterns of naturally-acquired immunity. The justification for including immigrants is that they are a high-risk group for malaria and comprise non-immune subjects of all ages. To investigate the duration of protection that occurs when boosting natural inoculations are uncommon, trials in areas of low transmission will be required.

#### 3.2 Operational criteria

In areas that meet the epidemiological criteria above, the selection of appropriate sites for community-based field trials will depend on a number of additional factors. The following operational criteria may assist in assessing the suitability of a particular site:

- (a) A genuine commitment of national authorities to the conduct of the trial will help in gaining the support and confidence of both community participants and health professionals.
- (b) Availability and involvement of national research institutions with interested national (and/or international) investigators and field/laboratory teams to provide local expertise and which have access to national and international resources to support the trial.
- (c) Reasonable expectation of social and political stability at the national and local levels.

- (d) A sufficiently well-established health service infrastructure to meet the primary health care needs of the population and to provide for referral of cases to hospital when required.
- (e) A well-established, effective programme for administration of existing vaccines against other diseases.
- (f) Availability of basic laboratory services in reasonable proximity to the proposed trial site.
- (g) Adequate transportation and communication infrastructure to provide access to the population and for the population to have access to the health care services on a year-round basis.
- (h) Availability of background data on the epidemiology of malaria, including information on the levels of malaria morbidity and mortality.
- (i) The potential for the site to serve as a field training centre.

#### 4. Background information required to design a field trial of a vaccine

In order to design a field trial it will be necessary to have some information on the epidemiology of malaria in the proposed study area. Data will also be required on the demography of the study population, and on patterns of antimalarial drug usage and on other malaria control measures. Such information may already be available; if not it may be necessary to conduct a longitudinal study in the area before the trial starts. The following 4 items will be required in order to plan the size of a trial.

##### 4.1 Mortality rates:

If mortality is to be used as an end-point of the trial, estimates will be required of death rates, from all causes and from malaria, among subjects in the age groups in which the trial is to be conducted. To estimate these rates it will be necessary to conduct a census of the study area, in order to have the denominator for rate calculations. A surveillance system must be set up to detect deaths (see Annex 1). Such surveillance should be conducted in a population including several thousand children so that reasonably reliable estimates of death rates are obtained.

##### 4.2 Incidence rates of malarial infection

In a sub-group of subjects representative of those to be included in the trial, all, or all with parasitaemia, should be treated with antimalarials to clear malaria parasites from their blood. These subjects should then be followed over an extended period (e.g. a year), with regular blood slides being taken to detect new infections with malaria. In this way, incidence rates of different species of malarial infection can be obtained that can be categorized by age, sex and season.

##### 4.3 Incidence rates of clinical malaria

A larger sub-group of subjects (than required for 4.2), also representative of those to be included in the trial, should be visited regularly (e.g. weekly) and any subject with fever should have a blood slide taken. Those with fever and malaria parasite levels over defined limits may be classified as cases of clinical malaria (see Annex 1) for the calculation of malaria attack rates. Rates can be calculated according to age, sex, season, etc.

#### 4.4 Migration

Data should be collected on out-migration of subjects from the study area in order to estimate what proportion of subjects in a longitudinal study might be lost to follow-up.

Additional information that would be essential for planning a field trial includes:

#### 4.5 Consumption patterns of antimalarials

Sources of antimalarials in the study area should be identified and surveys conducted of consumption patterns of antimalarials in those in the age groups to be included in a trial. Subjects, or their parents should be asked what actions they take and where treatment is obtained when they, or their children are ill.

#### 4.6 Sensitivity of malaria parasites to different drugs

This information will be required in order to select the chemotherapeutic regimens needed to clear parasitaemias and to treat malaria in the study area.

#### 4.7 Acceptability of vaccination

Studies should be conducted to confirm that a high proportion of the population targetted for vaccination would accept the vaccine. This might be done in pilot studies of vaccination in the study area (e.g. for immunogenicity testing).

#### 4.8 Other background data

As discussed in section 4.4, it would be desirable to have information for the proposed trial area on some or all of the features listed below in order to be able to compare and contrast the results of trials conducted in different areas:

- (a) The diversity of asexual blood-stage antigens in malaria parasites in the study area.
- (b) Immunological responses induced by natural infection in persons in different age groups to the antigens present in the vaccine.
- (c) The prevalence of genetic factors which affect host responses to P. falciparum, in particular Hb-S, thalassaemias, ovalocytosis and G-6-PD deficiency.
- (d) The prevalence of other diseases that might alter responses to malaria or antimalarial vaccines (e.g. HIV infection).
- (e) The coverage of the population with other vaccines, and the ages at which these are given.
- (f) Nutritional status of the study population.
- (g) Past and present malaria control measures; patterns of drug resistance.
- (h) The use of personal protection measures against malaria (e.g. bed nets, antimalarial drugs).

- (i) Behavioural and social factors that may affect exposure to malaria (e.g. occupations).
- (j) Entomological factors that may affect the outcome of a vaccine trial (e.g. sporozoite inoculation rate and behaviour of local vector mosquitoes).

## 5. Trial design

The trial should consist of a double-blind comparison between a vaccine and a placebo, randomized between individuals. Past experience with trials of other vaccines has shown that to avoid all ambiguity of results with respect to both protection and side-effects it is essential to use a randomized double-blind design. Selection of an appropriate placebo will need to await exact specifications of vaccine composition and the results of preclinical trials.

If several candidate vaccines are available more than one type of P. falciparum asexual blood-stage vaccine could be tested in the same trial.

### 5.1 Selection of age limits for trial participants

The choice of the age groups to be included in a trial will depend upon the epidemiological pattern of malaria in the proposed trial area. In areas where the major burden of malaria mortality is in infancy studies would be focussed on this group, whereas in other areas studies in older children or, in the case of non-immune immigrants, adults would be appropriate. It is important that the age groups included in the trial correspond to those that would form the target for vaccination in a control programme should an effective vaccine be found.

### 5.2 End-points measured in a vaccine trial

The end-points of interest in a vaccine trial have been given in section B.1. It is important that protocols are developed, before the trial starts, giving explicit definitions of how each endpoint will be measured. Such definitions may vary from trial to trial depending, for example, on what other diseases are prevalent in the area that may mimic some of the symptoms of malaria (e.g. that cause fever).

#### 5.2.1 Death

The ascertainment of all deaths in a study population involving several thousand children will require a carefully designed and tested surveillance system. Assignment of the probable causes of death will require the development and validation of a system of "verbal autopsy" (see Annex 1).

#### 5.2.2 Clinical malaria

There is no definition of clinical malaria that is universally accepted. In non-immune individuals the presence of fever with parasitaemia gives a definition with high specificity (i.e. it will not include many whose symptoms are not truly due to the malaria infection). However, in semi-immunes resident in areas where malaria is hyper or holoendemic and where many other infections cause fever, such a definition of malaria is likely to have poor specificity. In these areas it will be necessary to define cut-off levels for both fever and malaria parasitaemia above which an individual is classified as having clinical malaria.

### 5.2.3 Malaria infection

A small sub-group in the trial should be monitored for the incidence of malarial infection (as distinct from clinical malaria). Treating all children in the vaccinated and control groups with an effective blood schizonticide at the time of vaccination will facilitate the recognition of new infections.

### 5.2.4 P. falciparum gametocytaemia

To assess the impact of the vaccine on malaria infectivity, gametocyte counts should be performed on any blood slides taken to detect malaria infection or disease and, if possible, the infectivity of gametocytes to mosquitoes should be determined.

### 5.2.5 Side effects of vaccination

These may be minor (e.g. local reaction at vaccine site) or major (e.g. life-threatening). It is essential that a surveillance system be set up to detect any major or minor adverse reactions occurring within a few days of vaccination and that the incidence of possible adverse effects be recorded systematically and within a set period of time.

### 5.2.6 Immune response to vaccination

Measurement of the immune response to vaccination is an important end-point in vaccine trials. It may be possible to undertake separate analyses of vaccine efficacy in those who have shown a good immune response to the vaccine and in those who have not.

## 5.3 Trial size

The size that a trial should be in order to detect a difference in disease or infection risks between vaccinated and unvaccinated subjects will vary according to the endpoints chosen and their incidence in the trial area. Conventional statistical methods to estimate the required sample size for a trial require that the levels of four factors be specified. These are:

- (i) the estimated risk, or rate, of occurrence of the endpoint of interest in study subjects who will not be vaccinated.
- (ii) the minimum protective effect of vaccination that the trial should have a high probability (see (iv)) of detecting (see (iii)).
- (iii) the probability level ( $\alpha$ ) at which the null hypothesis (vaccine efficacy = 0) will be rejected. Conventionally taken as 0.05 or 0.01.
- (iv) the probability of declaring a significant difference if the true vaccine efficacy is that specified in (ii). This is known as the statistical power of the trial [ $-100(1-\beta)\%$ ].

## 5.3 Trial size

The size that a trial should be in order to detect a difference in disease or infection risks between vaccinated and unvaccinated subjects will vary according to the endpoints chosen and their incidence in the trial area. Conventional statistical methods to estimate the required sample size for a trial require that the levels of four factors be specified. These are:

- (i) the estimated risk, or rate, of occurrence of the endpoint of interest in study subjects who will not be vaccinated;

- (ii) the minimum protective effect of vaccination that the trial should have a high probability of detecting;
- (iii) the probability level ( $\alpha$ ) at which the null hypothesis (vaccine efficacy = 0) will be rejected. Conventionally taken as 0.05 or 0.01;
- (iv) the probability (1- $\beta$ ) of declaring a significant difference if the true vaccine efficacy is that specified in (ii). This is known as the statistical power of the trial, commonly expressed as a percentage [=100(1- $\beta$ )%].

In general, it is most efficient to have the same number of subjects in the vaccinated and unvaccinated groups in a trial, and the formulae below assume that this is the case.

For comparing risks in vaccinated and unvaccinated subjects the required sample size is calculated as:

$$n = [z_{\alpha}/(2pq) + z_{\beta}/(p_1q_1+p_2q_2)]^2/(p_2-p_1)^2$$

where n is the sample size in each group  
and  $p_2$  is the risk of the event of interest in the unvaccinated group during the trial period.

$p_1$  is the risk of the event in the vaccinated group. [If it is desired to detect a vaccine efficacy of v (measured as a proportion rather than a percent), then  $p_1 = (1-v)p_2$ ]

$$p = (p_1+p_2)/2; q=1-p; q_1=1-p_1; q_2=1-p_2$$

Values of z are taken from tables of the Normal distribution. For example, for  $\alpha=0.05$   $z_{\alpha}=1.96$  (two-sided test)

$$\alpha=0.01 \quad z_{\alpha}=2.32 \text{ (two-sided test)}$$

and for 95% power  $\beta=0.05$   $z_{\beta}=1.64$

for 90% power  $\beta=0.10$   $z_{\beta}=1.28$

for 80% power  $\beta=0.20$   $z_{\beta}=0.84$

#### Example 1

In a trial of a vaccine to protect against infant deaths, suppose the risk of infant death is 120/1000 births, and 20% of deaths are due to malaria. Suppose it is desired to detect a vaccine efficacy of 50% (at the 5% level of significance with 90% power). Then

$$p_2 = 24/1000 \text{ (the risk of malaria death among infants)}$$

$$p_1 = 12/1000$$

$$z_{\alpha} = 1.96$$

$$z_{\beta} = 1.28$$

$$n = (1.96/[2(0.018)(0.982)] + 1.28/[(0.024)(0.976) + (0.012)(0.988)])^2/(0.024-0.012)^2 = 2575$$

i.e. it will be necessary to include about 2600 infants in each group (followed for 1 year from birth).  
[In the same situation, to have 80% power to detect a vaccine efficacy of 70%, 714 infants would be required in each group.]

For the above computation it has been assumed that malaria deaths can be distinguished from other infant deaths. If only infant deaths are recorded, without attribution of cause of death, then a 50% reduction in malaria mortality would reduce the overall risk of infant death from 120/1000 to 108/100. The study size required to detect this difference would be 14 725 (i.e.  $p_2=0.120$  and  $p_1=0.108$ ) in each group. Thus, there is clearly much to be gained from attempting to assess cause specific mortality.

#### Example 2

If the child mortality rate is 25/1000/yr and 20% of deaths are from malaria, the study size to have 80% power to detect a 70% reduction in the malaria death rate, measured on malaria specific deaths, is 4,585 in each group, and measured on all deaths is 29,067 in each group. Thus, for malaria specific deaths, 4,600 children might be followed in each group for a year (or 2,300 in each group for 2 years - with the assumption that the vaccine efficacy remains constant for 2 years).

#### Example 3

If 50% of the unvaccinated children are expected to experience one or more clinical attacks of malaria during the rainy season and the vaccine efficacy is assumed to be at least 50%, such that only 25% will experience one or more attacks following vaccination, then the required sample size in each group is:

$$n = \frac{\{1.96/[2(0.375)(0.625)] + 1.28/[(0.5)(0.5) + (0.25)(0.75)]\}^2}{(0.5-0.25)^2} - 77$$

Note: If it is decided to test for a significant reduction in malaria in the vaccinated group in each of several strata (e.g. different age groups), the sample size has to be calculated for each of these groups.

#### 5.4 Duration of trial

The most useful vaccines will be those that give high protection for a long period. Thus, if a vaccine is found to protect against morbidity (or mortality) from malaria during the first year, follow-up of the trial group should continue, ideally for as long as there is a significant difference between vaccinated and unvaccinated, although the maintenance of a placebo group in this situation may raise ethical questions. However, it might be argued that in circumstances where the study group is subject to close surveillance it would be justifiable to continue follow-up provided that all episodes of malaria were subject to prompt detection and treatment. This argument cannot be sustained for studies of mortality and once a vaccine has been shown to reduce mortality it would be unacceptable to continue surveillance for deaths without offering vaccination to those who had received placebo.

An effect of a vaccine in reducing malaria morbidity might not necessarily be reflected in a corresponding impact on mortality. Thus, in trials in which

mortality was planned as an endpoint as well as morbidity, it might be considered justifiable to continue the mortality surveillance and follow-up beyond the time an impact on morbidity was shown. Clearly, this would require careful ethical review.

If no protection against malaria morbidity is shown during the first year of a study it might be decided to end the study at this point. However, if the trial also included mortality surveillance on a larger group it would be advisable to continue to follow the population for the time required in the trial protocol to detect a mortality effect of the magnitude sought, unless there were compelling reasons for supposing that the failure to find an impact on morbidity would also apply to mortality.

Provision should be made to stop the trial at once if an unacceptable number of serious adverse reactions arise as a consequence of vaccination. An independent clinical monitor should be appointed for the trial and advising in these circumstances would be one of his major responsibilities.

### 5.5 Exclusions

In so far as possible the trial should not exclude any persons who would be likely to receive vaccination if the vaccine were put into a control programme after efficacy had been demonstrated. It would be prudent, however, to exclude from an initial trial those likely to respond adversely to vaccination or liable to develop episodes of illness which might be difficult to distinguish from adverse reactions. It is recommended that the following groups of individuals be excluded:

- (i) those with clinical malaria at the time of vaccination
- (ii) those with any other severe acute illness at the time of vaccination
- (iii) those with any severe chronic disease
- (iv) those severely malnourished
- (v) those for whom informed consent is not obtained.

## 6. IMPLEMENTATION

### 6.1 Pre-vaccination

Prior to starting a field trial it will be necessary to obtain the approval of the appropriate ethical committee and approval from national and local administrative bodies. During the early phase of planning of a vaccine trial a trial committee should be established whose membership comprises representatives of interested parties and also independent experts. The function of the trial committee will include the provision of advice to the investigators on study design and monitoring the conduct of the trial. A trial monitor should be appointed who holds the key to the vaccine randomization and to whom cases and side effects are reported.

The purposes of the trial and the methods to be used should be discussed with representatives of the communities in which the trial is planned and those who will be eligible for entry into the trial should be properly briefed on possible adverse effects of vaccination and on possible benefits. It should be made clear that participation in the study is voluntary and those refusing to participate will receive all their routine vaccinations and will not be discriminated against in any way.

## 6.2 Vaccination

Those entering the trial will be vaccinated using recommended dose(s) and techniques at a time selected to produce maximal vaccine effect during intense transmission. Thus, residents in areas of seasonal transmission might be vaccinated at the beginning of the rains and non-immune immigrants just before entering areas of intense transmission. However, if the vaccine were expected to confer long-lasting protection, vaccination during the season of low transmission might be preferable, as this would facilitate laboratory evaluation of the immune response to the vaccine.

Trial participants should all be vaccinated within as short a period as possible. Vaccine specifications should be clearly defined. Trial vaccines must meet international standards and be the same as those to be used if the vaccine is to be licensed and marketed. Proper transport and storage of the vaccine must be ensured and monitored.

## 6.3 Associated chemotherapy

Early clinical trials may demonstrate the need for chemotherapy at, or shortly before, vaccination, to avoid immunosuppression due to current malaria infection (see above, section A.4.2). Whether or not such prior treatment is required to enhance vaccine immunogenicity, the sub-groups to be included in studies of the incidence of malaria infection and of clinical malaria should be treated with an effective blood schizonticide at the time of vaccination. This will facilitate the recognition of new infections. Whether it is desirable or practicable to treat all those included in studies of mortality will depend on local circumstances. The drug used should be selected on the basis of parasite sensitivity and drug toxicity studies. Chloroquine is the drug of choice in areas where *P. falciparum* is sensitive to it. Curative doses should be used.

Vaccination of a large fraction of a community and the associated treatment of a fraction twice as large might reduce transmission, and the incidence of infection, disease and death in the unvaccinated controls, to the point of reducing the power of the trial to detect a further significant decrease in the vaccinated group. In a trial comparing vaccinated and unvaccinated individuals in a community, it would be advisable to avoid vaccinating too large a fraction of the community. The safe maximum is unknown and will depend on the local epidemiological situation, but it may be prudent to vaccinate no more than 25% of a community.

## 6.4 Data collection and measurement of effects.

Methods for data collection and measurements are given in Annex I.

## 6.5 Analysis

Some endpoints of interest in a malaria vaccine trial affect each subject, at most, only once (eg, death, the occurrence of the first episode of clinical malaria), whereas others may consist of a count of a number of events for each subject (eg, the number of episodes of clinical malaria, the number of new infectious with malaria, the number of days with parasitaemia). For endpoints of the first kind there may be interest in the risk of the endpoint in a given period or the rate at which it occurs during the trial. A risk is calculated as the proportion of subjects who experience the endpoint in the defined period, whereas a rate is calculated as the number of subjects who experience the endpoint divided by the time they were at risk for the endpoint (ie, once a subject has experienced the endpoint he or she is no longer considered to be "at risk"). For rare endpoints (eg, death from malaria) the distinction between risks and rates is academic, as all but a few subjects are "at risk"

for the complete trial period, but in situations in which a high proportion of the trial population may experience the endpoint of interest (eg, infection with malaria in a holoendemic area) it may be of interest not only to compare the risks, say, of malaria infection, in vaccinated and unvaccinated subjects but also to compare the rates at which they are infected. For example, if all in the vaccinated and unvaccinated groups developed malaria (ie, risk = 1 in both groups), but the disease occurred later, on average, in the vaccinated subjects, this would be evidence of a possible protective effect of vaccination, indicated by a lower rate of infection in vaccinated subjects (which would be of scientific, if not of immediate public health, significance).

The possible results from a trial are shown in algebraic form in the table below:

Group	Size of Group	No. experiencing endpoint of interest	Proportion with endpoint*	Person time-at-risk	Rate of occurrence of endpoint
1. Vaccinated	$n_1$	$c_1$	$p_1 = c_1/n_1$	$y_1$	$r_1 = c_1/y_1$
2. Unvaccinated	$n_2$	$c_2$	$p_2 = c_2/n_2$	$y_2$	$r_2 = c_2/y_2$
Total	$n$	$c$	$p = c/n$	$y$	$r = c/y$

\* adjustments may be necessary if some subjects are lost to follow-up.

The table below summarizes methods that may be used to estimate vaccine efficacy and to test the statistical significance of differences in event rates or risks in those in the vaccinated and unvaccinated groups. Also given are methods to put confidence intervals on the estimate of relative risk.

	<u>Risk</u>	<u>Rate</u>
Relative risk/rate	$R_i = p_1/p_2$	$R_a = r_1/r_2$
Estimate of vaccine efficacy (%)	$100(1 - R_i)$	$100(1 - R_a)$
Test of significance (chi-square with 1df.)	$X^2 = ( c_1 - E(c_1)  - 0.5)^2 / V(c_1)$	
Where $E(c_1) =$	$n_1 p / n$	$n_1 r / n$
and $V(c_1) =$	$n_1 n_2 c (n - c) / n^2 (n - 1)$	$n_1 n_2 c / n^2$
95% confidence limits on		
$\log_e$ (relative risk/rate)	$\log_e R_i (1 \pm 1.96 / \sqrt{X^2})$	$\log_e R_a (1 \pm 1.96 / \sqrt{X^2})$
and hence confidence limits on vaccine efficacy may be derived.		

A discussion of the use of risk and rate measures in the context of vaccine trials is given by Smith et al (1984).

If each subject may experience the endpoint of interest more than once, the second column in the table displaying the results of a trial should be replaced by "total number of events in group" and the comparison of the groups is made by dividing these numbers by the person-time-at-risk in each group, which, in this instance, will be the total period of follow-up for the subjects in the respective groups. Thus, if  $e_1$  and  $e_2$  are the number of endpoints in the vaccinated and unvaccinated groups, respectively, the rates of occurrence are estimated by  $r_1 = e_1/y_1$  and  $r_2 = e_2/y_2$  and a measure of vaccine efficacy (%) is given by  $100(r_2 - r_1)/r_2$ .

Testing for statistical significance, and deriving confidence intervals on the estimate of vaccine efficacy, is not straightforward in this case and the advice of a statistician should be sought. In general, the methods outlined above for the comparison of rates are appropriate for multiple episodes only if it is reasonable to assume that once a subject has experienced one or more episodes he or she is no more or less likely to experience another episode than those who have experienced no episodes up to that time in the same group (vaccinated or unvaccinated). This assumption is rarely valid as susceptibility to disease events is usually very heterogeneous between individuals in a community, even among those of the same age and sex. In such circumstances statistical significance might be tested using a t-test or, preferably, a non-parametric test.

#### 7. Ethical considerations

The design and implementation of a trial should conform to both national and international ethical standards. Prior to implementation, the study design must be reviewed by a properly constituted local or national ethical committee, which must include representatives of the group to be vaccinated, responsible health authorities, and technical experts. There must be informed consent and a written statement of how it is to be obtained. There must be no pressure to participate and no differences between services offered to acceptors and non-acceptors. Health services for treatment of vaccine reactions and malaria infection and adequate referral and follow-up capability must be available. An independent clinical referee should be designated and given the authority to break the trial code on an individual basis, should that seem desirable.

Before embarking on a field trial, the safety of a candidate vaccine will have been assessed in subjects from both non-endemic and endemic areas. Only those candidate vaccines that have acceptable levels of safety and are produced according to internationally recognized manufacturing practices, such as those recommended by WHO, will proceed to testing in the field.

The field testing of a blood-stage vaccine raises several special ethical issues. Because of the difficulty in assessing adequately the efficacy of a blood-stage vaccine in non-immune volunteers, it may be necessary to conduct trials in partially immune subjects exposed to natural challenge (Phase IIb/III) without prior assurance that the vaccine provides any protection. Furthermore, because of the difficulty in identifying a reduction in symptoms in partially-immune adult volunteers who receive the vaccine, it may be necessary to undertake trials in children, in whom protection against clinical effects of malaria infection will be easier to measure, without definitive proof that the vaccine is protective. In such cases, the decision to proceed with trials will be based on evidence from earlier human trials (safety, immunogenicity) and the protective effect, if any, from trials in non-human primates.

C. SELECTED REFERENCES

1. Ethical Aspects

Proposed International Guidelines for Biomedical Research involving Human Subjects, CIOMS, Geneva, 1982.

Report of the International Conference on the Role of the Individual and the Community in the Research, Development and Use of Biologicals. Bulletin of the World Health Organization, 55(Suppl. 2): 167-177 (1977).

2. Standards for Production and Regulatory Issues

Acceptability of cell substrates for production of biologicals. WHO Technical Report Series, No. 747, (1987). World Health Organization, Geneva.

Certification Scheme on the Quality of Pharmaceutical Products moving in International Commerce and Text of Good Manufacturing Practice (GMP). Document WHO/PHARM/82.4 Rev. 3, (1987), World Health Organization, Geneva.

Proposed General Requirements for Manufacturing Establishments and Control Laboratories. Expert Committee on Biological Standardization, Document BS/89.1616, WHO, Geneva, (1989).

Sezaret, P. and Magrath, D.I. Quality assurance in vaccines. In: "The Assessment of the Safety and Efficacy of Vaccines to Regulate Fertility". Proceedings of a WHO Symposium, June 12-16, (1989), Geneva (in press).

The National Licensing and Control of Biological Products. Proposed revised WHO document, Expert Committee on Biological Standardization, Document BS/89.111, WHO, Geneva, (1989).

WHO Expert Committee on Biological Standardization, Thirty-ninth report. Technical Report Series, No. 786, (1989), World Health Organization, Geneva.

3. Asexual Blood-stage Vaccine Research

Anders, R.F., Murray, L.J., Thomas, L.M., Davern, K.M., Brown, G.V. & Kemp, D.J. Structure and function of candidate vaccine antigens in Plasmodium falciparum. Biochemical Society Symposia, 53: 103-114 (1988).

Aslund, L., Sjölander, A., Wahlgren, M., Wahlin, B., Ruangjirachuporn, W., Berzins, K., Wigzell, H., Perlmann, P. & Petersson, U. Synthetic gene construct expressing a repeated and highly immunogenic epitope of the Plasmodium falciparum antigen Pf155. Proceedings of the National Academy of Science, USA, 84: 1399-1403 (1987).

Chang, S.P., Kramer, K.J., Yamaga, K.M., Kato, A., Case, S.E. & Siddiqui, W.A. Plasmodium falciparum: gene structure and hydrophathy profile of the major merozoite surface antigen (gp195) of the Ugando-Palo Alto isolate. Experimental Parasitology, 67: 1-11, (1988).

Cheung, A., Leban, J., Shaw, A.R., Merkli, B., Stocker, J., Chizzolini, C., Sander, C. & Perrin, L.H. Immunization with synthetic peptides of a Plasmodium falciparum surface antigen induces antimerozoite antibodies. Proceedings of the National Academy of Science, USA, 83: 8328-8332 (1986).

- Cheung, A., Leban, J., Shaw, A.R., Merkli, B., Stocker, J., Chizzolini, C., Sander, C. & Perrin, L.H. Immunization with synthetic peptides of a Plasmodium falciparum surface antigen induces antimerozoite antibodies. Proceedings of the National Academy of Science, USA, 83: 8328-8332 (1986).
- Collins, W.E., Anders, R.F., Pappaioanou, M., Campbell, G.H., Brown, G.V., Kemp, D.J., Coppel, R.L., Skinner, J.C., Andrysiak, P.M., Favaloro, J.M., Corcoran, L.M., Broderson, J.R., Mitchell, G.F. & Campbell, C.C. Immunization of Aotus monkeys with recombinant proteins of an erythrocyte surface antigen of Plasmodium falciparum. Nature, 323: 259-262 (1986).
- Collins, W.E., Anders, R.F., Pappaioanou, M., Campbell, G.H., Brown, G.V., Kemp, D.J., Coppel, R.L., Skinner, J.C., Andrysiak, P.M., Favaloro, J.M., Corcoran, L.M., Broderson, J.R., Mitchell, G.F. & Campbell, C.C. Immunization of Aotus monkeys with recombinant proteins of an erythrocyte surface antigen of Plasmodium falciparum. Nature, 323: 259-262 (1986).
- Dubois, P., Dedet, J.-P., Fandeur, T., Roussilhon, C., Jendoubi, M., Pavillac, S., Mercereau-Puijalon, O. & Pereira da Silva, L. Protective immunization of the squirrel monkey against asexual blood stages of Plasmodium falciparum by use of parasite protein fractions. Proceedings of the National Academy of Science, USA, 81: 229-232 (1984).
- 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, WHO, Geneva. Document TDR/IMMAL/SWG(10)/88.3, (1988).
- Gentz, R., Certa, U., Takacs, B., Matile, H., Döbeli, H., Pink, R., Mackay, M., Bone, N. & Scaife, J.G. Major surface antigen p190 of Plasmodium falciparum: detection of common epitopes present in a variety of plasmodia isolates. EMBO Journal, 7: 225-230 (1988).
- Holder, A.A. The precursor to major merozoite surface antigens: structure and role in immunity. Progress in Allergy, 41: 72-97 (1988).
- Hui, G.S.N. & Siddiqui, W.A. Serum from Pf195 protected Aotus monkeys inhibit Plasmodium falciparum growth in vitro. Experimental Parasitology, 64: 519-522 (1987).
- Kabilan, L., Troye-Blomberg, M., Perlmann, H., Andersson, G., Högh, B., Petersen, E., Björkman, A. & Perlmann, P. T-cell epitopes in Pf155/RESA, a major candidate for a Plasmodium falciparum malaria vaccine. Proceedings of the National Academy of Science, USA, 85: 5659-5663 (1988).
- Mackay, M., Goman, M., Bone, N., Hyde, J.E., Scaife, J., Certa, U., Stunnenberg, H. & Bujard, H. Polymorphism of the precursor for the major surface antigens of Plasmodium falciparum merozoites: studies at the genetic level. EMBO Journal, 4: 3823-3829 (1985).
- Marsh, K. & Howard, R.J. Antigens induced on erythrocytes by Plasmodium falciparum: expression of diverse and conserved determinants. Science, 231: 150-153 (1986).
- Miettinen-Baumann, A., Strych, W., McBride, J.S. & Heidrich, H.-G. A 46 000 dalton Plasmodium falciparum merozoite surface glycoprotein not related to the 185 000 - 195 000 dalton schizont precursor molecule: isolation and characterization. Parasitology Research, 74: 317-323 (1988).

- Nguyen-Dinh, P., Berzins, K., Collins, W.E., Wahlgren, M., Udomsangpetch, R. & Perlmann, P. Antibodies to Pfl55, a major antigen of Plasmodium falciparum: longitudinal studies in humans. American Journal of Tropical Medicine & Hygiene, 37: 501-505 (1987).
- Ozaki, L.S., Jendoubi, M., Mattei, D., Braun-Breton, C., Jouin, H., Blisnick, T. & Pereira da Silva, L. Clones of DNA sequences coding for two polypeptide antigens of asexual blood stages of Plasmodium falciparum potentially related to protection. In: Agabian, N., Goodman, H. & Nogueira, N. (eds) Molecular Strategies of Parasitic Invasion (UCLA Symp. Mol. Cell. Biol. No. 42), Alan R. Liss, New York, 1987, pp. 683-690.
- Patarroyo, M.E., Romero, P., Torres, M.L., Clavijo, P., Andrew, D., Lozada, D., Sanchez, L., Del Portillo, P., Pinilla, C., Moreno, A., Alegria, A. & Houghten, R. Protective synthetic peptides against experimental Plasmodium falciparum-induced malaria. In: Chanock, R.M., Lemer, R.A., Brown, E. & Ginsberg, H., eds. Vaccines 87. Modern Approaches to New Vaccines, CSH Press, Cold Spring Harbor, NY, 1987, pp. 117-124.
- Patarroyo, M.E., Amador, R., Clavijo, P., Moreno, A., Guzman, F., Romero, P., Tascon, R., Franco, A., Murillo, L.A., Ponton, G. & Trujillo, G. A synthetic vaccine protects humans against challenge with asexual blood stages of Plasmodium falciparum malaria. Nature, 332: 158-161 (1988).
- Patarroyo, M.E., Romero, P., Torres, M.L., Clavijo, P., Moreno, A., Martinez, A., Rodriguez, R., Guzman, F. & Cabezas, E. Induction of protective immunity against experimental infection with malaria using synthetic peptides. Nature, 328: 629-632 (1987).
- Perlmann, H., Berzins, K., Wahlin, b., Hagstedt, M., Andersson, J., Högh, B., Petersen, E., Björkman, A. & Perlmann, P. Antibodies in human immune sera to amino acid repeats in Pfl55, a potential vaccine candidate antigen of the human malaria parasite Plasmodium falciparum. Scandinavian Journal of Immunology, 26: 325 (1987).
- Perrin, L.H., Merkli, B., Loche, M., Chizzolini, C., Smart, J. & Richle, R. Antimalaria immunity in Saimiri monkeys: immunization with surface components of asexual blood stages. Journal of Experimental Medicine, 160: 441-451 (1985).
- Perrin, L.H., Merkli, B., Gabra, M.S., Stocker, J.W., Chizzolini, C. & Richle, R. Immunization with a Plasmodium falciparum merozoite surface antigen induces a partial immunity in monkeys. Journal of Clinical Investigation, 75: 1718-1721 (1985).
- Peterson, M.G., Coppel, R.L., McIntyre, P., Langford, C.J., Woodrow, G., Brown, G.V., Anders, R.R. & Kemp, D.J. Variation in the precursor to the major merozoite surface antigens of Plasmodium falciparum. Molecular & Biochemical Pharmacology, 27: 291-302 (1988).
- Roger N., Dubremetz, J.F., Delplace, P., Fortier, B., Tronchin, G. & Vernes, A. Characterization of a 225 kilodalton rhoptry protein of Plasmodium falciparum. Molecular & Biochemical Parasitology, 27: 135-142 (1988).
- Siddiqui, W.A., Tam, L.Q., Kramer, K.J., Hui, G.S.N., Case, S.E., Yamaga, K.M., Chang, S.P., Chan, E.B.T. & Kan, S.-C. Merozoite surface coat precursor protein completely protects Aotus monkeys against Plasmodium falciparum malaria. Proceedings of the National Academy of Science, 84: 3014-3018 (1987).

- Tanabe, K., Mackay, M., Goman, M. & Scaife, J.G. Allelic dimorphism in a surface antigen gene of the malaria parasite Plasmodium falciparum. Journal of Molecular Biology, 195: 273-287 (1987).
- Wahlgren, M., Björkman, A., Perlmann, H., Berzins, K. & Perlmann, P. Anti-Plasmodium falciparum antibodies acquired by residents in a holoendemic area of Liberia during development of clinical immunity. American Journal of Tropical Medicine & Hygiene, 35: 22-29 (1986).
- Weber, J.L., Leininger, W.M. & Lyon, J.A. Variation in the gene encoding a major merozoite surface antigen of the human malaria parasite Plasmodium falciparum. Nucleic Acids Research, 14: 3311-3323 (1986).
4. Parasitology and Clinical Aspects
- Bruce-Chwatt, L.J. A longitudinal survey of natural malaria infection in a group of West African adults. West African Medical Journal, 12: 141-173 (1963).
- Bruce-Chwatt, L.J. Essential Malariaology. Second Edition. William Heinemann Medical Books Ltd., London, (1985).
- Immunological aspects of malaria immunology. Report of the Scientific Working Groups on the Immunology of Malaria and on Applied Field Research in Malaria. WHO, Geneva. Document TDR/IMMAL-FIELDMAL/SWG(2)/88.3 (1988).
- Malaria. Principles and Practice of Malariaology. Ed. W.H. Wernsdorfer and Sir I. McGregor. Churchill Livingstone, Edinburgh, London, Melbourne and New York, (1988).
- Miller, M.J. Observations on the natural history of malaria in the semi-resistant West African. Transactions of the Royal Society of Tropical Medicine and Hygiene, 52: 152-168, (1958).
- World Health Organization, Malaria Action Programme. Severe and complicated malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80(Suppl.): 1-50, (1986).
5. Epidemiology and Statistics
- Kahn, H.A. An Introduction to Epidemiologic Methods. Oxford University Press, New York, (1983).
- Levy, P.S. and Lemeshow, S. Sampling for Health Professionals. Lifetime Learning Publications, Belmont, California, (1980).
- Molineaux, L. and Gramiccia, G. The Garki Project. WHO, Geneva, (1980).
- Orenstein, W.A. et al. Field evaluation of vaccine efficacy. Bulletin of the World Health Organization, 63: 1055-1068, (1985).
- Pocock, S.J. Clinical Trials. A Practical Approach. John Wiley & Sons, Chichester, (1983).
- Radhakrishnan, S., Nair, N.G.K. and Jagabal, P. Implications of misdiagnosis in field trials on vaccines. Indian Journal of Medical Research, 80: 711-720 (1984).

World Health Organization, Geneva. Sample Size Determination. A User's Manual. WHO Epidemiology and Statistical Methodology Unit. Document WHO/HST/ESM/86.1.

Schlesselman, J.J. Sample size requirements in cohort and case-control studies of disease. American Journal of Epidemiology, 99: 381-384, (1974).

Schlesselman, J.J. Case-control Studies Design, Conduct, Analysis. Oxford University Press, New York, (1982).

Smith, P.G., Rodrigues, L.C. and Fine, P.E.M. Assessment of the protective efficacy of vaccines against common diseases using case-control and cohort studies. International Journal of Epidemiology, 13: 87-93, (1984).

## ANNEX I

### METHODS OF MEASUREMENT

#### 1. Introduction

Most asexual blood-stage parasite vaccine trials will involve making the measurements described below during the pre-intervention stage of the study and after vaccination has been undertaken. Other measurements which would greatly facilitate comparison between trials include measurement of the sporozoite inoculation rate, determination of the prevalence of haemoglobin abnormalities in the study population, analysis of the antigenic characteristics of the strains of *P. falciparum* prevalent in the study area and the immunological profile of the study population. The measurement of the sporozoite inoculation rate, while not indispensable, would be particularly useful, because it would provide an independent estimation of the challenge, and because of the non-linear relationship between entomological inoculation rate and human incidence rate.

The following aspects of measurement require critical review during the preparatory phase of a trial: standardization of all field, clinical and laboratory procedures; quality control monitoring in the trial; reproducibility of measurements within and between field workers and at different times; comparability of procedures and methods with other studies; sensitivity and specificity of any diagnostic methods; level of precision required for all measurements to be taken in the trial (not necessarily the highest possible); minimization of the numbers of specimens (e.g. of blood) to be collected; arrangements for the collection, transportation and preservation of specimens; procedures for recording of data and for the analysis of results.

#### 2. Detection and investigation of deaths

It is desirable that Phase III field trials should include mortality as an end-point. Even when the size of the trial is insufficient to make this feasible, surveillance for mortality among study subjects should be maintained.

The most appropriate method for determining mortality among trial subjects will be determined by local circumstances. In some situations, for example among migrants involved in a large industrial project, any deaths will be notified to project management or medical personnel and, thus, a passive surveillance system, adequate to determine mortality rates, will exist.

In other communities, including many areas of rural Africa, a more active mortality surveillance system will be required. This may be set up by stationing a reporter in each community in the trial area, possibly a member of the community, whose responsibilities will include the identification and reporting of any deaths among study subjects. In areas where post-mortem examinations are not feasible some information on cause of death may be obtained by asking questions of the bereaved family about the final illness and circumstances of death. If mortality from malaria, as established by the verbal autopsy technique, is used as an end-point for a vaccine trial it is essential that the criteria used in attributing death to malaria should be clearly established before the start of the trial and subsequently adhered to.

#### 3. Measurement of clinical malaria

A universally agreed definition of clinical malaria does not exist and the criteria used to make this diagnosis may vary from area to area. Provided that the relevant measurements are made it may be possible to study the effects of a

vaccine using definitions of clinical malaria based on more than one criterion, for example as defined by fever accompanied by high or moderate levels of parasitaemia.

Clinical episodes of malaria in trial subjects may be detected by either active or passive case detection procedures. It is likely that most trials will involve both approaches, the degree of emphasis placed on each will be determined by the access that those in the trial have to treatment facilities. In communities where most patients with even minor illnesses report to a single or a small number of treatment facilities, records from these clinics may provide all the information required on morbidity in a trial. In less privileged communities more active case detection will be required. One approach that has proved successful in other malaria intervention studies is a weekly visit to each trial subject, during which a brief morbidity questionnaire is completed, the subject's temperature is recorded and a blood film is made if a raised temperature is found. The level of temperature chosen to indicate abnormality should be determined in preliminary studies; it will be influenced by both the route by which the temperature is recorded and by the characteristics of the study community. In previous studies in malaria endemic areas of Africa, an axillary temperature in the range of 37.5 - 38.0°C has been found to provide a useful cut-off point. Electronic digital thermometers provide a convenient and safe way of measuring temperature in field trials.

Unless a trial is large, it is unlikely that the incidence of severe or complicated malaria, such as cerebral malaria, could be used as an end-point. However, the severity of clinical attacks of malaria identified by active and passive case detection techniques, for example an inability to work or attend school, should be recorded according to pre-determined criteria.

In areas where malaria is highly endemic, the detection of fever and malaria parasitaemia in the same child does not necessarily mean that malaria parasites were responsible for the child's fever, as a significant proportion of all children in the community will have malaria parasitaemia at any one time. The occurrence of a large number of cases of fever accompanied by parasitaemia, but in which malaria parasites were not the cause of the fever, could dilute the apparent protective effect of a malaria vaccine which had its main effect on fever and illness caused by malaria parasites rather than on the prevalence of infection. It might be possible to make some correction for this potential bias if measurements of the prevalence of parasitaemia were made in randomly selected afebrile trial subjects at various points during the post-vaccination observation period.

#### 4. Determination of the incidence of infection

In order to determine the incidence of new infections in trial subjects, parasitaemia should be cleared from all subjects included in this aspect of the trial by the administration of an effective schizonticidal drug, at the time of or shortly before immunization. Once vaccine or placebo has been given subjects in this cohort should be bled at regular intervals, perhaps once a week or once a month depending upon the level of malaria transmission, and blood films examined for asexual parasites and gametocytes. In this way it will be possible to determine the incidence of new infections in vaccinated and control subjects over a defined period. In some communities the development of splenomegaly provides strong evidence of a recent malaria infection.

The currently accepted method for detecting malaria infections is by microscopic examination of thick and thin blood films. The sensitivity of this technique depends on the volume of blood examined. The most commonly used substitutes for a standard volume of blood are 100 and 200 thick-film microscopic fields. Currently accepted measurements of density that allow

discrimination at high densities involve the counting of parasites either against white blood cells in the thick film or against red blood cells in the thin film. In transforming such relative counts into numbers per volume, it is usual to assume a fixed white or red blood cell count in the study population. Parasite density is measured most accurately by combining a red blood cell count with a parasite count made on a thin blood film but this approach is only suitable for infections with a high level of parasitaemia. Notwithstanding efforts to standardize all procedures involved, their performance continues to vary between investigators and with the same investigator over time, and this variation is not always clearly perceived or documented.

The following procedures are considered essential when blood film positivity is used as a criterion for assessing the efficacy of a malaria vaccine:

- (a) standardization of laboratory procedures within each trial and as far as possible between trials;
- (b) taking of blood films in duplicate;
- (c) "blind" microscopic blood film examination; (e.g. the microscopist should not know if a slide is from a vaccinated or unvaccinated individual or from a febrile or afebrile subject)
- (d) preservation of blood films for further reference;
- (e) quality control within each trial by re-examination of a coded sample of blood films;

The use of newer diagnostic techniques, such as DNA and RNA probes and antigen detection assays, might be considered to measure infection rates. Whilst these cannot at present replace microscopic examination they may well become valuable additions, permitting rapid, objective and specific evaluation of large numbers of blood samples.

#### 5. Measures of infectivity of subjects for vector

Gametocyte counts should be made routinely on positive blood films. However, this is an indirect measure of the infectivity of a subject for vectors. Alternative methods, such as membrane-feeding of laboratory-bred mosquitoes on blood taken from an infected individual, will provide a direct measure of infectivity. However, these methods are difficult and should only be undertaken by experienced workers.

#### 6. Measurement of side-effects

The measurement of side-effects should be considered in relation to the time following vaccination at which side-effects are expected. Current or past P. falciparum infection may increase the risk and severity of side-effects, so that early trials in non-endemic countries may not be adequate predictors. Side-effects should be assessed in all phases of vaccine trials.

Vaccinated and control (placebo vaccinated) persons should be observed immediately after vaccination to identify acute reactions. A follow-up visit to the homes of the study population should be made within a few days to identify early reactions, such as pain and abscess at the vaccination site, and constitutional symptoms, including fever. This can be done most readily by placing a project worker in each study community.

Delayed toxicity, including immunopathological phenomena, may not become apparent for many months, and observations for side-effects may have to continue beyond the period of vaccine protection and for long enough to cover the first expected challenge infection. Both case detection and population surveys should be used.

7. Immunological assays

It is unlikely that it will be possible to make extensive immunological measurements in all those in a field trial, which may include large numbers of subjects. However, it will be essential to make some immunological measurements in selected subjects to confirm that the batch of antigen(s) used in the vaccine induces an appropriate immune response, for example an antibody response to one or more important epitopes. It will also be important to determine in some subjects the duration of the immune response induced by vaccination, and the possible effects of boosting by natural infection by measuring immune responses in vaccinated and control subjects at various times after vaccination.

8. Drug utilization

Drug utilization during the period of post-vaccination follow-up should be assessed by (a) direct questioning of trial participants (b) review of dispensary records and those of shops selling drugs within the study area (c) random urine assays for the anti-malarial drugs which are known to be used frequently in the trial community.

## ANNEX II

## CANDIDATE ANTIGENS FOR INCLUSION IN VACCINES\*

The antigens of P. falciparum asexual blood-stages include a number of molecules which appear to play a role in the induction of protective immune responses. It is not yet clear which antigen(s) will prove to be the most suitable for vaccine development. Antigens currently considered to be candidates for inclusion in anti-P. falciparum asexual blood-stage vaccines are the following:

- a schizont surface glycoprotein (kDa 185,000 - 220,000) which is proteolytically processed at about the time of schizont rupture to generate the majority of the antigens detected on the surface of merozoites. This antigen has been referred to as PMMSA, Pf190, gp185, Pf185, PSA, MSPP and MSA-1. Monoclonal antibodies to this antigen can inhibit merozoite invasion in vitro. Immunization of monkeys with the protein purified from parasites, or with an N-terminal synthetic peptide, induced some protection against challenge infection. Another synthetic peptide, derived from the same N-terminal region, was used as a component of a multivalent synthetic vaccine, with encouraging results, in human volunteers. Considerable antigenic diversity exists in this molecule among different strains of P. falciparum. Sequencing studies on the gene from many strains have shown that the molecule contains alternating conserved and variable regions. The variable regions are mostly of two types and it appears that diversity has arisen, at least in part, as a result of intragenic recombinations. A vaccine incorporating conserved regions of this antigen would hopefully induce immune responses that are equally effective against all strains of P. falciparum.
- the ring-infected erythrocyte surface antigen (kDa 155,000), known as RESA or Pf155, is transferred to the erythrocyte membrane from the invading merozoite. The RESA/Pf155 gene has been cloned and sequenced.

The RESA polypeptide contains two blocks of repetitive sequences which encode immuno-dominant epitopes. These repeats are conserved among different strains and no antigenic diversity of this molecule has been observed. Polyclonal and monoclonal antibodies to various repeat epitopes in RESA/Pf155 inhibit merozoite invasion in vitro. In a vaccine trial in Aotus monkeys fragments of RESA/Pf155 provided partial protection against challenge with P. falciparum. Protection in this monkey trial correlated with the production of antibodies to two different repeat sequences of 8 and 11 amino acids, but not with antibodies to the dominant 4-amino-acid repeat.

- a merozoite surface antigen of kDa 45,000 - 56,000 termed MSA-2 or gp46-53. The MSA-2 gene from several strains of P. falciparum has been sequenced to reveal conserved C-terminal and N-terminal sequences flanking strain-variable regions which contain different repetitive sequences. Although there are a large number of MSA-2 alleles, there are a smaller number of allelic families, each characterized by a particular sequence repeat. Monoclonal antibodies to this antigen, one of which reacts with an epitope encoded by sequences within the repeat, inhibit merozoite invasion in vitro. A vaccine based on MSA-2 could include multiple repeat sequences or, alternatively, conserved regions of the molecule.

---

\* This section was prepared by Dr R.F. Anders and Dr O. Mercereau-Puijalon.

antigens present in the rhoptries of merozoites that are discharged onto the red cell surface during the invasion process. Several groups have described monoclonal antibodies which react with the rhoptries and immunoprecipitate antigenic complexes. One of the complexes contains three polypeptides of kDa 150,000, 130,000 and 110,000. The other consists of at least two polypeptides of kDa 82,000 and 41,000. The identity of the proteins in these complexes remains to be clearly established. A serine protease has been identified as a polypeptide of kDa 82,000-76,000 belonging to the complex 82,000-41,000 immunoprecipitated by one of the anti-rhoptry monoclonal antibodies. An inactive form of the protease is anchored into a membrane by a GPI moiety. The protease is enzymatically active only when excised from the membrane by a specific phospholipase. A protein of kDa 41,000 isolated from the same 82,000-41,000 complex provided partial protection when used to immunize Saimiri monkeys. Several genes coding for proteins belonging to these complexes have now been cloned. Rhoptry antigens appear to lack the extensive antigenic and structural diversity found in the merozoite surface antigens. Their potential as vaccine candidates derives from the demonstration that a P. yoelii rhoptry antigen protected immunized mice, and that monoclonal antibodies to P. falciparum rhoptry antigens inhibit merozoite invasion in vitro. No protection has yet been reported with cloned rhoptry antigens of human malaria parasites.

antigens exposed on the surface of infected erythrocytes which have been detected by surface labelling and immunoprecipitation, indirect immunofluorescence microscopy, agglutination and inhibition of cytoadherence. It is not clear whether these different procedures all detect the same antigen. Immune sera that contain antibodies capable of blocking cytoadherence recognize a polymorphic surface antigen (kDa 250,000 - 350,000) of the homologous isolate. This antigen has been called PfEMP1 (P. falciparum erythrocyte membrane protein 1). Other antigens have been shown to play a role in cytoadherence, in particular the knob-associated histidine-rich protein. Recently a human monoclonal antibody, 33G2, was found to interfere with cytoadherence in knobless parasites. Several antigens have been described in association with the membrane of the infected erythrocyte, and the corresponding genes have been cloned. None of these antigens has yet been used in vaccine trials. However, the titre of antibodies to neo-antigens on the surface of the infected erythrocyte correlates with resistance to infection with P. falciparum.

antigens of kDa 35,000 and kDa 55,000 of unknown location which, when isolated from infected erythrocytes, partially protected Aotus monkeys against infection with P. falciparum. N-terminal peptides from these antigens (conjugated to carrier protein) also partially protected Aotus monkeys. Protection was improved by immunizing monkeys with a mixture of these two peptides and a peptide from the PMMSA. A hybrid peptide combining these three peptides and other sequences, in the form of a disulphide linked polymer, protected human volunteers in the first clinical trial of an asexual blood-stage vaccine.

a histidine and alanine rich protein (PfHRP2) of kDa 70,000 which is secreted from infected erythrocytes. A fragment of this antigen expressed in E. coli partially protected Aotus monkeys.

a serine rich antigen, kDa 113,000 - 140,000, found in the parasitophorous vacuole. This antigen has been referred to as SERA, p113, p126 and Pf140. The antigen is a component of immune complexes formed when schizonts rupture in the presence of immune serum. Saimiri monkeys

immunized with Pf140 isolated from infected erythrocytes were partially protected against challenge infection with a heterologous strain of P. falciparum.

- antigen 96-R or GBP130. This thermostable antigen is released from the schizont at the time of merozoite release, and remains loosely associated with the surface of the merozoite. A good correlation has been found between acquired protective immunity and the humoral response to this antigen in monkeys. A fragment of this antigen expressed in E. coli failed to induce protection in monkeys.

ANNEX III

RESEARCH TOPICS RELEVANT TO VACCINE TRIALS

In addition to the collection of baseline data, against which to evaluate the effect of candidate malaria vaccines in different epidemiological situations, several other preliminary epidemiological investigations would be relevant for the further development and epidemiological evaluation of P. falciparum vaccines, including asexual blood-stage vaccines. The subject was reviewed by a joint IMMAL-FIELDMAL Scientific Working Group in September 1988, which identified and outlined the research required (1). Factors that might be studies include:

1. Diversity of human malaria parasites and of the immune response to them.

There is a need for further research on the uniformity/diversity of P. falciparum blood-stage antigens that are candidates for inclusion in vaccines and of human immune responses to them. Examples of important aspects of the research, would include: the dominant T- and B-cell epitopes; the different levels of parasite populations (within single humans or mosquitoes, within and between geographical areas); the relevant genetic mechanisms in parasites and man; the possible development of diversity under the selection pressure of immune responses.

2. The development of the immune response to malaria parasites and its correlation with protection.

There is a need for further research on:

- 2.1 the development of specified human immune responses to defined P. falciparum asexual blood-stage antigens under natural exposure and their correlation with natural protection, including interaction between different immune responses in the production of protection, variation in immune responses and protection associated with intensity, seasonality and duration of exposure and with individual characteristics, and the transition from passive (maternal) to active immunity, in situations of either perennial or seasonal transmission;
- 2.2 the methodology of epidemiological measurement of malaria morbidity (disease, including its grading by severity) and mortality;
- 2.3 the epidemiology of malaria morbidity (disease, including its grading by severity) and mortality, in different situations, and its relationship to the distribution of sporozoite inocula and of immune responses.

The development of immune responses and their correlation with protection is best investigated through longitudinal cohort studies, with emphasis on the detection of clinical episodes; they should preferably be conducted in contrasting epidemiological situations and consider simultaneously multiple facets of the immune response; cohorts of special interest include infants

---

(1) Scientific Working Groups on Immunology of Malaria and on Applied Field Research in Malaria.  
Immunological Aspects of Malaria Epidemiology.  
TDR/IMMAL-FIELDMAL/SWG(2)/88.3

and children in endemic areas, women in endemic areas, before and during their first pregnancy, non-immune immigrants into endemic areas, previously immune individuals returning to an endemic area after temporary residence in a non-endemic area. Cross-sectional studies may be useful for the selection of immune responses worth studying longitudinally. Case-control studies may be useful for the identification of risk factors of severe and complicated malaria, which may be too rare for conducting longitudinal studies at an acceptable cost.

3. Natural patterns of inoculation, their generation and their consequences.

Research is needed on the natural distribution of sporozoite inocula in different epidemiological situations, the generation of those distributions (including the effect of immune responses and drugs, not only in man but also in the vector) and their consequences in terms of pathology and of the development, maintenance, boosting of immune responses and protection; the research requires the development of measurement methodologies, and some of the pertinent investigations may be feasible only in animal models.

4. Use of seroepidemiological tests for the epidemiological characterization and classification of malaria situations.

The characterization and classification of malaria situations will be essential for the field evaluations of malaria vaccines. The potential of several recently developed immunological tests for the purpose needs to be evaluated.

\* \* \*