

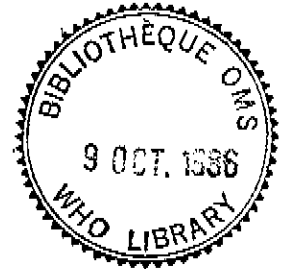


UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR
RESEARCH AND TRAINING IN TROPICAL DISEASES

Geneva, July 1986

*Plasmodium falciparum - immuno-deficient
malaria - prevention and
control
vaccines*

GUIDELINES FOR THE EPIDEMIOLOGICAL EVALUATION OF
PLASMODIUM FALCIPARUM SPOROZOITE VACCINES:



PREPARED JOINTLY BY THE WHO MALARIA ACTION PROGRAMME
AND THE UNDP/WORLD BANK/WHO SPECIAL PROGRAMME FOR
RESEARCH AND TRAINING IN TROPICAL DISEASES

CONTENTS

	<u>Page</u>
INTRODUCTION	2
A. GENERAL BACKGROUND	3
1. Current Status of Development of <u>Plasmodium falciparum</u> Sporozoite Vaccines	3
2. Mode of Action of <u>P. falciparum</u> Sporozoite Vaccines and Their Potential Effects	3
3. Sporozoite Vaccines and Malaria Control	4
4. Factors Likely to Affect the Outcome of <u>P. falciparum</u> Sporozoite Vaccine Trials	6
B. FIELD TRIALS IN MALARIA-ENDEMIC COUNTRIES	9
1. Information Required from Early Clinical Trials (Phase I and Phase II)	9
2. Criteria for the Selection of Areas/Populations for Field Trials	9
3. Study Design for the Measurement of Protection in a Resident Population under Natural Challenge (Phase III Trial)	10
4. Study Design for Measuring the Impact of a Sporozoite Vaccine on Transmission: Salient Points	16
5. Methods of Data Collection and of Measurement	17

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C.	PRELIMINARY OR CONCOMITANT RESEARCH RELEVANT TO THE EPIDEMIOLOGICAL EVALUATION OF <u>P. FALCIPARUM</u> SPOOROZOITE VACCINES	24
1.	Methods of Measurement	24
2.	Epidemiology of <u>P. falciparum</u> Malaria	24
3.	Expected Effects of Sporozoite Vaccines	25
4.	Simulation Models	25
D.	SELECTED REFERENCES	25

INTRODUCTION

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 Geneva in February 1985⁽¹⁾. The present document considers the epidemiological evaluation of Plasmodium falciparum sporozoite vaccines in malaria-endemic areas. It is concerned mainly with the measurement of protective efficacy in a resident population under conditions of natural challenge. Measurement of its potential impact on transmission is also briefly discussed.

Epidemiological evaluation should be carried out only after meticulous early clinical studies (Phase I and Phase II) are completed in selected volunteers in both nonendemic and endemic areas. These early trials should establish the basic safety, immunogenicity and efficacy of the vaccine, the appropriate dosage, and the effect of prior antimalarial treatment. Before conducting an epidemiological trial in an endemic area, the results of early trials require confirmation in the same or a similar endemic area.

This document is addressed to national health authorities, in particular to those of malarious countries interested in the potential use of P. falciparum sporozoite vaccines for the control of malaria and to research scientists interested in the epidemiological 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 epidemiological (Phase III) trials, but also the required earlier clinical trials.

The document was produced by 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 Steering Committees of the TDR Scientific Working Groups on the Immunology of Malaria (IMMAL), on Applied Field Research in Malaria (FIELDMAL), and on Epidemiology (EPID).

In this report, the terms "immune" and "immunity" indicate resistance to infection, resulting from previous infection or vaccination; "immune response", a process either humoral or cellular of the body's immune system which follows exposure to antigens, e.g. following infection or vaccination, but which does not necessarily reflect or correlate with a state of protection.

(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/85.3.

A. GENERAL BACKGROUND

1. Current Status of Development of Plasmodium falciparum Sporozoite Vaccines

Peptide vaccines for use in human subjects must contain the relevant immunological information within their amino acid sequences. To induce an efficient, long-lasting antibody response, which can be boosted by subsequent infections, peptide antigens must contain structures (epitopes) recognized by the antibody-producing B cells and by T cells which regulate antibody production. The molecular structures which react with B cells are frequently distinct from those which react with T cells. Therefore, the T epitopes must be supplied either from a sequence in the native protein other than the B epitope or from unrelated molecules (e.g. tetanus toxoid). The implication of these immunologic principles for a sporozoite vaccine based on epitopes of the circumsporozoite (CS) protein is that boosting of vaccinated individuals through naturally acquired sporozoite inoculation may require that the vaccine contain T epitopes from the CS protein. If the vaccine T epitope is unrelated to the CS protein (e.g. tetanus toxoid), then the potential advantage of boosting by sporozoites could be lost.

In addition to their obligatory role in antibody production, T cells may block sporozoite infectivity by an antibody-independent mechanism (cellular immunity). To induce such immunity, malaria-specific T epitopes are crucial.

Anti-P. falciparum sporozoite vaccines now being developed are based on part of the CS protein on the surface of the parasite. The epitope concerned is recognized by antibodies which mediate the circumsporozoite precipitation reaction and neutralize sporozoite infectivity. The gene encoding the CS protein has been cloned and sequenced. The immunodominant epitope of the CS molecule contains tandemly repeated sequences of four amino acids (Asn-Ala-Asn-Pro), which form the basis of two vaccines now being developed. In one case, the vaccine antigen is produced by chemical synthesis of a twelve mer (3 sequential tetramers). The synthesized sequence is linked to a tetanus toxoid carrier molecule. Thus, the T epitope derives from a molecule unrelated to the CS protein. The other vaccine is based on the repetitive epitope expressed by recombinant DNA in Escherichia coli. In this case the molecule is larger and may contain both B-cell and T-cell epitopes. However, 32 amino acids in this recombinant peptide (tet32) are unrelated to the CS protein and may function as a T-cell epitope. In both vaccines, aluminium hydroxide is used as adjuvant. Clinical trials of vaccine safety and immunogenicity (Phase I) in adult volunteers have begun in the United States.

2. Mode of Action of P. falciparum Sporozoite Vaccines and Their Potential Effects

2.1 Mode of action of sporozoite vaccines

There is evidence from animal models that immunization against sporozoites can prevent the establishment of liver infection. Immunity to sporozoites is in part antibody mediated, and antibodies have been shown to neutralize sporozoite infectivity in animals and in cultured liver cells. Less is known about cell-mediated mechanisms in ant sporozoite immunity. Limited studies in animal models suggest that they may play an important role. There is as yet no information about naturally acquired or vaccine-induced cellular immunity to sporozoites in human subjects.

Some other hypothetical aspects of the mode of action of sporozoite vaccines are worth considering: if the sporozoite inoculum injected by a mosquito were too large for a given level of immunity, only a certain proportion of the sporozoites may be prevented from gaining access to the

liver. A reduction in the number of sporozoites able to develop may reduce the severity of subsequent infection and disease by prolonging the prepatent and incubation periods, allowing more time for an immune response to be mounted against the blood stages and for treatment to be sought. No direct evidence exists on these points. Second, natural inoculations of sporozoites may usefully boost artificial ant sporozoite immunity.

2.2 Potential effects of *P. falciparum* sporozoite vaccines

These are summarized in the following Table:

Variable affected	Probable effects	Other possible effects
Infection (parasitaemia)	Decreased incidence Decreased prevalence	Decreased density of <i>P. falciparum</i> (1) Increased prevalence & density of <i>P. malariae</i> , <i>P. vivax</i> , <i>P. ovale</i> (2)
Immune response	Increased response to sporozoites Decreased response to other <i>P. falciparum</i> stages	
Morbidity (disease)	Decreased incidence	Decreased severity(1)
Mortality rate(3)	Decreased mortality rate(1)	Decreased case fatality

- (1) Assuming that vaccination could produce partial immunity against sporozoites, which in turn might reduce the severity of infection; on the other hand, a decreased immune response to blood stages could enhance the severity of infection.
- (2) Assuming that *P. falciparum* infection itself suppresses or masks infections with the other malaria parasites.
- (3) 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 latter may decrease more than the former.

3. Sporozoite Vaccines and Malaria Control

The major purpose of the epidemiological evaluation of a vaccine should be to determine its potential usefulness for disease control. A discussion of the potential uses of sporozoite vaccines is therefore important for planning their epidemiological evaluation. Two broad categories of indications can be distinguished: the protection of vaccinated individuals and the control of transmission. This distinction is not absolute: if a sufficiently high proportion of individuals is protected, there may be a significant impact on transmission. An appreciation of the distinction is nevertheless essential for the design of vaccine trials.

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 are the major target group for eventual vaccine use. Among permanent residents, newborn infants, with or without passive (maternal) immunity, and pregnant women are important subgroups. Infants, young children and other vulnerable groups that have incomplete or variable levels of naturally acquired immunity may require special study.

In some situations nonimmune immigrants who are newly settling into endemic areas may be of particular interest for evaluation of the vaccines.

As these vaccines may give only time-limited protection, periodic revaccination may be required for continuous protection. On the one hand, one-time vaccination might be sufficient to modify the first attack (e.g. by delaying it from infancy to childhood); on the other, after vaccination, immunity may be maintained by natural exposure to sporozoites. It will be critically important to determine the duration of protection, which may vary with age and intensity of natural sporozoite inoculation.

3.1.2 Protection of nonimmune temporary visitors to endemic areas

A sporozoite vaccine giving total protection for the duration of the visit would be the ideal form of protection of nonimmune temporary visitors to endemic areas, especially as effective chemoprophylaxis becomes increasingly problematic.

Although nonimmune visitors represent a relatively small fraction of those exposed, they are for social and economic reasons likely to represent a significant target group for vaccination. This could affect vaccine design, as the requirements of nonimmune visitors may differ from those of permanent residents of endemic areas. For example, convenience of mass administration is less important for temporary visitors than for permanent residents, and partial protection, if it exists, may be of greater interest for permanent residents than for temporary visitors. The acceptable cost of a vaccine may be higher for visitors.

Certain classes of temporary visitors are likely to be useful for the evaluation of malaria vaccines, in particular sporozoite vaccines. Organized groups have specific advantages: they can be closely monitored; chemoprophylaxis may be withheld, since treatment can be made readily available; they may be nonimmune; they may be exposed to a wide variety of epidemiological situations; prolonged follow-up may be possible. Close medical supervision will be essential.

3.2 Control of transmission

There are three possible indications for the control of transmission through vaccination, used in combination with other methods:

- Interruption of transmission for malaria eradication in situations in which vaccination is likely to make the difference between success and failure: this might occur in areas with moderately intense transmission and favourable social, behavioural and economic factors, where other methods, especially vector control, can reduce transmission to a low level without interrupting it.

- Reduction in transmission in situations in which interruption of transmission cannot be achieved or maintained but where the vaccine could effectively contribute to improved control.
- Control of epidemics in areas previously freed from malaria and in areas of normally low endemicity.

Some of the key issues concerning the control of transmission by malaria vaccines include the following.

There are important but imperfectly understood relationships between intensity of transmission, coverage and effectiveness of vaccination, and expected impact on transmission: a first approximation of the proportion of the population that must be effectively covered to interrupt transmission is given by the function $(1 - 1/R_0)$, where R_0 is the "basic reproduction rate"⁽¹⁾ of malaria, i.e. the average potential number of secondary cases generated by one primary case. R_0 is difficult to estimate and varies between population sections. It can be much larger (e.g. a hundred or more) in malaria than in smallpox (4 or 5). If $R_0 = 100$, the effective coverage required to interrupt transmission (approximately 99%) is virtually impossible to achieve. Fortunately, R_0 is not always so high and can usually be reduced greatly by vector control.

The effective vaccine coverage that can be achieved will be affected by the degree of compliance, the existence of contraindications, groups affected by the contraindications and their importance as reservoirs of infection.

3.3 Conclusion: priorities

Evaluation of the degree of protection of vaccinated persons will normally precede evaluation of the impact on transmission. The former requires smaller trials and must be performed before vaccination is permissible on large numbers of people.

Those persons to be protected include residents and temporary visitors. Among residents of endemic areas, two types of population are of immediate interest: permanent residents and nonimmune immigrants. Among temporary visitors, trials in organized groups are probably easiest to evaluate.

Of the different possible kinds of "protection" that sporozoite vaccines might confer, protection against the acquisition of infection (parasitaemia) and the onset of symptomatic malaria should be evaluated first. Subsequent studies should determine effects on morbidity and mortality.

The remainder of this document mainly concerns the evaluation of protection of residents of endemic areas against the acquisition of infection and the onset of symptomatic malaria.

4. Factors Likely to Affect the Outcome of P. falciparum Sporozoite Vaccine Trials

A number of factors other than vaccination itself might affect the outcome of the trials, either because they interfere with the effectiveness of the vaccine or because they have an independent effect on exposure to or manifestations of malaria, or both. Variation of such factors may bias

(1) The formula is derived as follows: let V be the proportion of the population to be vaccinated; for the ultimate disappearance of malaria, it is necessary that $R_0(1 - V) < 1$, which gives $V > 1 - 1/R_0$.

comparisons between places, times and persons. It is important to review these factors, in order to decide which, if any, should be taken into account either in planning (e.g. by stratification and/or randomization) or in analysis (e.g. by stratification).

Potentially relevant factors are listed below, with some indications of how they can be measured, dealt with in the trials and studied. These factors may be equally or even more important at earlier stages of vaccine evaluation, and studies designed to elucidate their importance may need to be carried out as soon as possible.

4.1 Parasitological factors

4.1.1 Antigenic diversity and variation

This has not so far been discovered with the CS protein of P. falciparum, but further research concerning possible geographic diversity is justified. If P. falciparum parasitaemia appears in a vaccinated person in the presence of high antisporezoite antibody levels, samples should be preserved for study of the CS protein and/or gene.

4.1.2 Diversity and variation of drug responses

These are common, important and relatively easy to measure.

4.2 Human host factors

4.2.1 Effects of malaria in the host

- Pre-existing acquired active immunity: a problem partly controlled by prior stratification by age. In addition, prevaccination immunological testing will provide essential information and allow a stratified analysis of the results of the trial.
- Passive immunity in infants: on the basis of experience with other vaccines, including animal experiments with blood-stage malaria vaccines, it is suspected that maternal antisporezoite antibody might suppress the immune response of infants to a sporezoite vaccine. Research on this topic should receive high priority.
- Suppression of the immune response to sporezoites by a concurrent blood-stage infection: observed in a rodent malaria model. If there is such an effect in human subjects, it could be removed by treatment, possibly a few weeks before vaccination. Questions relating to the occurrence of immunosuppression and its correction by treatment should be investigated during early clinical trials in endemic areas. In the meantime, for the evaluation of personal protection in a population previously exposed to a relatively high inoculation rate, it may be recommended to treat the whole study population with a safe and effective blood schizonticide at the time of vaccination. This would also facilitate the detection of new infections and hence the assessment of protection.
- P. falciparum infection is commonly accompanied by infection with one or more other species of malaria parasites in the same population. It is generally believed that, within the human host, P. falciparum parasitaemia suppresses or masks the parasitaemias due to other malaria parasites. Parasitaemia due to other species of malaria must therefore be measured in a P. falciparum vaccine trial. P. malariae is of special interest because of the serious, delayed quartan malarial nephrosis complication.

4.2.2 Other human host factors

- Genetic factors affect host responses to P. falciparum, in particular Hb-S, thalassaemias, ovalocytosis and some types of G-6-PD deficiency. They are detectable by standard tests and their geographical distribution is relatively well known. Their expected effect is relatively small in comparison with the expected effect of an effective P. falciparum sporozoite vaccine. In a study of personal protection by such a vaccine it is probably not necessary to stratify for these factors before randomization. Stratification of results according to locally common relevant markers should indicate whether a more thorough investigation is required.
- Age and sex may affect exposure to malaria, response to malaria and response to the vaccine. They may act independently of immunity. Stratification or matching by age and sex should help to take into account their effects.
- Human behaviour may influence vaccine trials in several ways: occupational, social or other activities may affect exposure to the vector; personal protective measures may reduce exposure to malaria; treatment-seeking and drug usage patterns may greatly alter the apparent effect of the vaccine. These factors must be taken into account during the design of protocols for vaccine trials.
- Other diseases, either endemic or epidemic, may alter the response to malaria infection or to the vaccine and their presence may complicate evaluation of a malaria vaccine trial.
- Interactions between a malaria vaccine and other vaccines: one vaccine may change the acceptability of other vaccines according to how well it is tolerated, affect the development of an immune response to other vaccines and increase the incidence of side-effects to other vaccines. These potential interactions will need to be considered in evaluating a sporozoite vaccine. It may be prudent, at least initially, not to give malaria vaccines within two months before or after the administration of any other vaccine. All vaccinations should be included in the records of individuals of the study population.
- Diet and nutritional status: a milk diet, iron deficiency and protein deficiency may all have some suppressive effect on malaria parasitaemia, although protein deficiency may also decrease the immune response. It is probably prudent, at least initially, not to conduct vaccine trials in severely malnourished populations and individuals. Nutritional status can be monitored by standard anthropometric measurements. Such monitoring may be useful not only for the detection of a possible effect of nutritional status on vaccine-induced protection but also for the evaluation of the indirect effect of such protection on nutritional status itself.

4.3 Entomological factors

The entomological factors which could affect the outcome of sporozoite vaccine trials are the magnitude and distribution of the local intensity of natural challenges/boosters in the form of sporozoite inoculations: reasons for and ways of measuring them are discussed below (see section 5.8).

4.4 Control measures

Malaria control measures, i.e. vector control, control of man-vector contact (bednets, etc.) and antimalarial drugs may all have an independent

effect on the endpoints (infection, disease) of malaria vaccine trials. They may change during the course of a trial. It is therefore recommended that vaccine trials be conducted in areas and populations in which no significant change in malaria control measures is planned, except as part of the trial. It is also recommended that the measures applied both on a communal and on an individual basis be monitored, in particular the use of antimalarial drugs from whatever source. Methods for monitoring drug use may include interviews and chemical tests.

B. FIELD TRIALS IN MALARIA-ENDEMIC COUNTRIES

1. Information Required from Early Clinical Trials (Phase I and Phase II)(1)

Early clinical trials are required to produce information needed for the planning of epidemiological trials, in particular information regarding: safety, acceptability, immunogenicity and efficacy (protection, including its duration), by age, sex, past malaria experience and current malaria infection; formulation, storage, dosage; booster effect of revaccination or sporozoite inoculation.

There are major differences between nonimmune subjects in nonendemic countries and populations living in endemic areas which could influence the response to malaria vaccines and the evaluation of the results of vaccine trials. These include the existence of varying degrees of immunity in the local population of malarious countries, genetic differences affecting susceptibility to malaria and differing nutritional and health levels. The results of clinical trials in nonendemic areas cannot therefore provide an adequate basis on which to begin epidemiological trials in endemic areas. Early clinical trials conducted in nonmalarious areas must be repeated in residents in endemic areas. Some additional clinical trials in special high-risk groups, e.g. pregnant women and infants, can be carried out only in malarious areas. Any proposal to circumvent early clinical trials in endemic areas should be rejected.

It is possible that epidemiological trials will be conducted from which certain groups, such as infants and pregnant women, would be excluded, while preliminary trials in those groups would be conducted concurrently or later.

2. Criteria for the Selection of Areas/Populations for Field Trials

2.1 Epidemiological criteria

It is preferable to conduct vaccine trials in situations in which vaccination is likely to be effective and relevant to control. Potentially eligible situations are likely to be many and varied (see section A.3). Priority for vaccine trials should be given to situations likely to give most information for a given investment. The following populations probably merit highest priority: residents of areas of intense seasonal transmission; residents of areas of intense perennial transmission; nonimmune (previously little or not exposed) immigrants of different age-groups settling into areas of intense transmission. The preference for intense transmission is justified on two grounds: challenge and protection are easy to measure and if a vaccine protects against intense transmission, it is likely to protect against less intense transmission, while the reverse may not be true. The inclusion of both

(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/85.3.

seasonal and perennial transmission is justified because they are likely to induce a different pattern of naturally acquired immunity. The justification for including immigrants is that they comprise nonimmune subjects of all ages.

The availability of prior knowledge of the local epidemiology of malaria is an advantage.

2.2 Operational criteria

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

- Government commitment to the conduct of the trial: genuine commitment of national authorities will help in gaining the support and confidence of both the community participants and the health professionals.
- Involvement of national research institutions with interested national (and/or international) investigators and field/laboratory teams to provide local expertise and with access to national and international resources to support the trial.
- Reasonable expectation of social and political stability at the national and local levels.
- 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.
- Availability of basic laboratory services: ideally the community-based epidemiological evaluation would take place in populations in reasonable proximity to clinical trial sites.
- A reasonable 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.
- Availability of background epidemiological data.
- Additional advantages would include the potential for the site to serve as a regional centre and particularly to serve as a field training centre.

3. Study Design for the Measurement of Protection in a Resident Population under Natural Challenge (Phase III Trial)

3.1 Objectives

- (i) To measure the rate of protection induced by a P. falciparum sporozoite vaccine against P. falciparum infection.
- (ii) To measure the rate of protection induced by a P. falciparum sporozoite vaccine against symptomatic P. falciparum malaria.
- (iii) To determine the duration of protection.
- (iv) To identify and estimate the frequency of side-effects attributable to vaccination.
- (v) To investigate malarial disease and mortality as it occurs in vaccinated and unvaccinated individuals. (This is an ancillary objective since the study is not primarily designed to evaluate the effects of the vaccine on the severity of disease and on mortality, although it may yield some information on those aspects.)

There are some difficulties in combining objectives (i) and (ii); an alternative approach would be to address them sequentially in different trials. They have been tentatively combined here for the following reasons: once protection against infection is demonstrated, a double-blind evaluation of protection against illness may become unacceptable ethically; in terms of evaluation methods, whether one is primarily interested in parasitological or in clinical protection, it would be very ill-advised to dismiss either parasitological or clinical evaluation.

3.2 Epidemiological situations, study populations

As already indicated, initial field trials of a P. falciparum sporozoite vaccine should preferably be carried out in resident populations under intense perennial transmission; resident populations under intense seasonal transmission; nonimmune populations migrating into areas of intense malaria transmission. The latter type of trial raises specific problems, especially in terms of follow-up and of expected incidence of severe malaria.

3.3 Intervention

Vaccination: The trial is a comparison of rates of P. falciparum infection and symptomatic malaria in vaccinated persons compared with the corresponding rates in nonvaccinated controls.

Selected persons are 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 may be vaccinated at the beginning of the rains and nonimmune immigrants just before entering areas of intense transmission. However, if the vaccine effect were expected to last long enough, vaccination during the season of low transmission might be preferable, because it would facilitate laboratory evaluation of the immune response to the vaccine.

Vaccinations are given to the randomly selected group, 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 in the product to be licensed and marketed if the trial is successful. Proper transport and storage of the vaccine must be ensured.

More than one type of P. falciparum sporozoite vaccine could be tested in the same trial. That would indeed be the best way to compare different vaccines initially. If more than one induces significant protection, further comparison would require larger trials.

The trial should consist of a randomized double-blind comparison between a vaccine and a placebo. Even though the main outcome variable, infection (parasitaemia) with P. falciparum, can be measured objectively without knowledge of vaccination status, the use of a placebo is justified. A trial of a vaccine of such potential major public health importance must be performed as rigorously as possible. 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, for which a placebo is required. Selection of an appropriate placebo will need to await exact specifications of vaccine composition and the results of pre-clinical trials. Tetanus toxoid is a possible choice as a placebo for vaccines that use it as the carrier. The choice of placebo may also depend on the population group involved in the study.

Associated chemotherapy: Early clinical trials may demonstrate the need for chemotherapy a few weeks before vaccination, to avoid immunosuppression due to current malaria infection (see above, section A.4.2). Whether or not such prior treatment is required, vaccinated and control groups among residents of malarious areas should probably be treated with an effective blood schizonticide at the time of vaccination. This will facilitate the recognition of new infections. The drug used should be selected on the basis of drug sensitivity and toxicity studies. Chloroquine is the drug of choice in areas where *P. falciparum* is sensitive to it. In other areas, mefloquine is the first choice. Curative doses should be used. Amodiaquine and pyrimethamine-sulfadoxine cannot be recommended because of the risk of serious toxicity. Chloroquine prophylaxis reduces the antibody response to human diploid cell rabies vaccine(1). The significance of this finding with respect to malaria vaccines requires investigation.

Pretreatment of the study population (vaccinated and nonvaccinated) can be further justified by the fact that in areas of intense malaria transmission many persons will have parasitaemia when first examined and must be treated. Drugs are usually readily available in these areas and self-medication may be widely practised. In holo- and hyperendemic areas, low levels of parasitaemia cannot be excluded on the basis of a single blood examination. Persons clearly suffering from clinical malaria should be excluded from the trial, at least until they recover.

3.4 Study groups

As stated above, the trial should consist of a randomized double-blind comparison between vaccine and placebo. Both vaccine and control groups should live in the same community. The best sampling unit is the individual. Random sampling of individuals allocated to vaccine or control groups will be most conveniently carried out at the time of vaccination. Stratification by age or other variables can also be done then. An optimal plan consists of the following steps:

- A complete population census is carried out of the community or communities (e.g. villages) in which the trial is to be conducted.
- Individuals are excluded from participation based on clearly defined criteria, such as age, illness, malnutrition, etc. Exclusion can be made from census information or at the time of vaccination.
- Informed consent is obtained. Persons are informed that they may receive either a vaccine or a placebo. Only volunteers are retained.
- Prior antimalarial treatment is administered, if preliminary investigations show the need for it (see section A.4.2).
- The volunteers are randomly allocated to vaccine or control groups; if the trial is stratified, for instance by age and/or occupation, random allocation is used within each stratum.
- Injection with vaccine or placebo is given, together with antimalarial treatment.

(1) Pappaioanu, M., Fishbein, D.B., Dreesen, D., Schwartz, I.K., Campbell, G.H., Sumner, J.W., Patchen, L.C. and Brown, W.J. Antibody response to pre-exposure to human diploid-cell rabies vaccine given concurrently with chloroquine. The New England Journal of Medicine, 314: 280-284 (1986).

The initial census allows a better assessment of the relationship of vaccinated individuals to the community as a whole and of possible epidemiological heterogeneity within the community. It also facilitates follow-up and extension of the trial, if required.

3.4.1 Relationship between groups included in Phase III vaccine trials and special target groups

Two important target groups for a *P. falciparum* vaccine will be pregnant women and infants, and children under five years of age. These are high-risk groups for malaria mortality and morbidity. There is no justification for including pregnant women, infants and children in trials carried out in non-malarious areas, but because of their public health importance, trials will be required in these groups. Such trials will be carried out subject to proof of vaccine safety and efficacy in other groups. Early clinical trials of safety, immunogenicity and efficacy will be required in pregnant women, infants and young children in endemic areas before they can be included in epidemiological trials.

Early clinical trials in adult males conducted in nonmalarious areas will also have to be repeated in endemic areas. Early clinical trials in children could begin after similar trials in adult males have shown safety and efficacy (However, because of the subjects' semi-immune status, it may not be possible to demonstrate efficacy in this group.). The corresponding trials in infants (less than one year of age) and pregnant women will follow trials in older children, since infants and pregnant women are theoretically at most risk from untoward effects of the vaccine.

3.5 Sample size

Four factors should be taken into account in calculating sample size:

i) the estimated incidence rate of infection (or of symptomatic malaria) in the absence of vaccination; let p_1 = the cumulative incidence rate, which is the proportion of the unvaccinated expected to acquire the infection within a given period of follow-up; $q_1 = 1 - p_1$ = the proportion expected not to acquire the infection in this period;

ii) the minimum protective effect which it is desired to detect: it is proposed to set that minimum at a 50% reduction in the incidence rate; let p_2 = the corresponding proportion of the vaccinated = $0.5 p_1$; $q_2 = 1 - p_2$;

iii) the acceptable probability (α) of declaring the vaccine effective when in fact it is not (type I error); α is usually set at 0.05 or 0.01: this probability is the desired level of statistical significance for the trial.

(iv) the acceptable probability (β) of declaring the vaccine ineffective when in fact it is effective (type II error); β is often set at 0.2 or 0.1. The power of a study is defined as $(1 - \beta)$ and provides the probability that a study will detect a difference if it actually exists.

The required sample size is calculated as follows:

$$n = \left[z_{\alpha} \sqrt{2\bar{p}\bar{q}} + z_{\beta} \sqrt{\frac{p_1 q_1}{1} + \frac{p_2 q_2}{2}} \right]^2 / \left(\frac{p_1 - p_2}{1} \right)^2$$

where n = the sample size (1)

$p_1, p_2, q_1, q_2, \alpha, \beta$ are defined as above

$\bar{p} = (p_1 + p_2)/2$; $\bar{q} = (q_1 + q_2)/2$

values of z are found in tables of normal distribution.

The following Table gives the required sample sizes for high to very high values of the cumulative incidence rate of *P. falciparum* infection (parasitaemia) (p_1), similar to those found in Northern Nigeria over an 80-day period(2), given $p_2 = 0.5 p_1$, $\alpha = 0.05$ and $\beta = 0.1$.

P_1	$P_2 = 0.5 P_1$	n
0.15	0.075	371
0.30	0.15	161
0.40	0.20	109
0.60	0.30	56

These numbers are only indicative. The following points should also be considered:

- Numbers given refer to persons initially negative and adequately followed; if a proportion r_1 is expected to remain positive after the initial treatment and a proportion r_2 to be lost to follow-up, the sample size should be multiplied by $1/(1-r_1)(1-r_2)$.
- With the numbers given, percent protection would be estimated only within relatively wide confidence limits: narrowing the limits to preset values would require an increase in the sample size.
- The incidence of symptomatic malaria is less well known and its assessment less well standardized: it is expected to be lower than the incidence of infection and the discrepancy is expected to increase with immunity; the evaluation of protection against symptomatic malaria will thus require either a larger sample size or a longer period of follow-up.
- Numbers given assume that there are no false positives: while that assumption may be acceptable with respect to parasitaemia, false positives are expected to be relatively common with respect to symptomatic malaria; this will reduce the power of the trial (and the calculated protection rate), and may be compensated only by an increase in sample size.

3.6 Phases

After study sites have been selected, community relations established and field teams organized, the following approximate time schedule could be implemented.

i) Preparatory phase: About 12 months may be required to finalize the study design, establish facilities, and test and standardize all field, laboratory and data recording methods.

ii) Baseline phase: Background information on the area, the population, the local risk of malaria and its seasonal variation, etc. will have been collected during the preparatory phase or even before. The need for true baseline data however (i.e. incidence data against which to measure change), in particular the effect of vaccination, is questionable in a trial of personal protection, which will be measured against simultaneous incidence in the controls. Randomization, after stratification if appropriate, for instance by

(1) The number required in each of the groups, i.e. vaccinated and unvaccinated; if one wants to determine separately the value of vaccination for different strata, such as age or occupation groups, n has to be calculated for each of those groups.

(2) The Garki Project. L. Molineaux & G. Gramiccia. WHO, Geneva, 1980.

age and/or occupation, should ensure that vaccinated and control subjects are indeed submitted to the same risk. In nonimmune immigrant populations vaccinated on arrival in a malarious area, incidence data before vaccination will by definition not be available.

iii) Intervention and evaluation phase: This phase begins with vaccination. Its duration should be kept flexible and will depend on results as the trial proceeds. A complete estimation of the duration of protection would require follow-up until incidence rates in vaccinated and controls are no longer significantly different. Prolonged follow-up may also be required for detecting possible late side-effects. If revaccination and its evaluation are to be considered, this will further prolong the study. It is suggested to plan for an initial intervention and evaluation phase of one year, renewable as often as useful.

iv) Final analysis phase: Analysis will be continuous during the preceding phase. The final analysis and the production of the final report may require an additional year.

3.7 Evaluation

3.7.1 Data collection and measurements

Methods for data collection and measurements will be selected among those reviewed below in section B.5.

The key measurements are the incidence of new P. falciparum infections and of symptomatic P. falciparum malaria. They will be detected by surveys with home visits to every household every week or two in order to enquire about symptoms possibly attributable to malaria or to the vaccines and about the use of antimalarial drugs, to take temperatures, and to obtain blood by finger prick for parasitological and immunological tests on those with signs or symptoms suggestive of malaria. Blood will also be obtained from all asymptomatic persons during some surveys. The frequency of such surveys or the possibility of using population samples instead of the entire population will depend upon the epidemiological situation. Drug use may also be assessed by biochemical tests (perhaps on a sample basis and not necessarily at every survey). Case detection between surveys will be done by health personnel at health stations designed for accessibility, acceptability and maximum coverage; cases will be assessed clinically, parasitologically and immunologically. All individuals with malaria detected at or between surveys will be treated immediately with appropriate antimalarials. Their follow-up will continue as before, thus allowing detection of possible residual protection after breakthroughs.

3.7.2 Analysis of results

The analysis of results will correspond to the objectives of the trial (see section B.3.1). The following discussion is concerned with the first two objectives, namely the measurement of protection and its duration.

Percent protection given by the vaccine can be calculated as follows:

$$P(t) = 100[p_1(t) - p_2(t)]/p_1(t)$$

where $p_1(t)$ = cumulative proportion positive in the controls at time t ; $p_2(t)$ = cumulative proportion positive in the vaccinated at time t ; and $P(t)$ = percent protection at time t after vaccination(1).

(1) $P(t)$ calculated in this way is based on the cumulative incidence of the first infection during the time period and may be quite sensitive to the length of the time period used.

There are other useful ways of looking at incidence and protection rates: person-times at risk rather than persons can be used as denominator, by counting each episode of infection instead of individual persons in the numerator or by considering successive intervals separately rather than cumulatively. The most suitable method may also depend on the mechanism of action of the vaccine.

Significance: The probability that the difference observed between those vaccinated and the controls is explainable by chance alone can be evaluated by chi-square analysis.

Confidence limits (CL) of percent protection are calculated as follows: variance $\ln(p_2/p_1) = (1-p_2)/p_2n_2 + (1-p_1)/p_1n_1$, which allows calculation of the 95% CL of $\ln(p_2/p_1)$, hence the CL of the percent protection.

3.8 Ethical considerations

The design and implementation of the trial must 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 and of the responsible health authorities as well as technical experts. There must be informed consent and a written statement of how that 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.

4. Study Design for Measuring the Impact of a Sporozoite Vaccine on Transmission: Salient Points

If trials of personal protection give encouraging results, and if vaccination of infants, young children and pregnant women proves feasible and permissible, it will become important to evaluate the impact of vaccination on transmission. Salient features of a study designed to assess this impact and that distinguish it from a study on protection of vaccinated individuals, include the following:

- The unit of evaluation is a relatively closed area of transmission, e.g. the total population of a stable village. Preliminary entomological investigations, in particular surveys of breeding places along transects between villages, are recommended in order to evaluate the degree of isolation between evaluation units.
- Evaluation of the impact of vaccination on transmission requires both baseline data (i.e. measurement of baseline transmission and an estimation of its natural variability) for at least one year before vaccination and one or more unvaccinated evaluation units to be observed throughout the study. In unstable situations, one year's baseline data may be insufficient.
- With respect to sample size, the number of evaluation units (villages) is the critical factor rather than the number of persons. Several vaccinated units and several unvaccinated units should be studied. A limiting factor is likely to be the capacity for high quality entomological follow-up.
- It is unlikely that areas of identical intensity of transmission will be found. The allocation of units to vaccinated and control groups should

be made after the units have been stratified according to intensity of transmission.

- The areas of transmission will only be relatively closed, i.e. some people or vectors will certainly move between areas. The consequences of such movement can be minimized by surrounding each evaluation unit with a buffer zone treated in the same way. In addition, the movement of persons into and out of the areas should be monitored and taken into account in the analysis.
- In the vaccinated units, vaccine coverage should be as high as possible. A high degree of compliance is essential for this kind of trial.
- Other interventions, including vector control and the use of anti-malarials, likely to affect transmission or its assessment should be taken into account in establishing the objective, as well as in monitoring and analyzing the results of the trial. The objective could be to evaluate vaccination vs. no control of transmission, or the combination of vaccination with other methods vs. vaccination alone, or the combination vs. no control. The possibility of making several of these comparisons simultaneously is constrained by sample size.
- The use of a double-blind design using placebo may not be possible and not even necessary. It will be essential to ensure the unbiased assessment of outcomes attributable to the vaccine.
- Evaluation will depend on selection and application of some of the measurement methods reviewed below (see section B.5). The key measurements will be the incidence of new infections in human subjects, detected by parasitological surveys and by case detection, and the infection rate in the vector.
- The objective of the intervention may be either the reduction or the interruption of transmission; the same principles of evaluation apply to both cases.

5. Methods of Data Collection and of Measurement

The following review does not imply that all the methods listed should be used in every trial. The selection of the variables to be measured and of methods to be used will depend on the specific objectives of each particular vaccine trial.

The following aspects of measurement require critical review during the preparatory phase of a trial: standardization; quality control; reproducibility within the study, including comparability over time; comparability with other studies; sensitivity and specificity; level of precision actually required (not necessarily the highest possible); minimization of the numbers of specimens (e.g. of blood) to be collected; collection, transportation and preservation of specimens; recording of data; analysis of results.

5.1 Methods of data collection in the human population

Different variables are of interest in the human population: infection, disease, immune response, side-effects, mortality. The relevant measurement methods are considered in subsequent sections (see 5.2 to 5.7), while this section considers methods of data collection.

5.1.1 Population surveys

Periodic surveys of the total study population will enable the regular follow-up to be conducted on all study subjects in terms of: symptoms and

signs possibly attributable to malaria or to vaccination, parasitology, immunology, and, if appropriate, human behaviour concerned with exposure to and the use of antimalarials or other means of personal protection. The most effective survey method is by house-to-house visits. The frequency will depend on the precise objectives of a particular trial and on what is feasible and acceptable. In the study design for a personal protection trial, outlined above (see section B.3), weekly or two-weekly surveys are proposed for enquiry into signs and symptoms of malaria, with finger-prick blood taken from those with symptomatic malaria. Finger-prick blood should also be taken from asymptomatic persons, either from all at some surveys or from a sample at each survey. Different laboratory tests might be performed at different frequencies.

Population surveys conducted through frequent house visits are similar to the active case detection (ACD) of the malaria eradication strategy. The major difference is that blood for laboratory tests will also be taken from asymptomatic persons.

5.1.2 Detection of cases between surveys

Governments are committed to provide adequate diagnostic and treatment facilities to the whole population, and it would be appropriate to strengthen the peripheral health services in order to make case detection as sensitive as possible. This would involve the early identification, and enrolment in the trial, of all services, public or private, to be consulted by the study population. It may be necessary to set up new health stations especially for the trial. Participants should be encouraged to use the health stations for any symptoms that develop. Monitoring of health care utilization patterns may need to be incorporated in the study design.

The same system of case detection should also detect serious side-effects of vaccination. This system is similar to the passive case detection (PCD) of the malaria eradication strategy.

5.1.3 Detection and investigation of deaths

Even though some trials may not be designed for the adequate measurement of mortality (see below), it is desirable in all trials to set up a system of detection of all deaths occurring in the study population and to determine, on the basis of history, the probable cause of death.

5.2 Infection in man

The currently accepted method for detecting malaria infections is by microscopic examination of thick and thin blood films. Sensitivity depends on the volume of blood examined. The most commonly used convenient substitutes for a standard volume are 100 and 200 thick-film microscopic fields. A complete examination includes identification of parasite species and of *P. falciparum* gametocytes, and measurement of parasite density. 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. Notwithstanding efforts to standardize all procedures involved, their performance continues to vary between investigators and with the same investigator over time, and the variation is not always clearly perceived or documented.

The following procedures are considered essential, and are applicable to all trials:

- standardization of procedures within each trial and as far as possible between trials;

- taking of blood films in duplicate;
- "blind" microscopic blood film examination;
- preservation of blood films for further reference;
- quality control within each trial by reexamination of a coded sample of blood films;
- comparability between trials by exchange and re-examination of coded samples of blood films;
- with respect to density, the recording and storage of actual parasite numbers counted, even if some analyses will use only density classes.

In addition, parasitaemia may be more accurately determined either by measuring the volume of blood examined or by combining a white and/or red blood cell count with the parasite count. The use of newer diagnostic techniques, using DNA probes or monoclonal antibodies, should be considered. These cannot at present replace microscopic examination but may well become valuable additions, permitting rapid, objective and specific evaluation of large numbers of blood samples. In particular, the use of DNA probes may be helpful for species-specific diagnosis of parasitaemia.

5.3 Immune response

5.3.1 Measurement of antisporeozoite immune response

Immunological assessment will be required to assess the prevaccination immune status of individuals, to characterize the immune response to vaccination and its duration, and to correlate the immunological response with protection.

Immunological assessment of antisporeozoite vaccines will depend essentially on measurements of antibody levels. The necessity for inclusion of cell mediated immunity (CMI) assays should be assessed during early clinical trials. The inclusion of CMI measurements in field trials will involve important logistic and ethical questions due to the need for venipuncture, and therefore the justification for their inclusion must be established in advance.

5.3.1.1 Antibody measurement

Methods

Several methods are currently available for the measurement of antisporeozoite antibodies. In increasing order of sensitivity, these are:

- circumsporozoite precipitation (CSP) reaction
- indirect immunofluorescent antibody test (IFAT)
- enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA)

In addition, a functional assay measures the inhibition of sporozoite invasion into liver cells in vitro.

No crossreaction has been found so far between sporozoite antigens of the different species of human malaria.

It is not yet clear whether anti-asexual blood-stage antibodies may crossreact with sporozoites. One P. falciparum asexual-stage antigen

crossreacts with the CS protein, but the practical importance of this finding for serology has not yet been evaluated.

Samples, storage and transportation

All the antibody assays can be carried out using small amounts of serum obtained from finger-prick blood samples. Studies published to date have all used freezing as the method of storage: liquid nitrogen containers are required for transportation of samples unless they reach the laboratory within 24 hours. Long-term storage at -70°C is recommended.

Recent findings suggest that samples collected on filter paper may be satisfactory for subsequent elution and assay of antisporezoite antibodies.

Recommended tests

As an initial screening method, both for the prevaccination population studies and for measurement of the immune response to vaccine, the ELISA is the method of choice. A relevant peptide based on the repetitive sequence of the CS molecule should be used in the assay. Large numbers of samples can be processed rapidly at low cost and the results are quantitative.

Selected samples, based on the ELISA results, should be further tested for CSP reaction (using live sporozoites) and by the IFAT (using slides of glutaraldehyde-fixed sporozoites). In addition, a proportion of samples should be tested for *in vitro* inhibition of sporozoite invasion into liver cells, which may be a more direct assay of functionally relevant antibody.

Standardization of the assays used in the trial is essential. The use of a standard reference antibody is recommended.

For certain carrier-based vaccines, pre- and post-vaccination immune status to the carrier must be assessed during the early clinical trials in the endemic area.

5.3.1.2 Evaluation of cell-mediated immunity

As noted above, the value of CMI measurements in the case of sporozoite vaccine testing should be established during Phase I and II trials.

T cell regulatory function

The proliferative response of blood T cells, stimulated with the same defined natural antigens or peptides included in the vaccine, may be assayed before and after vaccination (and challenge).

Cell-mediated effector activity

The classical test for cell-mediated effector activity is a skin test for delayed-type hypersensitivity (DTH) by intradermal injection of antigen. If a skin test is included in the trial, the same antigen as used in the vaccine should be used for skin testing.

Samples, storage and transportation

Blood samples for lymphocyte proliferation assays and lymphokine measurement must be taken by venipuncture, in order to obtain sufficient blood and to ensure sterility. Samples which can be transported to a laboratory within 24 hours can remain at ambient temperature. Samples of separated cells which cannot reach the laboratory within 24 hours will require liquid nitrogen storage facilities. Lymphocytes can be collected and then stored in liquid nitrogen for testing at a later date.

5.3.2 Antibodies to blood-stage parasites

Antibodies to whole parasites are considered to provide an estimate of the cumulative incidence of parasitaemia over a period of time (e.g. between two serological surveys). Titres are expected to be affected indirectly by a sporozoite vaccine: in comparison with unvaccinated controls, those vaccinated are expected to have a lower conversion rate from negative to positive, possibly a higher rate from positive to negative and generally lower antibody titres. The most appropriate test is probably the IFAT.

5.4 Malaria morbidity (disease)

Morbidity can be considered in terms of incidence of infection, incidence of disease and incidence of severe disease. It is important to distinguish these concepts.

Different types of malaria vaccines could reduce one or more of these forms of morbidity. A P. falciparum sporozoite vaccine is expected to reduce the incidence of infection and thereby the incidence of disease, including severe disease. It is not known whether a P. falciparum sporozoite vaccine will act by reducing the number of sporozoites reaching the liver and thereby reducing the probability that infection will produce disease, severe disease and death.

The assessment of malaria disease and its severity in a vaccine trial raises several problems. The definitions and diagnostic criteria used in clinical assessment are not adequately standardized. With respect to severe malaria, standards have been proposed at a recent WHO meeting⁽¹⁾. One method of evaluating the effectiveness of vaccination is likely to be rapid case detection. As it would be unethical to leave detected cases untreated, there will be little opportunity to assess the severity and duration of disease. If the required sample size is calculated on the basis of percent protection against the acquisition of infection, it is likely to be too small to measure the impact on relatively rare complications, such as cerebral malaria. A vaccine might be very effective in preventing most infections but selectively fail to prevent some of the most dangerous infections, and this differential effect may not be detected if the evaluation is in terms of infection. On the other hand, a vaccine might substantially reduce morbidity with very little reduction in prevalence of parasitaemia (but probably with reduction in parasite density). Larger trials at a later stage (Phase IV) may be needed for assessment of morbidity.

A vaccine which induced only partial immunity to sporozoites might reduce the severity of infection through prolongation of the prepatent and incubation periods. It would therefore be important to know whether such prolongation occurs. It is unlikely that this can be assessed in field trials under natural challenge, but it is an important research question for artificial challenge studies, in animals and in human subjects.

Although the epidemiological evaluation of P. falciparum sporozoite vaccines is likely, at least initially, to be made mainly in terms of infection and early symptoms, it is also advisable to assess illness and hence to standardize clinical definitions, diagnostic criteria and records.

(1) World Health Organization. Severe and complicated malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80(Suppl.): 1-41 (1986).

5.5 Malaria mortality

In considering mortality, it is important to distinguish the mortality rate (from all causes combined), the malaria-specific mortality rate and the malaria case fatality rate.

Malaria vaccines could reduce all three rates. A P. falciparum sporozoite vaccine is expected to reduce the malaria-specific mortality rate through a reduction in the incidence of infection. Through its indirect effects, it is likely to reduce to an even greater extent the mortality rate from all causes combined, and it might also reduce the severity of infection and hence the case fatality rate.

The assessment of malaria mortality in a vaccine trial raises problems similar to those of assessment of disease and its severity. Death may not be registered and its cause may be difficult or impossible to determine. Early detection and treatment of cases should virtually prevent death from malaria. Evaluation of effects on mortality requires a much larger sample size than evaluation of effects on infection. Even if a vaccine showed a reasonable rate of protection against parasitaemia, vaccinated individuals who nevertheless developed parasitaemia might include those who were destined to die from severe malaria, because of either intense infection or inadequate host defenses. In this situation the vaccine might show a high protection rate against infection but little or no effect on mortality. As with the assessment of serious morbidity, further studies may be required at a later time to determine the impact of a malaria vaccine on mortality.

Even though the epidemiological evaluation of a P. falciparum sporozoite vaccine is likely to be made, at least initially, mainly in terms of infection and early symptoms, it is advisable to set up an effective, standardized system of registering deaths, of investigating their causes and of recording the information.

5.6 Measurement of side-effects (safety)

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 nonendemic countries may not be adequate predictors. Side-effects should be assessed in all phases of vaccine trials. Moderately frequent and infrequent adverse effects are more likely to be detected in epidemiological (Phase III and IV) trials.

5.6.1 Immediate side-effects

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 done within a few days to identify early reactions, such as pain and abscess at the vaccination site, and constitutional symptoms, including fever. Health services and facilities for careful clinical examination should always be available.

5.6.2 Delayed effects

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. Both case detection and population surveys should be used.

5.6.3 Laboratory examinations

The need for haematological, immunological and biochemical examinations should be assessed on the basis of earlier trials. Extensive testing for urinary protein may permit the detection of incipient renal immunopathological manifestations. Studies requiring a venous blood sample may be difficult to carry out frequently or in the whole population. Samples from the controls are necessary for comparison.

5.7 Human behaviour

The aspects of human behaviour most relevant to the early epidemiological evaluation of a *P. falciparum* vaccine are: occupation and travel that may affect exposure to the vector; personal protective measures, such as bednets, which may reduce exposure to malaria; and factors that affect the probability of detecting an infection, such as the use of diagnostic facilities and of antimalarial drugs.

Information regarding exposure and use of personal protective measures can be collected through standardized short interviews and by observation of activities. Treatment-seeking and drug-use patterns can be determined by questionnaire and observation. Urine and blood samples can be examined for the presence of antimalarial drugs.

5.8 Entomological measurements

5.8.1 Relevant variables

The entomological variables most relevant to epidemiological evaluation of malaria vaccines, in particular *P. falciparum* sporozoite vaccines, are those related to intensity of transmission: frequency of infection in the vector (sporozoite rate), the entomological inoculation rate (the number of sporozoite-positive bites received per person per day) and distribution of intensities of infection within the vector population. Measurements of these variables can be used to calculate the challenge faced by vaccination, the impact of vaccination on transmission and the degree of natural exposure to sporozoites.

While entomological measurements are essential for transmission studies, they are also useful for personal protection studies. The main measurement of challenge is incidence rate in controls, but the entomological inoculation rate provides additional information because it allows better discrimination at the higher levels of transmission.

5.8.2 Methods

The estimation of entomological indicators used in malaria epidemiology involves three steps: a sampling scheme, i.e. the selection of times and places at which to collect mosquitos; a collection method, e.g. all-night collection from human subjects; and the examination of mosquitos collected, e.g. for species and infection. Only some particularly relevant issues can be discussed here.

For the three variables identified above, the preferred method of collection is all-night direct collection from human subjects. This raises several problems, including the availability of collectors, the ethics of exposing them to infection, and the cost, especially of efficient supervision. In the past, the ethical question has usually been answered either by using as collectors adults who are exposed in any event and presumed to be relatively immune or by the use of chemoprophylaxis (questionable in situations where no completely safe and effective prophylactic drug is available). If a substitute

collection method is used (e.g. man-baited net traps or collection from human subjects for only part of the night) it should be checked periodically against the standard method.

In planning and interpreting measurements of the man-biting rate, it is important to be aware of the non-uniformity of the true man-biting rate and of the biases implicit in its measurement.

Vector infection is now best determined using monoclonal antibodies to the CS protein in an ELISA. Sporozoite detection is species specific and quantitative. The method can be used to determine the distribution of parasite loads in the vector population. This may be related to the risk of severe disease and to that of breaking through vaccine-induced immunity.

C. PRELIMINARY OR CONCOMITANT RESEARCH RELEVANT TO THE EPIDEMIOLOGICAL EVALUATION OF P. FALCIPARUM SPOROZOITE VACCINES

The following is a tentative list of topics of research that could, and for some topics indeed should, be conducted before or at the same time as the epidemiological evaluation of P. falciparum sporozoite vaccines.

1. Methods of Measurement

New tests would be useful to measure protection, distinguish natural from vaccine-induced immune responses to P. falciparum sporozoites and to confirm radical cure of a P. falciparum infection.

Standardization of measurement methods will be essential to ensure comparability of data within and between trials.

Adaptation of existing tests for epidemiological use: it would be useful to investigate which tests for antibody measurement can be performed on blood collected on filter paper and to what extent refrigeration of specimens is indispensable.

2. Epidemiology of P. falciparum Malaria

It would be relevant to investigate:

- Natural patterns of the antibody response to P. falciparum sporozoites, in relation to age, season, infection, disease, entomological inoculation rate; the transition from passive (maternal) to active antibody response to P. falciparum sporozoites; the detection of the effects of subsequent exposure to sporozoites.
- The existence of naturally acquired cell-mediated immunity to P. falciparum sporozoites.
- The effect of chemoprophylaxis on antibody levels against P. falciparum sporozoites.
- The existence of antigenic diversity of P. falciparum sporozoites.
- Natural patterns of P. falciparum challenges presented by the vectors, including the distribution of intensity of infection in vector populations.
- The relationship between the number of sporozoites inoculated and the severity of subsequent disease in animal models.

3. Expected Effects of Sporozoite Vaccines

The following may merit further investigation:

- The existence and characteristics of suppression of the immune response to P. falciparum sporozoite vaccines by blood-stage infections and the effect of chemotherapy on such suppression if it occurs.
- The existence and significance of cell-mediated immunity following vaccination against sporozoites.
- The existence and significance of suppression of the immune response to the vaccine by chloroquine, by analogy with such an effect with respect to rabies vaccine.
- The existence and significance of partial immunity to sporozoites.
- Effect on sporozoites in the vector of ant sporozoite antibodies present in the vector's blood-meal.

4. Simulation Models

These may assist in the planning and evaluation of vaccine trials and of vaccine utilization in public health programmes.

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