SOME CHARACTERISTICS OF THE IMMUNE RESPONSE TO SPOROZOITES OF SIMIAN AND HUMAN MALARIA

Ruth S. Nussenzweig, M.D., Ph.D.; and David Chen, M.A., Ph.D.

The quest for a vaccine against malaria has focused considerable attention on sporozoite-induced immunity in recent years. This article reviews our present knowledge of this subject and summarizes the results of current research, concentrating especially on the degree to which sporozoite antisera are specific for particular human and simian malaria strains and species.

Introduction

The purpose of this article is to review the state of the art of immunization against malaria using the organism’s sporozoite stage, and to summarize some recent progress in this area, mainly by defining the specificity of the immune response to sporozoites of human and simian malaria. It also reports preliminary results obtained by immunizing Rhesus monkeys with irradiated sporozoites of Plasmodium cynomolgi and attempts to correlate these findings with the results obtained in preliminary human trials (Clyde, et al., 1973). Finally, it seeks to point out a number of the gaps in our knowledge of sporozoite-induced immunity.

To summarize the state of the art on immunization with sporozoites is not a difficult task, since it has been an "unorthodox" approach followed by few investigators. In fact, most immunization attempts have used blood stages of the parasite, partly because of difficulties involved in working with infected mosquitoes, but also because of the quite deeply rooted, erroneous idea that sporozoites are not immunogenic.

On the other hand, immunization with sporozoites is not really a new approach, since it was shown quite convincingly in the 1940’s that avian malaria sporozoites which have been exposed to ultraviolet radiation induce antisporeothe bodies and a partial, although very significant, protection in immunized birds (Mulligan, et al., 1941).

Immunization of Rodents

The work on immunization with sporozoites of rodent malaria began about seven years ago, when we observed that a single intravenous injection of irradiated sporozoites totally protected a variable proportion of injected mice against an otherwise invariably lethal P. berghei infection (Nussenzweig, et al., 1967). The percentage of mice protected against sporozoite challenge after this single immunizing dose varied from 15 to 100 per cent in different experiments.

It should be pointed out that two of the basic questions raised by these experiments, namely, (1) the reasons for this considerable variation in the immunogenicity of different sporozoite preparations, and (2) the related matter of how to increase this protection so as to obtain resistance in 100 per cent of the animals, still remain to be answered. The closest
approach to the latter goal was achieved by treating the mice with *Corynebacterium parvum* before immunization. *C. parvum*, a potent stimulator of the reticuloendothelial system (RES), can substantially increase non-specific resistance to sporozoite-induced infections (Nussenzweig, 1967).

When administration of this RES stimulant was combined with injection of a single dose of X-irradiated sporozoites, we observed a considerable increase in the percentage of protected animals. Whether this was simply an additive effect of non-specific plus specific resistance, or whether *C. parvum* acted as an adjuvant, amplifying the protective response induced by X-irradiated sporozoites, is still unclear. Meanwhile, the effects of *C. parvum* on the simian malaria system are being explored in order to see if it also enhances the immune response to *P. cynomolgi* sporozoites.

If one increases the number of immunizing sporozoite injections per mouse to four or five, the results become very reproducible, close to 100 per cent protection being observed in most of our experiments (Nussenzweig, et al., 1969a). This experimental model has permitted us to determine a number of characteristics of sporozoite-induced immunity in rodent malaria, characteristics which were reviewed in a report to the Inter-American Malaria Research Symposium organized by the Pan American Health Organization in 1972 (Nussenzweig, et al., 1972).

Recent Work on Human and Simian Malaria Sporozoites

This initial work is what led us into our more recent investigations of immune responses that can be produced against sporozoites of various human and simian malaria species.

**Vaccination of Monkeys**

Attempts to vaccinate simian hosts by using irradiation-attenuated sporozoites are still in a somewhat preliminary phase. Findings reported by others (Collins and Contacos, 1972), as well as our own unpublished observations, provide some evidence of partial immunity resulting from several of these attempts. However, optimal conditions for simian immunization still remain to be established.

Apart from the need to find the best dosage, route, and schedule for immunization, the success achieved by these vaccination attempts will probably depend heavily on selection of an appropriate antigen preparation. The data reported on the pages that follow provide information concerning the immunogenicity of different sporozoite preparations that relates directly to this point.

The subject has been investigated by examining the antibody response (a) to sporozoites of different strains and species of simian and human malaria, and (b) to different developmental stages of sporozoites. In addition, we have obtained initial data on the infectivity of some of these developmental stages.

**Induction of Anti-Sporozoite Antibodies in Rats**

Investigation of the immune response to simian and human malaria sporozoites was facilitated by using rats as antisporeozoite antibody producers. This was made possible by the finding that injection of simian malaria sporozoites into this unnatural host causes parasite development to stop and induces a very rapid and consistent circumsporozoite (CSP) antibody response (Nussenzweig, et al., 1973). These antibodies can be detected by incubating the sporozoites with immune serum, which causes a thread-like precipitate to form, usually at one end of the sporozoite. The reaction can be observed easily under a phase contrast microscope and is essentially similar to the rodent malaria CSP reaction previously described (Vanderberg, et al., 1969).

We have recently found CSP antibody formation to result from injection of rats with a total dose of 1.0-2.0 x 10^5 irradiated or non-irradiated sporozoites administered in one or two intravenous injections. The antibody is detectable less than two weeks after the initial immunization.
This pattern of antibody response has been found consistently in all our attempts to immunize rats with sporozoites of various simian and human malarial species. Furthermore, intravenous immunization of Rhesus monkeys with irradiated sporozoites of Plasmodium cynomolgi has shown these animals to produce a similar but delayed CSP antibody response.

**Determination of Antigenic Maturity of P. cynomolgi Sporozoites**

The development of certain antigens in sporozoites of simian malaria has been investigated by testing the immunogenicity and infectivity of the various stages of P. cynomolgi (strain B) during sporogony.

For this purpose infected Anopheles stephensi mosquitoes were sectioned, separating the thorax from the abdomen, and sporozoites were collected from these abdominal and thoracic regions as well as from dissected salivary glands and midguts. This was done under conditions designed to minimize reciprocal contamination of the various sporozoite populations. Rats were then immunized with the sporozoites from these different locations, the sporozoites having been obtained at different time intervals following the mosquito's infective blood meal—i.e., from day seven up to day 25 after infection. The purpose of these experiments was to determine whether sporozoite maturation at any given location was time-dependent or, alternatively, whether parasite populations from the midguts ever became infective and antigenically mature before moving to the hemocele and later to the salivary glands.

All the sporozoite populations were analyzed in terms of the following characteristics: (1) their infectivity for Rhesus monkeys, (2) their capacity to induce the formation of CSP antibodies, and (3) their capacity to serve as antigenic material in the CSP reaction.

It was initially observed that the immunogenicity of the sporozoite populations varied considerably, depending on their location in the mosquito. It was further observed that a prolonged period of mosquito infection failed to prompt additional sporozoite maturation, unless migration of the sporozoites toward the salivary glands had also occurred.

Midgut sporozoites only induced minimal amounts of CSP antibodies and only in exceptional cases. Moreover, they consistently failed to react with known positive antisporozoite antisera. The basic antigenic characteristics of sporozoites from the hemocele of the abdominal region were rather similar. They induced only minimal amounts of antibodies, and very few produced a tail-like precipitate characteristic of the CSP reaction.

Midgut sporozoites so far have been non-infective to monkeys. Sporozoites from the hemocele of the abdominal regions were also non-infective in several instances. In other experiments these hemocele sporozoites produced patency after a significantly prolonged incubation period, indicating a considerable loss of infectivity.

Quite different results were obtained with sporozoites from the thoracic hemocele. These parasites were infective, though apparently less so than salivary gland sporozoites. They induced a considerable CSP antibody response, but relatively few individual sporozoites (no more than 1 per cent) yielded a positive CSP reaction.

Finally, all three characteristics were fully present in salivary gland sporozoites. Their infectivity was also considerable, although it varied a great deal in different batches of mosquitoes. This variability made it difficult to compare the infectivity of sporozoite populations from different regions. Comparisons became meaningful only when parallel studies were conducted using the same batches of mosquitoes.

The time elapsed between infection of the mosquito and its dissection did not seem to play a major role in determining the degree of sporozoite infectivity. Thus the earliest salivary gland sporozoites (appearing 10 to 11 days after infection) were on some occasions just as infective as sporozoites obtained 25 days after infection.

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4 The general body cavity of the mosquito.
However, we did notice a progressive degree of antigenic maturation in salivary gland sporozoites obtained at differing lengths of time after infection. Early salivary gland sporozoites (collected 10 days after the mosquitoes' blood meal) reacted poorly upon incubation with immune sera. Maximum reactivity was obtained from parasites collected 17 and 21 days after infection. Further experiments on the comparative infectivity and immunogenicity of these different sporozoite populations are currently underway.

The Species Specificity of Antibodies Produced against Sporozoites of Simian Malaria

Antisera produced against salivary gland sporozoites of a number of different simian malaria species have been tested for CSP reactivity. In each instance the sera were first screened, using homologous sporozoites as antigen, to confirm the presence of antisporeo- zoite antibodies. When positive, these sera were tested with sporozoites of other human and simian malaria species and strains to detect any possible cross-reactions.

The results obtained (see Table 1) indicate that positive reactions occurred only between homologous sporozoites and antisera. Even when the simian malaria species were believed to be rather closely related, as in the case of the two "ovale-type" parasites *P. simiovale* and *P. fieldi*, no cross-reactions were observed. Nor did antisera produced against sporozoites of simian malaria cross-react with sporozoites of human malaria. Thus, antisera prepared against the "vivax-type" parasite *P. cynomolgi* did not cross-react with either the Rio Meta or the Sal II strain of *P. vivax*. The antisera produced against sporozoites of other simian malarias also failed to react with sporozoites of either *P. falciparum* or *P. vivax*. Additional data of a more detailed nature, including information about the strain-specificity of these antibodies, are presently being obtained.

The Species Specificity and Strain Cross-Reactivity of Antibodies Produced against Sporozoites of Human Malaria

We have also investigated the specificity of antisera produced by intravenous immunization of rats with sporozoites of various types of human malaria. So far we have obtained antibodies against the Thau strain of *P. falciparum* and the Sal II strain of *P. vivax*. These antisera have produced positive CSP reactions only when incubated with sporozoites of the same species. No cross-reactions have been observed between sporozoites of *P. vivax*, *P. falciparum*,

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<th>TABLE 1 – Circumsporozoite (CSP) reactivity observed in sera of rats immunized with various simian malaria sporozoites.</th>
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<td><strong>Sporozoite antigen</strong></td>
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<sup>a</sup> This antiserum was also tested against sporozoites of the Sal II and Rio Meta strains of *P. vivax* and the Thau strain of *P. falciparum*, but did not cross-react with any of these.

<sup>b</sup> No positive reactions occurred when these antisera were tested against sporozoites of *P. vivax* (Sal II) and *P. falciparum* (Thau strain).

<sup>c</sup> No positive cross-reactions were observed when this antiserum was tested against *P. falciparum* (Thau strain).
or any of several simian malaria species (see Table 2). Sporozoite strains from different geographic regions have reacted as strongly with heterologous as with homologous antisera.

These serologic findings have recently been confirmed in human volunteers immunized by the bite of X-irradiated *P. falciparum*-infected mosquitoes (Clyde, et al., 1973a). One of these volunteers is reported to have developed detectable anti-sporozoite antibodies and resistance to repeated sporozoite challenge. After a long period of repeated immunization his serum yielded a positive CSP reaction with sporozoites of the homologous Thau strain as well as with sporozoites of three other *P. falciparum* strains. No cross-reaction with *P. vivax* sporozoites was observed (Clyde, et al., 1973b).

The protective immunity acquired by this volunteer paralleled these serological results. He was shown to be totally resistant to challenge with sporozoites of the three other *P. falciparum* strains, but was fully susceptible to sporozoites of *P. vivax*.

**Conclusions**

These results agree closely with our earlier data (Nussenzweig, et al., 1969b, and Vanderberg, et al., 1969) on the correlation between cross-reactivity of CSP antibodies and cross-protection in sporozoite-induced rodent malaria. Further findings on the cross-reactivity of different simian and human malaria sporozoites might therefore help to predict the range of cross-protection to be obtained from these antigen preparations.

The finding that sporozoites are subject to a process of antigenic maturation during the sporogonic cycle of *P. cynomolgi* is certainly noteworthy. This maturation process shows some similarity to what had previously been observed in rodent malaria (Vanderberg, et al., 1972). Antigenic maturation, therefore, seems to follow a similar pattern and to represent a common feature of sporogonic development in all mammalian malaria parasites.

This still leaves open the basic question of what factors prompt the appearance and/or expression of certain sporozoite antigens. To the extent that their expression parallels the capacity of sporozoites to induce protective immunity, characterization of these antigens becomes a problem of fundamental importance. In this regard there is hope that comparative antigenic analysis of different sporozoite populations might lead to characterization of the "protective antigens" involved.

It was recently demonstrated that sporozoites concentrated and purified by gradient centrifugation retain their immunogenicity and infectivity (Krettli, et al., 1973). Thus characterization of "protective antigens" might be achieved using this approach. In addition, the considerable yield of parasites collected by this method makes it easier to obtain large sporozoite doses for purposes of vaccination.

From the point of view of immunization it is of paramount importance to use immunolog-
ically mature sporozoites that are equipped with the "protective antigens." For vaccination attempts these parasites should therefore be harvested at the stage of infection when maximum maturation and migration to the hemocele and salivary glands have occurred; this time does not necessarily coincide with the time of maximum parasite yield.

**SUMMARY**

Previous work on rodent malaria has demonstrated that intravenous vaccination with X-irradiated sporozoites frequently results in total protection against an otherwise lethal sporozoite inoculum. Similar vaccination attempts are being pursued in simian hosts and more recently in human volunteers.

The purpose of the experiments reported here was to establish (1) some of the antigenic characteristics of various simian and human malaria sporozoites, (2) the stage specificity of their antigen or antigens, and (3) the extent of parasite infectivity of the different developmental stages.

Rats were immunized with a number of preparations, providing antisera specific for various human and simian malaria species and strains. Rhesus monkeys were injected with different developmental stages of sporozoites to determine their infectivity.

Only sporozoites collected from the thoracic region (hemocele and salivary glands) of mosquitoes induced significant antibody formation and were consistently infective. An additional degree of maturation took place in the salivary glands. Sporozoites obtained from the latter site were the most infective and the best inducers of circumsporozoite (CSP) antibody formation. These sporozoite populations also contained a higher percentage of parasites which produced CSP precipitates detectable upon incubation with immune serum.

It was further established that CSP (anti-sporozoite) antibodies to the simian and human malaria species involved are strictly species-specific, in that we observed no cross-reaction, even between malarial species believed to be closely related. However, intense reactions were obtained with antisera from different strains of the same species and from different geographic isolates. This knowledge should be valuable in choosing the type of sporozoite preparation to be used in developing a malaria vaccine.

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**REFERENCES**


(7) Nussenzweig, R. S., and D. Chen. Characteristics of the antibody response to sporozoites of simian and human malaria: Its stage and


