Antibodies to Sporozoites: Their Frequent Occurrence in Individuals Living in an Area of Hyperendemic Malaria

Abstract. *Serum samples from 158 West Africans were tested for antibodies against sporozoites, the vector stage of the malaria parasite. Antibodies specific for* Plasmodium falciparum *sporozoites were detected by means of the circumsporozoite precipitation assay and indirect immunofluorescence. More than 90 percent of the serum samples from adults gave positive immunofluorescent reactions against* falciparum *sporozoites, whereas most of the samples from children gave low or negative reactions.*

The sporozoite stage of the malaria parasite, injected by the bite of irradiated infected mosquitoes, induces total protection against challenge with homologous viable sporozoites in sporozoite-immunized rodents and man. The ability of the sporozoite to evoke an immune response has been demonstrated by the detection of sporozoite precipitating and neutralizing antibodies in the serum of these protected animals (*1*).

Whether the sporozoite plays a role in the natural resistance developed by individuals living in areas of hyperendemic malaria is not known. In the past it was thought that the sporozoites, introduced into the host by the bite of infected *Anopheles* mosquitoes, were present in the circulation for too short a period and in too small numbers to be immunogenic. In fact, previous attempts to demonstrate sporozoite-neutralizing activity in the serum of West African adults were not successful (*2*). We now report that a large proportion of individuals living in areas of endemic malaria do develop antibodies against the sporozoite stage of *Plasmodium falciparum*.

A total of 158 serum samples from West Africans of four age groups (5 to 9 years, 10 to 15 years, 20 to 49 years, and more than 50 years old) were studied. The serum donors were residents of the rural village of Keneba in the West Kiang district of The Gambia, an area hyperendemic for *P. falciparum* malaria. The villagers have been monitored by yearly health surveys since 1949, and records for each individual's malaria experience and general health have been maintained (*3*). Serum was obtained from finger-prick blood samples taken approximately 2 to 3 months after the peak period of malaria transmission. So that we would examine some aspects of the malaria infection that might affect the antisporozoite response, in selecting samples for circumsporozoite precipitation (CSP) and immunofluorescent testing, we included the serum of individuals with either high or low antibody titers against malaria-infected erythrocytes (*4*).

The serum samples were tested against viable and glutaraldehyde-fixed *P. falciparum* sporozoites. The parasites were obtained from a laboratory colony of *Anopheles gambiae* (species A) infected by membrane feeding (*5*) on blood obtained from patients in the Medical Research Council Clinic who had between 50 and 3000 *P. falciparum* gametocytes per cubic milliliter of blood. The mosquito salivary glands were dissected 12 to 17 days after the infective blood meal and the sporozoites were maintained in cold tissue culture Medium 199. Fixed parasite preparations were obtained by incubating the sporozoites in 0.1 percent glutaraldehyde solution for 10 minutes at room temperature (*6*). The antibodies to sporozoites were measured by the CSP assay (*7*) and indirect immunofluorescence (*8*).

The percentage of serum samples from each age group that gave positive CSP reactions when incubated with viable sporozoites is shown in Fig. 1A. In contrast to previous findings (*2*), 18 percent (28 out of 158) of all the undiluted serum samples had positive CSP reactions. We observed that the percentage of samples containing antibodies to sporozoites increased with age. Thus, 51 percent of the undiluted serum samples from individuals over 50 years of age gave positive CSP reactions with viable *P. falciparum* sporozoites. In comparison, only 2.5 percent and 5 percent of the children in the 5- to 9- and 10- to 15-year age groups, respectively, had antisporozoite antibodies detectable by the CSP assay.

Serum samples obtained from the older groups had higher levels of CSP reactivity. At a 1:4 dilution, samples from 29 percent of the adults over 50 years of age were still CSP positive (Fig. 1A). None of the samples from children in the 5- to 9- and 10- to 15-year age groups gave positive reactions at a 1:4 dilution.

The same serum and sporozoite mixtures were also examined by using indirect immunofluorescence; this technique has recently been shown to be more sensitive than the CSP assay for detecting stage- and species-specific antibodies to sporozoites in animal models (*6*). We found that 70 percent (104 out of 148) of all the undiluted Gambian serum samples were positive when tested by immunofluorescence (Fig. 1B). In comparison, the CSP assay had detected antisporozoite reactions in only 18 percent of all the undiluted serum samples.

Again, a higher percentage of positive reactions was found in adults when the serum samples from the various age groups were assayed by immunofluorescence. More than 90 percent of the undiluted serum samples of individuals 20 to 49 years of age or older gave positive fluorescence with viable sporozoites. Only 47 percent of the samples

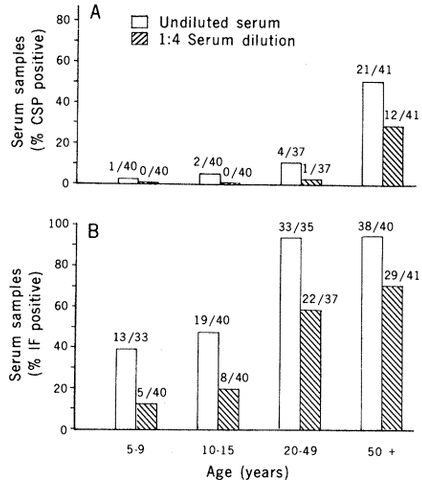

Fig. 1. All reactions were carried out with viable *P. falciparum* sporozoites (4 to 5.0 × 10^5/ml) maintained in cold Medium 199. Equal volumes (20 to 30 μl) of the parasite suspension and serum (undiluted or a 1:4 dilution) were incubated together for 30 to 45 minutes at 35°C. (A) For the CSP reactions, a small sample (approximately 5 μl) of the parasite and serum mixture was removed and examined by phase microscopy. A minimum of 20 parasites were counted and the number of sporozoites with the characteristic tail-like precipitate (CSP) was recorded for each serum sample. Serum was considered positive when a minimum of 10 percent (2 out of 20) of the parasites had detectable CSP reactions. Because of the limited availability of sporozoites, it was not possible to titrate the CSP end points for each serum sample. (B) For the indirect immunofluorescence (IF) assay, the remaining parasite and serum mixture was washed twice in cold Medium 199, reduced to approximately 20 to 30 μl, and air-dried on multiple well slides. The slides were then rinsed in phosphate-buffered saline, fixed in acetone, washed and stained with fluorescein isothiocyanate (FITC)-conjugated antiserum specific for human γ chains (Behring), and counterstained with Evans's blue. All reactions were coded and examined at ×25 magnification with a Leitz epi-illuminated microscope equipped with standard filters for FITC fluorescence.

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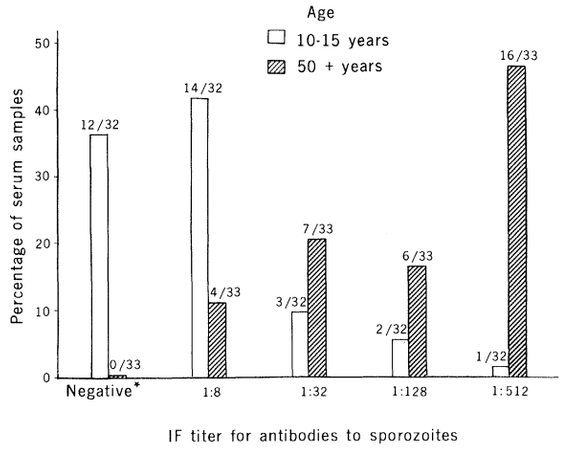

Fig. 2. *Plasmodium falciparum* sporozoites were fixed for 10 minutes at room temperature in 0.1 percent glutaraldehyde (Sigma) prepared in 0.1*M* cacodylate buffer and 4 percent sucrose. After the sporozoites were washed, the concentration was adjusted to 3 to 5×10^5 sporozoites per milliliter. Ten microliters of the fixed sporozoite preparation were distributed on multiple well slides that were air-dried and stored at −70°C until used in the IF assay. Fourfold dilutions of serum from Gambians, starting at a 1:8 dilution, were tested from individuals in the 10- to 15-year age group and adults in the 50+ group. Ten microliters of each dilution were incubated with the fixed parasites for 30 to 45 minutes at 35°C. After the mixture was rinsed, fixed in acetone, and washed, the sporozoites were stained for immunofluorescence as described for Fig. 1B. Most of the samples (63 out of 65) gave consistent IF reactions with both fixed and viable sporozoites. The asterisk indicates a negative response at a 1:8 serum dilution.

from children 10 to 15 years old and 39 percent of the samples from the youngest age group (5 to 9 years) contained sporozoite antibodies detectable by indirect immunofluorescence.

The immunofluorescent technique also detected a higher percentage of positive reactions in diluted serum samples. At a 1:4 serum dilution, 70 percent of the 50+ age group and 59 percent of the adults aged 20 to 49 years old had antibodies to sporozoites detectable by immunofluorescence. At the same dilution, 20 percent of the samples from the 10- to 15-year group and 12.5 percent of the samples from the 5- to 9-year-old children gave positive reactions.

The trend toward higher concentrations of antibodies in the older age groups was also reflected in the end-point titrations (Fig. 2). Serum from 32 individuals in the 10- to 15-year age group and 33 adults over 50 years of age were titrated against a preparation of glutaraldehyde-fixed sporozoites. As shown in Fig. 2, a high percentage of the serum samples from the 10- to 15-year-old Gambians had either negative reactions or low titers (1:8) when tested against fixed *P. falciparum* sporozoites. In contrast, most of the samples from adults (50+ years of age) had end-point titers of 1:128 and 1:512.

A significant positive relation was found between high antisporozoite titers, as detected by immunofluorescence with the glutaraldehyde-fixed antigens, and positive CSP reactions with viable sporozoites ($P < .001$). Consistent with earlier studies demonstrating the stage specificity of the CSP reaction (*1*), there was no correlation between CSP positivity and immunofluorescent titers against *P. falciparum*-infected red blood cells ($P < .7$).

The species and stage specificities of the immunofluorescent reactions with glutaraldehyde-fixed sporozoites were determined by using heterologous hyperimmune antiserums. Serum of sporozoite-immunized hosts protected against simian (*Plasmodium knowlesi* and *P. cynomolgi*) and human (*P. vivax*) malaria and normal human serum from the New York City Blood Bank failed to react with the fixed parasites. Serum from Balb/c mice immunized with *P. falciparum*-infected red blood cells, which gave high immunofluorescent titers with blood-stage antigen, failed to cross-react with the fixed *P. falciparum* sporozoites. Consistent with this stage specificity was the finding that in the serum from Gambians no correlation was found between antisporozoite immunofluorescent titers and antibody titers against the blood-stage parasites. This corroborated our findings in animal models that only stage- and species-specific antibodies are detected when intact sporozoites, either viable or glutaraldehyde-fixed, are used in the immunofluorescent assay (*6*, *8*).

The reason for the differences in the concentrations of sporozoite antibodies among the various age groups is unclear. The variation in the antibody response might simply reflect the increasing amount of exposure to sporozoite antigen with time. Observations on mosquito feeding behavior in The Gambia have shown that adults are bitten at a rate approximately seven times higher than the rate for children (*9*).

Another possibility is that the malaria infection itself might influence the immune response to sporozoites. In animal models, acute malaria suppresses the antibody response to numerous antigens (*10*) including sporozoites (*11*). Acute episodes of malaria are usually experienced in childhood, with little clinical manifestations among adults living in endemic areas. With increasing age and decreasing frequency of patent malaria infections, the immunosuppression induced by the parasite might decrease, which would allow the development of a higher antibody response against sporozoites.

The role of the sporozoite antibody response in resistance to malaria remains to be fully defined. Serum from sporozoite-immunized animals neutralizes sporozoite infectivity and mediates an enhanced clearance of the parasite from the circulation (*1*). The ability of immunized simian and human hosts to resist challenge is associated with high serum concentrations of antibodies to sporozoites. Circumsporozoite reactivity was correlated with functional immunity in sporozoite-immunized volunteers protected against the bite of *P. falciparum*- and *P. vivax*-infected mosquitoes (*12*, *13*).

Whether the sporozoite antibodies detected in the serum samples from individuals in The Gambia contribute to the development of resistance against malaria is difficult to ascertain. It is noteworthy that a number of the adult Gambians (10 out of 33) had high sporozoite antibody immunofluorescent titers while possessing low or negligible antibody titers against infected red blood cells. These individuals failed to present a patent malaria infection in the absence of a significant antibody response against the blood stages of the parasite. It is conceivable that the presence of high levels of sporozoite antibodies in these individuals might have resulted in the elimination of the infective sporozoites, thus preventing the development of patent infections. In the absence of blood-stage antigen boosters, the level of antibodies against the erythrocytic parasite would fall to low levels, while continued exposure to the bite of malaria-infected mosquitoes would increase the antisporozoite response. Challenge by viable sporozoites, introduced into the host by the bite of infected mosquitoes, enhanced both resistance and the sporozoite antibody response in a sporozoite-immunized volunteer (*13*).

A sporozoite vaccine would ideally induce immunity in children, thereby eliminating the long period required for the development of antisporozoite response under natural conditions. Protection induced by a sporozoite vaccine might be expected to be maintained for longer pe-

riods under endemic conditions, since the bite of infected mosquitoes would reinforce the vaccine-induced immunity.

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Solid Electrolyte Behavior of $NaMgF_3$: Geophysical Implications

Abstract. *In the solid state, $NaMgF_3$ transforms smoothly with temperature into a solid electrolyte phase; the conductivity is 130 siemens per meter just below the melting point. The isostructural compound $MgSiO_3$ should behave similarly under conditions obtaining in the earth's lower mantle, and so it is expected that the electrical conductivity in that region is ionic rather than electronic.*

It has been known for some years that many binary fluorides are good solid electrolytes ("superionic" conductors) with ionic conductivities in the solid state at high temperatures comparable to those of molten salts. The transition from the poorly conducting to the solid electrolyte state may be abrupt as in YF_3 and LuF_3 (*1*) or continuous as in PbF_2 and other salts with the fluorite (CaF_2) structure (*2*) and in salts with the tysonite (LaF_3) structure (*3*).

A number of regularities in the transition have been noted (*4*). The more important of these for the present discussion are as follows.

1) The occurrence of a solid electrolyte transition of a particular type is closely related to crystal structure. Without exception, it has been found that, if a material with a given structure type (for example, CaF_2 or LaF_3) undergoes a continuous transition, that is, is in class III (*4*), then so do all other crystals with the same structure.

2) The entropy increment associated with the transition, whether discontinuous or continuous (*5*), is comparable to the entropy of melting of the salt. This finding suggests the concept of "sublattice melting."

3) The ionic conductivity of the salt is typically 10^{-1} S m^{-1} just below the transition temperature and $\sim 10^2$ S m^{-1} in the solid electrolyte range. For normal salts (not solid electrolytes) these are the ionic conductivities typically found just below and above the melting temperature. Thus ionic conductivities in the range 10^{-1} to 10^2 S m^{-1} are to be associated with solid electrolytes of class III in the transition region.

It has also been shown recently (*6*) that the structures of YF_3 and LaF_3 are very closely related to that of the orthorhombic "perovskites" typified by $YFeO_3$ and $CaTiO_3$ (perovskite). It was thought likely therefore that perovskites of this type might prove also to be anion-conducting solid electrolytes. The oxides generally have high (> 2000°K) melting temperatures. Because high-temperature conductivity measurements are subject to considerable experimental difficulties and uncertainties (*7*), we have investigated first the ionic conductivity of $NaMgF_3$ (*8*) which has the same structure type (*9*) and is isoelectronic with $MgSiO_3$. The experimental techniques were the same as those described in (*1*).

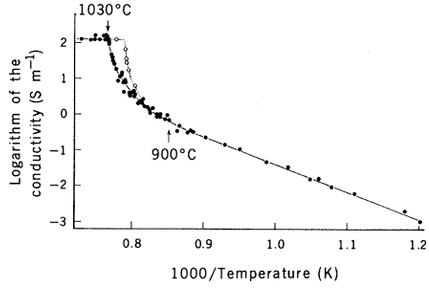

Fig. 1. The logarithm of the conductivity of $NaMgF_3$ as a function of reciprocal temperature. The arrow at 1030°C indicates the melting point, and the arrow at 900°C designates the temperature above which the salt becomes cubic. The closed circles represent four measurements (heating and cooling) on two different samples. The open circles and dotted line represent data obtained with a supercooled (~ 100°K/hour) melt.

The experimental results are displayed in Fig. 1. The conductivity of solid $NaMgF_3$ at high temperatures is in the solid electrolyte range (10^{-1} to 10^2 S m^{-1}), it is a continuous function of temperature, and it undergoes very little change at the melting temperature (1030°C). It is clear then that there is a continuous solid electrolyte transition in this compound that starts at about 900°C, the temperature at which the crystal becomes cubic (*9*).

Although of great interest in itself in establishing the occurrence of a solid electrolyte transition in a new structural type, this result also has geophysical implications of considerable consequence. It is a long-established principle in geophysics and geochemistry that fluorides can be used as model systems for isostructural oxides. We have discussed elsewhere (*10*) the correspondence between $NaMgF_3$ and the high-pressure perovskite form of $MgSiO_3$ (*11*), which is very likely a major constituent of the earth's lower mantle (that is, that region between 3500 and 5700 km from the center, the bulk of the earth) (*12*). Thus it is likely that perovskite $MgSiO_3$ is a high-temperature solid electrolyte and that the lower mantle is a solid electrolyte phase.

In connection with the above proposal, the following points are very relevant. (i) Heat flow considerations suggest very strongly that the material in the lower mantle is very close (within perhaps 10°K) to the melting temperature (*13*). (ii) The best estimates (*14*) of the electrical conductivity in this region place it in the range 10^{-1} to 10^2 S m^{-1}, that is, exactly in the "molten sublattice" or solid electrolyte range.

In short, the evidence is strong that the lower mantle consists of a solid electrolyte phase rather than an electronic conductor, as is usually assumed (*15*). As solid electrolytes differ in important ways from normal salts in many of their properties (thermodynamic, elastic, and rheological) (*16*), detailed models of the

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