Immune responses to sporozoite antigens and their relationship to naturally acquired immunity to malaria

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This paper reviews some of the epidemiological approaches that have been explored and gives some examples of how these methods have been used to investigate naturally acquired immunity to sporozoite infections.

Introduction

It is clear that man can naturally develop immunity to malaria because in areas of high challenge most adults remain free from severe clinical attacks and experience only occasional, low-level infections. Protective immunity to malaria is achieved in a progressive manner. Firstly, protection is acquired against death or severe clinical disease, then against milder clinical attacks, and finally against infection itself, although the latter kind of immunity is usually only partial. The immune mechanisms that are responsible for bringing about these different degrees of protection remain uncertain although the classical transfer experiments done in the Gambia (*3*) showed that a component or components of γ-globulin preparations, probably antibodies, play an important role. More information as to which antibodies are involved could be obtained by further passive transfer experiments using affinity purified antibodies or, better still, human monoclonal antibodies, but such studies raise difficult ethical problems. So far, most attempts to define the components of the immune response that are responsible for naturally acquired protective immunity have involved indirect epidemiological studies.

Methods and results

Epidemiological approaches to the investigation of the protective role of individual immune responses in malaria

The following three types of study of increasing complexity can be used to investigate the possible role of a specific immune response to a malaria antigen in protection against the infection.

1. *Study of adult immune subjects and non-immune controls*

The first approach to the characterization of an immune response considered to be a possible mediator of protective immunity is to show that the response can be found in the majority of healthy adults living in an area of high malaria transmission but not in non-exposed controls. Investigation of the latter group is important, especially for peptide antigens, because cell-mediated immune responses to malaria peptides have been found in some subjects who have never been exposed to the infection, perhaps reflecting exposure to a cross-reacting antigen. If the response under investigation cannot be demonstrated in immune adults it is unlikely to play a part in naturally acquired protective immunity although the reverse is not necessarily the case.

2. *Cross-sectional surveys of an exposed population*

A cross-sectional survey of a randomly selected sample of a population exposed to malaria is relatively easy to do and it can give some useful information about the possible protective role of an individual immune response. Cross-sectional surveys are of most value when immunological measurements are combined with conventional malariometric recordings such as the spleen and parasite rate. Detection of a positive correlation between the immunological test under investigation and the presence of parasitaemia or splenomegaly suggests that it simply reflects exposure whilst a negative correlation is compatible with a protective role.

In populations where malaria is endemic the incidence rate of attacks of clinical malaria and the prevalence of malaria parasitaemia and of splenomegaly are strongly age-determined. Comparison of the age-dependent profile of the immune response being investigated with variations in the prevalence of malaria by age may provide useful information on the

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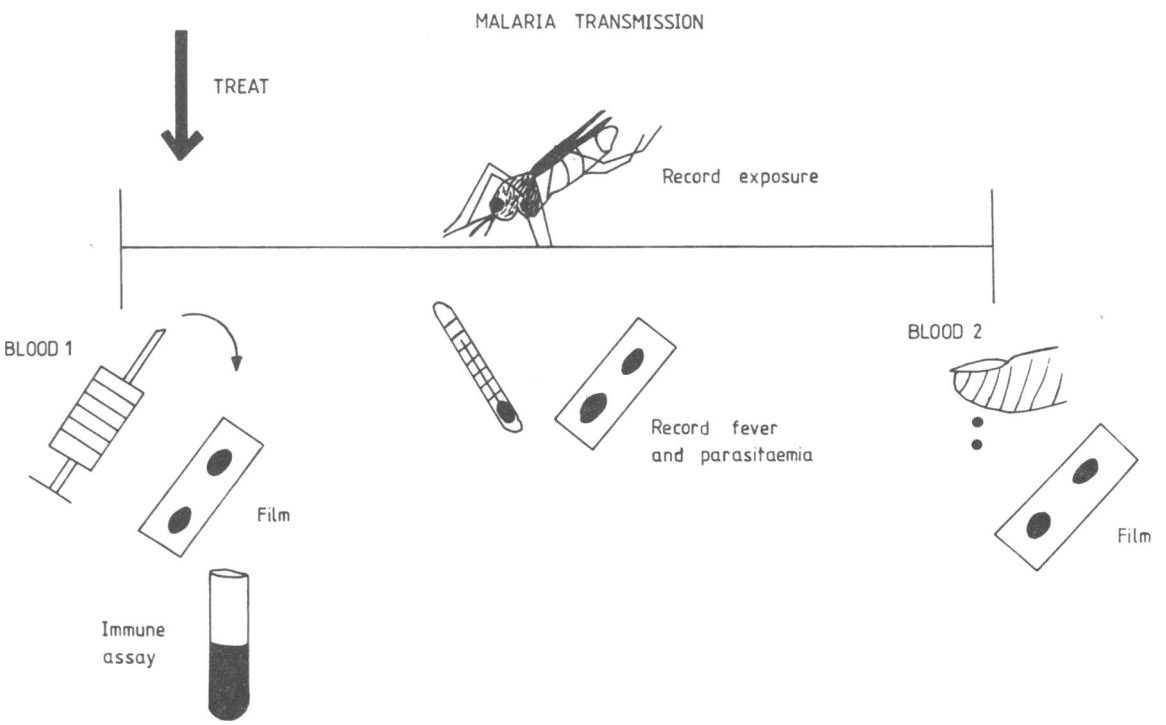
likely role of this response in protective immunity. If the age-dependent pattern of an immune response parallels that of parasitaemia, declining with increasing age, it is probable that it reflects only exposure to the infection. A response which rises in parallel with a decline in parasitaemia and which is sustained is more likely to have a protective role.

3. *Longitudinal cohort studies*

Longitudinal cohort studies are probably the most satisfactory epidemiological approach used so far to investigate the possible protective role of individual immune responses to malaria antigens but they are subject to many potentially confounding variables. The basic design of such studies is simple (Fig. 1); measurements of the immune response under investigation are made in a group of volunteers exposed to malaria and the results obtained related to the subsequent malaria experience of the study subjects. In their simplest form, studies of this kind assume that all the individuals entering the trial have the same genetic susceptibility to malaria and a similar exposure to infection throughout the period of observation. Neither of these conditions is ever likely to be met under natural conditions. It may be possible to reduce variations in genetic susceptibility among the study subjects by excluding those with genetically determined characteristics that influence the susceptibility to malaria, e.g., a haemoglobinopathy or α-thalassaemia, or by making allowance for specific genetic factors in the final analysis. This is difficult to do and studies undertaken so far have relied on randomization to distribute equal numbers of subjects with genetically determined resistance to infection to groups with and without the immune response under investigation. It may be possible to take some account of variations in exposure by measuring this directly and accounting for it in the final analysis. We are currently experimenting with this approach in the Gambia by recording the numbers of mosquitos inside the bed nets of study individuals.

If the immune response under investigation is directed at a variable part of a malaria antigen, strain variation will pose a further potentially confounding

Fig. 1: Design for a longitudinal cohort study to investigate the influence of an immune response on protective immunity to malaria.



factor because assays done with material derived from a parasite which differs considerably in its antigenic profile from the parasite circulating in the study community at the time of the investigation may not give meaningful results.

Despite the many potential pitfalls to a longitudinal cohort study we found that a study of this kind undertaken in a small group of Gambian children was able to demonstrate convincingly an association between the use of bed nets and the possession of haemoglobin genotype AS with protection against malaria (*12*).

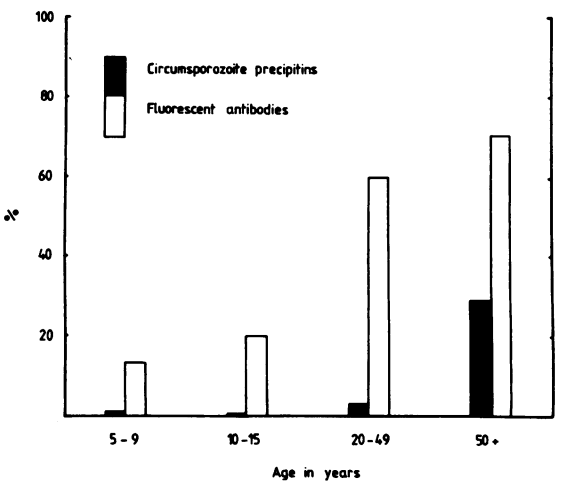
The immune response to sporozoite antigens and naturally acquired protective immunity to malaria

In this section, the results of studies carried out in the Gambia using the epidemiological approaches described above are summarized.

1. *Immune responses to sporozoite antigens in immunes and non-immunes*

Early attempts to demonstrate antibodies to sporozoites in adult malaria-immune Gambians were unsuccessful (*1*), but in 1979 Nardin et al., reported that antibodies to the circumsporozoite protein (CSP) could be demonstrated in the sera of many Gambian adults both by the circumsporozoite precipitation reaction and, more frequently, by immunofluorescence (Fig. 2). Antibodies were found much less frequently in children than in adults, except during the first 6 months of life when maternally transmitted antibodies could be found (*14*). Similar results have been obtained subsequently using radioimmunoassays or ELISAs to the CSP repeat peptides $(NANP)_3$ or $(NANP)_{40}$ (*12, 17*). However, antibodies to CSP repeat peptides cannot be detected in sera from a small proportion of apparently immune adults suggesting that the immune response to this antigen might be genetically restricted and that it is not essential for protection. Antibodies to NANP repeats are not found in non-exposed adults. In a study of a small group of expatriates resident in the Gambia anti-CSP antibodies were found, with only one exception, only in those who had been resident in the Gambia or another malaria endemic area for 5 years or more (K. Marsh et al., unpublished).

Fig. 2: **The prevalence of sporozoite antibodies in a rural area of the Gambia (sera were diluted 1:4).**



Data from ref. *15*.

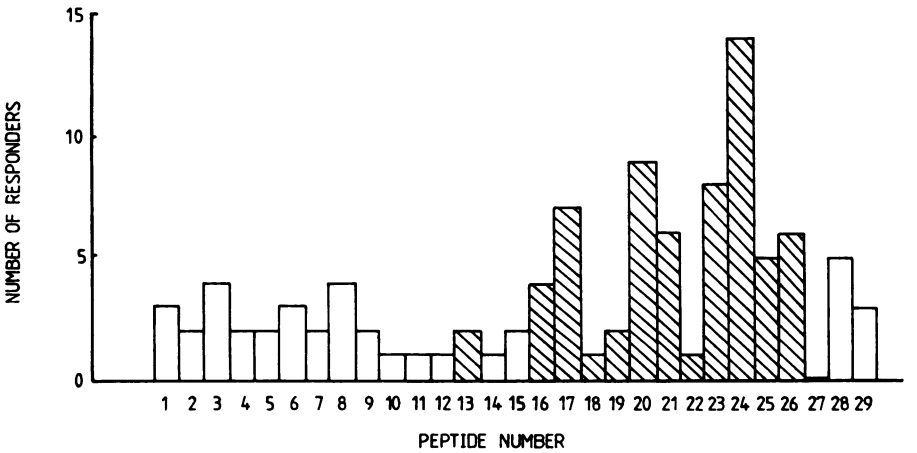
Recently studies of immune responses to CSP in adult immune Gambians and in non-immunes have been extended to assays of cell-mediated immunity (CMI). Good et al. (*9*) measured lymphocyte proliferative responses to 29 overlapping CSP peptides spanning the entire length of the CSP molecule in 35 healthy adult Gambians; 21 responded to at least one peptide. Analysis of the response of the whole group to each peptide showed three immunodominant sites, each located within the variable region of the molecule (Fig. 3). No significant proliferation was observed with lymphocytes obtained from six non-immune controls. To investigate further the importance of strain variation De Groot et al. (*4*) measured in the same adult donors the lymphocyte proliferative response to six peptides. Three peptides corresponded to the 326–345 region of the CSP molecule (TH2R) of three different strains of *Plasmodium falciparum* (7G8, Wellcome and LE5) and three to the 361–380 region (TH3R) of the same strains. Considerable variability in responsiveness was observed, some subjects responding to the peptide of only one strain whilst others responded to all three (Table 1). Two of nine non-exposed control subjects showed a modest proliferative response to one or other peptide.

In a further series of studies we are investigating the possible protective role of CD8 cells against sporozoites by studying the ability of lymphocytes obtained from adult immune Gambians to kill B cells expressing CSP peptides as a result of infection with a recombinant vaccinia virus. So far, specific killing has not been demonstrated but experiments are continuing.

2. *Cross-sectional surveys of immune responses to sporozoite antigens*

The prevalence of antibodies to $(NANP)_3$, as determined by radioimmunoassay, was studied in a rural Gambian community by Marsh et al. (*12*) (Fig. 4). The prevalence of anti-$(NANP)_3$ antibodies began to rise

Fig. 3: **Lymphocyte proliferative responses of 35 adult immune Gambians to 29 overlapping peptides spanning the entire length of the CS protein.**



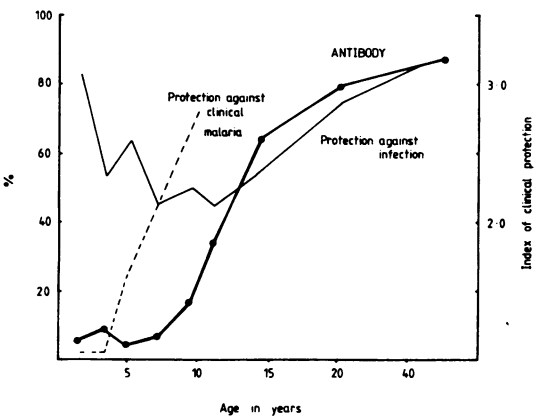
Data from ref. *9*.

Table 1: **Lymphocyte proliferative responses of 40 adult immune Gambians to two CSP peptides corresponding to the sequence found in three strains of *Plasmodium falciparum*[a]**

No. of subjects responding	Peptide	
	TH 2 R (326–345)	TH 3 R (361–380)
Three strains	13	10
Two strains	8	10
One strain	9	4
None	10	12
Not tested	–	4
Total	40	40

[a] Data from ref. *4*.

Fig. 4: **The relationship between sporozoite antibodies and protection against clinical malaria and against malaria infection in a rural area of the Gambia.**[a]



Data from ref. *12*.

[a] Protection against clinical malaria is expressed as the reciprocal of the number of clinical attacks per child per year determined by weekly morbidity surveillance. Protection against infection is indicated by the percentage of subjects with a negative blood film at the end of the rainy season.

only at about the age of 10 years. Comparison of the prevalence curve for antibodies to $(NANP)_3$ with the age-dependent pattern of other features of malaria showed that the incidence of clinical attacks of malaria declined well before the appearance of antibodies to $(NANP)_3$ but that the appearance of those antibodies corresponded more closely with a decline in the prevalence of parasitaemia. These findings suggest that antibodies to $(NANP)_3$ could play a part in protection against infection but not in protection against disease severity.

In 1988, CMI responses to the CSP peptides TH1R and TH2R were measured in approximately 500 Gambian subjects aged 1–70 years during a cross-sectional survey undertaken just before the malaria transmission season. Results from this study are now in press (Riley, E.M. et al. *Trans. Roy. Soc. Trop. Med. Hyg.*).

3. *Longitudinal cohort studies*

Two longitudinal studies of the possible role of antibodies to CSP peptides in protection against malaria have been done in the Gambia. In the first study, antibodies to $(NANP)_3$ were measured by radioimmunoassay in 124 Wollof children aged 1–11 years and related to the malaria experience of these children during the following rainy season (*12*). Clinical attacks of malaria were recorded a little less frequently in children who had anti-$(NANP)_3$ antibodies than in children who had not (Table 2) and, at the end of the rainy season, the prevalence of parasitaemia was significantly lower in children who were seropositive at the beginning of the rainy season than in those who were seronegative. This difference was not observed in adults.

A second study was undertaken in a group of Fula children aged 1–9 years resident in a different group of villages who were participating in a trial of bed nets (*17*). In this study, in which antibodies were measured by an ELISA to $(NANP)_{40}$ (*6*), the overall prevalence of antibodies in children was higher than that recorded in the first study. Children who were seropositive at the beginning of the rainy season were again found to experience fewer clinical attacks of malaria during the following months than children who were not (Table 3) but, at the end of the rainy season, the overall prevalence of parasitaemia was similar in the two groups.

In 1988, a third longitudinal cohort study was done in which the relationship between CMI responses to CSP peptides and protection against malaria was investigated. At the beginning of the rainy season lymphocyte proliferative and γ-interferon responses to the CSP peptides TH1R and TH2R of 3 strains were measured in 380 children aged 3–8 years. Between 10% and 30% of children responded to individual peptides. During the subsequent few months about half the children developed a clinical attack of malaria and others developed an asymptomatic parasitaemia. The results of the clinical and laboratory data sets are now being analysed.

Discussion

Comparison of the results of Gambian studies of sporozoite immunity with those of studies done elsewhere

The prevalence of anti-sporozoite antibodies has been measured in a number of communities in different

Table 2: **Antibodies to $(NANP)_3$ measured by radioimmunoassay in rural Gambians at the beginning of the rainy season and their relationship to subsequent malaria infection**[a]

	Age 1–11 years		Age 12 years or more	
	Positive ($n=15$)	Negative ($n=109$)	Positive ($n=46$)	Negative ($n=18$)
Clinical attacks of malaria (mean per child)	0.43	0.67	ND[b]	ND
Parasitaemia at end of rainy season (%)	20	59	29[c]	28[c]
	$P=0.01$			

[a] Data from ref. *12*.
[b] ND: not detected.
[c] Age-standardized.

Table 3: **Antibodies to $(NANP)_{40}$ measured by ELISA in Gambian children aged 1–9 years and their relationship to subsequent malaria infection**[a]

	Antibody status	
	Seropositive	Seronegative
Clinical attacks of malaria (mean per child)	0.17 (21/124)	0.22 (42/193)
Clinical attacks of malaria with high parasitaemia (mean per child)	0.07 (9/124)	0.17 (33/193)
Parasitaemia at the end of rainy season (%)	66.1 (74/112)	50.0 (90/180)
Parasitaemia ≥ 5000 per μl at the end of the rainy season (%)	17.0 (19/112)	19.4 (35/180)

[a] Data from Snow et al., unpublished.

Table 4: **Summary of cohort studies investigating the relationship between sporozoite immunity and protection against infection**

Country and reference	Age group	Measurement	Findings
Burkina Faso (*8*)	Adults	Antibody to $(NANP)_3$	Decreased incidence of infection in low transmission periods; no protection in high transmission periods
Gambia (*12*)	1–11 years	Antibody to $(NANP)_3$	Fewer clinical attacks (borderline) and reduced incidence of infection
Gambia (*17*)	1–9 years	Antibody to $(NANP)_{40}$	Fewer clinical attacks (high parasitaemia), no reduction in incidence of infection
Kenya (*11*)	Adults	Several antibody assays	No protection against infection
Kenya[a]	Adults	CMI responses to CSP peptides	Reduced incidence of infection in responders
Thailand[b]	All ages	Antibody to R32tet32	No protection against infection
Thailand (*16*)	5–15 years	Antibody to R32tet32	No protection against infection

[a] See footnote *b* below for reference.
[b] See footnote *a* below for reference.

malaria endemic areas. These studies have shown that the age at which antibodies first appear is closely related to the level of exposure. Thus, Druihle et al. (*7*) showed that in an area of Burkina Faso where adults are exposed to at least 100 infective bites per year most children aged 2–5 years had modest titres of fluorescent antibodies to sporozoites. In contrast, in an area of northern Senegal where exposure is only about one infective bite per year the antibodies were found only in older children and adults. Similarly, Esposito et al. (*8*) detected in Burkina Faso a direct relationship between the prevalence and titre of antibodies to $(NANP)_3$ in children and the entomological inoculation rate recorded in the community in which the children lived. In areas of high transmission in Kenya (*2*) and in the United Republic of Tanzania (*5*) antibodies were found in a high proportion of young children. In contrast, in communities in Indonesia (*10*) and in Thailand (*18*)[a] with a lower level of transmission a similar pattern to that observed in the Gambia was recorded, only a few children under the age of 10 years having high titres of antibody to sporozoites or CSP peptides. Surprisingly, a recent study in U.S. Peace Corps volunteers working in Africa showed that up to one half developed antibodies to $(PNAN)_5$ after only two years of exposure (*13*). Perhaps adults can more readily mount an antibody response to CSP peptides than children.

Several studies have investigated the relationship between antibodies to sporozoites and protection against malaria (Table 4) and some consensus is beginning to emerge. It is clear that antibodies to CSP peptides at the titres produced as a result of natural infection have, at the most, a very modest protective effect against malaria infection. Clear negative results have been obtained with cohort studies done in Kenya (*11*) and in Thailand (*16*).[a] The latter study, performed in Karen children, used a case–control technique. The results of the investigation in Kenya are particularly convincing, because in this investigation the sporozoite antibodies were measured by several different methods; none showed any suggestion of a protective effect. In Burkina Faso, cross-sectional surveys showed no protection against infection during the main malaria transmission season but some protection was seen in adults at the beginning and at the end of the rainy season when the pressure of infection was less (*8*). Our findings in the Gambia of some protection for seropositive subjects is in keeping with these observations because in this country, the pressure of infection, even at the height of the malaria transmission season, is less than that which prevails in the areas of Kenya and Burkina Faso where comparable studies have been done.

The results of only one longitudinal cohort study relating the CMI response to sporozoite antigens to protection against infection have been reported so far.[b] This study, which was carried out in an area of high transmission in Kenya, showed that of 25 adult volunteers who were infected with malaria during the

[a] **Hoffman, S.L. et al.** *Identification of a potentially protective T-cell epitope on the* Plasmodium falciparum *CS protein.* Paper presented at a meeting on the immunological aspects of malaria epidemiology, World Health Organization, Geneva, September 1988.

[b] **Webster, H.K. et al.** *Epidemiology of anti-sporozoite immunity in Thailand.* Paper presented at a meeting on the immunological aspects of malaria epidemiology, World Health Organization, Geneva, September 1988.

observation period only two responded to peptide 361–380 and only one to peptide 371–390 at the onset of the trial. In contrast, all three subjects who did not become infected responded to peptide 361–380 and two responded to peptide 371–390. These preliminary results are exciting but they need confirmation in trials involving larger numbers of subjects, such as the one which was done in the Gambia in 1988 in which 380 children were studied.

Conclusion

It is unlikely that epidemiological studies of the kind that have been described in this paper will ever be able to demonstrate convincingly that any specific immune response plays a critical role in protective immunity to malaria because such studies are inevitably subject to many potentially confounding factors. Furthermore, when an immune response is shown to be related to clinical protection this does not establish cause and effect since the immune response being studied may be linked to another more important immune mechanism which was not investigated. Epidemiological studies are likely to be better at demonstrating a negative rather than a positive effect and they have already provided good evidence that a CSP vaccine, which could only induce antibody levels comparable to those seen as a result of natural infection, would be very unlikely to provide any useful protective immunity in areas of high malaria transmission. Epidemiological studies may provide some helpful guidelines to molecular biologists and immunologists engaged in vaccine development on the directions that their work should take.

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