



Afro-immunoassay Network

1.0 Background:

1.1 Global Malaria Burden

Malaria is one of the most important tropical parasitic diseases. It is caused by protozoan parasites of the genus *Plasmodium* of which four species *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale* are responsible for the disease in man. These parasites are transmitted by the *Anopheles* mosquitoes which act as vectors of the disease. *Falciparum* malaria is the most lethal and frequently occurring form of the disease throughout the tropics and subtropics. It is a significant cause of anaemia, child mortality, of abortion, still birth, and of death in pregnant women. It causes impaired growth in children and loss of productive activity in adults. In Africa, extrapolations from epidemiological studies suggest that the disease is responsible for not less than 1.0 to 2.8 million deaths annually, mainly among children below the age of five years. The enormous number of lives and labour lost, the cost of treatment of patients, and the negative impact of the disease on development make malaria a major social and economic burden.

1.2 Strategy for malaria vaccine development

In malaria endemic regions most adults are protected from clinical malaria but such immunity is not sterile and adults often harbour asymptomatic parasitaemia. Infection with *P.falciparum* may result in uncomplicated malaria, severe malaria with the accompanying high mortality, or protection from disease. Clinical immunity, however, is slowly acquired after a number of years of several disease episodes, and the burden of morbidity and mortality are mainly in children less than 5 years of age, in addition to increased susceptibility to disease in primi-gravid women.

It has been observed by several workers that the antibodies produced are directed against all the stages of the parasite that occur in the mammalian host, namely the sporozoites, blood stages and gametocytes (*Nardin et al., 1979; Cohen and Butcher, 1971; Mendis et al., 1987*). However, no

significant correlation has been found between total anti-plasmodium antibody levels and malaria infection or clinical disease in individuals studied over long time periods. Thus, in general antibody levels have been found to be more indicative of previous infection than of functional acquired immunity (*Voller, 1971; Marsh et al., 1989*).

Development of a malaria vaccine as one of the interventions against malaria must therefore be focused on understanding the mechanisms and elements involved in acquisition of natural immunity in adults, and the enhancement of such in children and primi-gravid women. Immune responses against the malaria parasite, however, are exceedingly complex and not completely understood. Protection against the disease is thought to be effected through both antibody and cell mediated mechanisms, and to date, no standardized laboratory measure has been established to identify individuals protected against or susceptible to clinical malaria. The importance of antibody is evident from the protection conferred to neonates and infants by maternally derived malaria specific antibodies, and also from effective clinical treatment trials with purified immunoglobulins (*Cohen et al., 1961, McGregor et al., 1963, Sabchareon et al., 1991*).

Clinical malaria is caused by the multiplication of parasites of the blood stage above clinical thresholds, and a lot of research efforts have thus been concentrated on the identification of antigens of this stage. The rationale for narrowing down the antigens to be studied include the location and functions associated with the particular antigen, and of special interest is, whether such antigens are involved in the invasion of cells. Immune responses directed against such antigens may protect by blocking the invasive process or through other mechanisms, leading to a reduction in parasite numbers below clinical threshold. By various means, several blood stage antigens have been identified as putative malaria vaccine candidates antigens, which include the merozoite surface protein 1 (MSP1), the merozoite surface protein 2 (MSP2), apical membrane antigen 1(AMA1), the erythrocyte binding antigen (EBA), the merozoite surface protein 3 (MSP3), glutamate rich protein (GLURP), the serine rich protein (SERA), and more recently the erythrocyte membrane protein1 (PfEMP1). These blood stage antigens have been identified by a variety of means and are supported by a variety of rationales. It is a reasonable assumption that some of the antigens presently under consideration will work, at least in part, through antibody-mediated responses as well as cell mediated responses. It is therefore certain that in any vaccine trial the antibody and cellular responses to the vaccine antigen will need to be measured. It is also a reasonable assumption that if antibodies mediate protective effects, there is a relationship between concentration, isotype or function of the antibodies produced, and outcome. That being

the case, studies of the role of such responses in naturally developing immune protection are important: whilst the absence of a demonstrable relationship with protection would not necessarily imply lack of potential as a vaccine, demonstration of protective responses would give strong support for inclusion, and in some cases would be important in focusing attention on specific parts of potential vaccine molecules.

However, it must be remembered that linkage of protection to antibody levels to a particular antigen does not necessarily indicate that antibody is the means by which protection is achieved - it could reflect linkage to another type of immune response to the same antigen or even linkage to an immune response to another antigen. The determination of the kinetics of immune response in endemic populations is part of the essential characterization of potential vaccine molecules. Patterns of response are likely to vary with malaria ecology and establishing these is a useful and necessary prelude to study their potential role in natural acquired immunity or to the interpretation of responses in a vaccine study. Given the natural history of malaria (continued susceptibility to infection and episodes of illness, declining in frequency and severity over time), studies which attempt to relate immune response to protection must be longitudinal and have clearly specified definitions of immune status. We consider that it is important that such studies take place in a range of malaria transmission settings, have high priority for support. Co-ordination and standardization of designs and definitions should be facilitated.

Malaria immuno-epidemiological studies have been used to study the role of malaria antigen specific antibody in protecting from clinical malaria (*Egan et al., Dodo et, 1999, 2000, 2001 Theisen et al 2002*). Such studies tend to measure antibody levels at the beginning of a transmission season, follow subjects throughout the season, and attempt to associate particular pre-existing antibody responses with clinical immunity using various statistical methodologies that correct for age related exposure (*Egan et al., Dodo et, 1999, 2000, 2001 Theisen et al 2002*).

Clinical manifestations malaria parasite infections include chills, fever, headache, nausea, vomiting and diarrhoea. The definition of clinical malaria has usually been based on the microscopic detection of malaria parasitaemia in addition to either reported or measured febrile temperatures $\geq 37.5^{\circ}\text{C}$. The morbidity surveys that are essential part of these studies tend to presumptively drug treat individuals for clinical malaria prior to determination by microscopy whether parasites are present due to ethical considerations in clinical practice. It is well established that malaria fever is induced by the release of exogenous malaria antigens when

schizonts rupture to release merozoites (*Kwiatkowski et al., 1989*). Thus malaria fevers are directly caused by parasite multiplication and the inclusion of febrile temperature in defining the disease increases the sensitivity as well as specificity of case detection (*Smith et al., 1994, Schellenberg et al., 1994*). On the other hand, such case definitions may be rendered quite non-specific since in highly endemic malaria areas since children who suffer the bulk of malaria frequently carry malaria parasites in the absence of febrile temperatures. In such instances, it is extremely difficult to determine whether fever is attributable to the presence of malaria parasites or other febrile diseases like pneumonia and influenza. It is therefore imperative to obtain a case definition sensitive and specific enough to detect the fevers due to malaria and exclude as much as possible fevers of indeterminate sources.

In view of the difficulties in defining malaria, recent approaches have been to use febrile temperature $\geq 37.5^{\circ}\text{C}$ and parasitaemia above a certain threshold, since the proportion of malaria attributable fever increases with parasitaemia. Such cut-offs of parasitaemia have been obtained by statistical approaches that use available parasitological and clinical information, and may differ in different malaria endemic areas. Fever being a dichotomous variable, logistic regression approach will be used on the morbidity data, i.e., clinical and parasitological data that has accrued from morbidity surveys can be used to estimate the proportion of febrile episodes attributable to malaria parasites (ref), exploring for sensitivities and specificities that define clinical malaria as fever plus parasitaemia above different parasite cut-off levels, and help determine a case definition of malaria as fever plus parasitaemia above a certain threshold of parasites per microlitre of blood with a good sensitivity and specificity (at least 90%). This will enable the classification of study subjects as protected against or susceptible to clinical malaria and afford valid relational assessment with measured immune responses. Other potential pitfalls in analytical approaches to immuno-epidemiological studies include the fact that antibody may indicate immune status as well as age exposure and it has been difficult to establish a causal relationship between antigen specific antibody and protection from clinical malaria. An appropriate cohort study design and statistical modelling may correct for age related exposure, presumptive drug treatments, sickle cell type, G-6-PD deficiency, ethnic group, area of residence, socio-economic status, duration of residence in the community and thus enable relevant conclusions with regards to the protective effects of naturally induce antibodies to malaria vaccine candidate antigens. A longitudinal cohort study design with a nested case control aspect is the most appropriate, and will enable characterization of naturally acquired malaria antigen induced antibody levels into protective antibodies and that which is only indicative of exposure to the parasite during the

transmission. The establishment of a good malaria case definition from epidemiological data and the use of appropriate statistical models that corrects for all possible confounding factors, will enable better assessment of antibodies to malaria vaccine candidate antigens in relation to protection from malaria.