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The antibody response to sporozoites of simian and human malaria parasites: its stage and species specificity and strain cross-reactivity *

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Attempts to vaccinate against malaria are currently being pursued in both simian hosts and human volunteers, using X-irradiated sporozoites as antigen. The present experiments provide information on the developmental stage during which simian malaria sporozoites acquire certain antigen(s) and become infective, and on the antigenic similarities between sporozoites of different strains and species of simian and human malaria parasites. Such knowledge should prove of value in the choice of sporozoite preparations for future attempts at vaccination.

Studies on the immune response to sporozoites of simian and human malaria parasites have only recently been initiated, stimulated mainly by previous observations that total protection frequently resulted from the immunization of mice with X-irradiated sporozoites. The characteristics of this immune response were recently reviewed by Nussenzweig et al. (1), and have in fact certain similarities with the earlier, classical observations on sporozoite-induced immunity in avian malaria reviewed by Russell & Mohan (2).

Attempts to vaccinate simian hosts using sporozoites attenuated by irradiation are still in a somewhat preliminary phase. The results reported by Collins & Contacos (3), as well as our own observations (Chen & Nussenzweig, unpublished data), have provided some evidence of partial immunity in several of these trials. However, optimum conditions of immunization in this system still remain to be established. Apart from the optimum dosage, route, and schedule of immunization, which must be determined, possibly the most important factor for successful vaccination is the choice of an appropriate antigen preparation. Our present data provide information regarding this latter aspect, i.e., the immunogenicity of different sporozoite preparations. This has been investigated by examining the antibody response to (*a*) sporozoites of different strains and species of simian and human malaria parasites, and (*b*) different developmental stages of sporozoites. In addition, we have obtained initial data on the infectivity of some of these sporogonic stages.

Investigations of the immune response to sporozoites of simian and human malaria parasites were made easier by the use of rats as antisporozoite antibody producers. This was based on the finding by Nussenzweig et al. (4) that the injection of sporozoites of simian malaria parasites into this unnatural host led to an arrest in parasite development and induced a very rapid and consistent circum-sporozoite antibody response. These antibodies can be detected by the formation of a thread-like precipitate, which appears usually at one end of the sporozoites upon their incubation with immune serum. The reaction can easily be observed under a phase contrast microscope and is essentially similar to the circum-sporozoite (CSP) reaction described by Vanderberg et al. (5) in rodent malaria.

Recently, we observed that one or two intravenous injections of a total of $2\text{–}4 \times 10^5$ irradiated or non-irradiated sporozoites resulted in CSP antibody formation, which was detectable in less than 2 weeks after the initial immunization. This pattern of antibody response has so far been consistent in all attempts to immunize rats with sporozoites of the various simian and human malarias. Furthermore, the intravenous immunization of Rhesus monkeys with irradiated sporozoites of *P. cynomolgi* showed that these animals produce a similar, although delayed, CSP antibody response.

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ANTIGENIC MATURATION OF SPOROZOITES OF SIMIAN MALARIA PARASITES

The development of certain antigen(s) in the sporozoites of simian malaria parasites was investigated by determining the immunogenicity and infectivity of the various stages of *P. cynomolgi* (B strain) during sporogony.

For this purpose we sectioned infected *Anopheles stephensi* mosquitos, separating their thoraces from their abdomens, and collected sporozoites from the abdominal and thoracic regions as well as from dissected salivary glands and midguts. This was done under conditions that resulted in a minimal degree of reciprocal contamination of these sporozoite populations. Rats were immunized with sporozoites from these various locations, obtained at different time intervals after the infective blood meal, i.e., from day 7 up to the 25th day after mosquito infection.

The purpose of these experiments was to determine if sporozoite maturation in any given location was time-dependent, or alternatively, if parasite populations from the midguts ever became infective and antigenically mature before moving to the haemocele, or even to the salivary glands.

All sporozoite populations were analysed by the following three criteria: (*a*) their infectivity for Rhesus monkeys, (*b*) their capacity to induce the formation of CSP antibodies, and (*c*) their capacity to serve as antigen in the CSP reaction.

First, it was observed that the sporozoite populations varied considerably in their immunogenicity according to their location in the mosquito. It was further observed that a prolonged infection period failed to bring about additional sporozoite maturation, unless this was accompanied by migration of the sporozoites toward the salivary glands.

Midgut sporozoites induced only in exceptional cases a minimal amount of CSP antibodies, but consistently failed to react with known positive antisporozoite antisera. The basic antigenic characteristics of sporozoites from the abdominal region (haemocele) were rather similar. They induced a minimal amount of antibodies, but very few produced the tail-like precipitate characteristic of the CSP reaction. Furthermore, most of these parasites were noninfective. The results were quite different with sporozoites from the thoracic region (haemocele). These parasites were infective, although apparently less so than salivary gland sporozoites. They induced a considerable CSP antibody response, but relatively few individual sporozoites ($\leqslant 1\%$) gave a positive CSP reaction. Finally, all three characteristics were fully present in salivary gland sporozoites. Their infectivity was considerable, although quite variable in different batches of mosquitos. This variability, in fact, made any comparison of the infectivity of sporozoite populations from different regions rather difficult. Comparisons became meaningful only when parallel studies were made using the same batches of mosquitos.

The time of mosquito infection did not seem to play a major role in determining the degree of sporozoite infectivity. Thus the earliest salivary gland sporozoites (10–11th day of infection) were on some occasions as infective as sporozoites obtained on the 25th day after the infective blood meal.

In addition, we noticed a progressive degree of antigenic maturation in salivary gland sporozoites obtained after different periods of infection. Early salivary gland sporozoites (10 days) were poorly reactive; whereas maximum reactivity was obtained when 17- and 21-day-old parasites were incubated with immune sera.

Further experiments on the comparative infectivity and immunogenicity of these different sporozoite populations are now being completed.

SPECIES SPECIFICITY OF CSP ANTIBODIES TO SIMIAN MALARIA PARASITES

Antisera produced against salivary gland sporozoites of a number of different simian malaria species have been tested in the CSP reaction. In each instance, the sera were first screened, homologous sporozoites being used as antigen, to evaluate the presence of antisporozoite antibodies. When positive, these sera were tested with sporozoites of other species and strains of simian and human malaria parasites, to detect any possible cross-reactions.

The results (Table 1) indicate that positive reactions occurred only within the homologous system. Even in those instances in which the simian 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. Antisera produced against sporozoites of simian malaria parasites also did not cross-react with sporozoites of human malaria parasites. 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

Table 1. Specificity of the circum-sporozoite (CSP) reactivity observed in the sera of rats immunized with various simian malaria sporozoites.

Sporozoite antigen		Antisera against sporozoites of:			
		P. cynomolgi [a]	*P. fieldi* [b]	*P. simiovale* [b]	*P. knowlesi* [c]
vivax-type	*P. cynomolgi*	pos.	neg.	neg.	neg.
	P. gonderi	neg.	neg.	neg.	neg.
ovale-type	*P. fieldi*	neg.	pos.	neg.	neg.
	P. simiovale	neg.	neg.	pos.	neg.
other types	*P. knowlesi*	neg.	neg.	neg.	pos.

[a] This antiserum also did not cross react with sporozoites of the Sal II and Rio Meta strains of *P. vivax* and the Thau strain of *P. falciparum*.

[b] No positive reactions occurred with sporozoites of *P. vivax* (Sal II) and *P. falciparum* (Thau strain).

[c] No positive cross reactions were observed with sporozoites of the Thau strain of *P. falciparum*.

produced against sporozoites of other simian malaria parasites also failed to react with sporozoites of either *P. falciparum* or *P. vivax*. Additional and more detailed data, including the strain specificity of these antibodies, will be presented elsewhere (Chen, Collins, & Nussenzweig, in preparation).

SPECIES SPECIFICITY AND STRAIN CROSS-REACTIVITY OF SPOROZOITES OF HUMAN MALARIA PARASITES

These matters were investigated by using antisera of different specificities produced by the intravenous immunization of rats with sporozoites of the different human malaria parasites. So far we have obtained antibodies against the Thau strain of *P. falciparum* and the Sal II strain of *P. vivax*. These antisera produced positive CSP reactions only when incubated with sporozoites of the same species. No cross-reactions were observed between sporozoites of *P. vivax* and *P. falciparum*, or any of several simian malaria species (Table 2). Sporozoite strains consisting of isolates from different geographic areas reacted as strongly with homologous as with heterologous antisera.

These serologic results have recently been confirmed in human volunteers immunized by the bite of mosquitos infected with X-irradiated *P. falciparum*. One of these volunteers was reported by Clyde et al. (6) to have developed resistance to repeated sporozoite challenge. He also had detectable antisporozoite antibodies. After a long period of repeated immunization, his serum gave a positive CSP reaction with the homologous Thau strain as well as with sporozoites of three other strains of *P. falciparum*. No cross-reaction with sporozoites of *P. vivax* was observed (Clyde et al., personal communication, 1973). The protective immunity acquired by this volunteer paralleled these serologic 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*.

These results are in close agreement with earlier data of Nussenzweig et al. (7) and Vanderberg et al. (5) on the correlation between cross-reactivity of circum-sporozoite antibodies and cross-protection in sporozoite-induced rodent malaria. Further determinations of the cross-reactivity among sporozoites of different simian and human malaria parasites might therefore help to predict the range of

Table 2. Species specificity and strain cross-reactivity of anti-sporozoite (CSP) antibodies induced in rats by the i.v. injection of sporozoites of *P. falciparum* and *P. vivax*.

Sporozoite antigen		Antisera against sporozoites of:	
Species	Strain	*P. falciparum* (Burma)	*P. vivax* (Sal II)
P. falciparum	Burma	pos.	—
P. falciparum	Mark	pos.	—
P. coatneyi		neg.	—
P. vivax	Sal II	neg.	pos.
P. cynomolgi	B	neg.	neg.
P. knowlesi	H	neg.	—

cross-protection to be obtained from these preparations.

The finding that sporozoites are subject to a process of antigenic maturation during the sporogonic cycle of *P. cynomolgi* is certainly noteworthy. This process of maturation presents certain similarities with what had previously been observed by Vanderberg et al. (8) in rodent malaria. Antigenic maturation, therefore, seems to follow a similar pattern and to represent a common feature of the sporogonic development of all mammalian malaria parasites. This leaves open the basic question concerning the factors that lead to the appearance and/or expression of certain sporozoite antigens. In so far as the expression of these antigens parallels the capacity of sporozoites to induce protective immunity, their characterization becomes a problem of fundamental importance. It may be hoped that the comparative antigenic analysis of these different sporozoite populations will lead to the characterization of the " protective antigens ".

It was recently demonstrated by Krettli et al. (9) that sporozoites, when concentrated and purified by gradient centrifugation, retained their immunogenicity. This approach might make it possible to characterize these " protective antigens ". The considerable parasite yield obtained by this method also facilitates the use of large sporozoite doses for vaccination purposes.

From the point of view of immunization, it is of paramount importance to use immunologically mature sporozoites, equipped with the " protective antigens ". For vaccination tests, these parasites should therefore be harvested at the stage of infection when maximum maturation and migration to the haemocele and salivary glands has occurred, which may not necessarily coincide with the time of maximum parasite yield.

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RÉSUMÉ

RÉPONSE IMMUNITAIRE ENVERS DES SPOROZOÏTES DE PARASITES DU PALUDISME SIMIEN OU HUMAIN: SPÉCIFICITÉ DE STADE ET D'ESPÈCE ET RÉACTIVITÉ CROISÉE À L'ÉGARD DE DIVERSES SOUCHES

De précédents travaux sur le paludisme des rongeurs ont montré que l'immunisation par voie intraveineuse au moyen de sporozoïtes traités par rayonnement X confère fréquemment une protection totale contre un inoculum sporozoïtaire habituellement létal. Des essais similaires de vaccination sont actuellement en cours chez des hôtes simiens et, plus récemment, chez des volontaires.

Le but de la présente expérimentation était d'établir *a*) certaines des caractéristiques antigéniques de divers sporozoïtes des paludismes simien et humain; *b*) la spécificité de l'antigène en fonction du stade évolutif; et *c*) des données relatives au pouvoir infectant du parasite. Des rats, immunisés à l'aide de différentes préparations ont fourni des antisérums de spécificité variable. Des signes rhésus ont été inoculés au moyen de différents stades évolutifs des sporozoïtes afin de déterminer le pouvoir infectant de ces derniers.

Seuls les sporozoïtes prélevés dans la région thoracique (hémocèle et glandes salivaires) ont suscité une production d'anticorps et se sont montrés constamment infectieux. Un stade supplémentaire de maturation antigénique est réalisé dans les glandes salivaires; les sporozoïtes qui y ont été recueillis ont fait preuve d'un pouvoir infectant maximal et se sont révélés les plus aptes à provoquer la formation d'anticorps antisporozoïtaires. Ces formes renferment aussi la plus forte proportion de parasites produisant des précipités reconnaissables lors de l'incubation dans un immunsérum. On a établi que

les anticorps dirigés contre les sporozoïtes des paludismes simien et humain étaient strictement spécifiques d'espèce dans la mesure où aucune réaction croisée n'a été observée, même parmi des espèces parasitaires considérées comme très voisines. Cependant, des isolats d'origine géographique différente ont réagi avec les antisérums actifs contre une souche homologue.

Ces observations pourront être mises à profit pour déterminer le type de préparation de sporozoïtes à choisir pour la mise au point d'un vaccin antipaludique.

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DISCUSSION

BRAY: The problems in the use of irradiated sporozoites for vaccination will be: the supply of sporozoites, the storage of sporozoite so as to ensure the sterility of the preparations, the possibility of hypersensitivity, the number of injections required, the route of injection, and the use of adjuvants.

COHEN: Professor Nussenzweig's results indicate the potential of vaccination. Merozoite vaccination may be superior to sporozoite vaccination in practical terms and protective effect. I should like to know if any trials have been made of sporozoite viability following storage, if the duration of effective immunity after one vaccination and after challenge has been determined, if specificity has been examined immediately after vaccination to see if interferon plays a part, if any adjuvant has been investigated, and what results Professor Nussenzweig obtained with the immunization of monkeys.

NUSSENZWEIG: Cryopreservation of sporozoites has given poor results. Mice were protected for 2–3 months, after which patent infections appeared with prolonged incubation periods. Interferon may be a factor in the first 24–48 hours but seems unlikely to be a cause of protection thereafter. Neither Freund adjuvant nor pertussis adjuvant gives protection, but some success has been obtained with killed *Corynebacterium parvum*, which is a well-known nonspecific stimulator of the reticuloendothelial system. Two out of six rhesus monkeys have been protected against *P. cynomolgi*.

BRAY: In nature, the sporozoites inoculated were of the order of $\log^3$, whereas these experiments involved sporozoites of $\log^6$ to $\log^8$.

MEUWISSEN: Dr Verhave of the University of Nijmegen infected chloroquine-treated Wistar rats repeatedly with normal sporozoites of *P. berghei* Anka strain; 43 h after inoculation, the number of exoerythrocytic forms in the liver was counted. A second infection with normal sporozoites within 24 h induced a lower number of EE forms. The mechanism of this interference is being studied.

NUSSENZWEIG: No protection is achieved by vaccination with mosquito tissue alone.

BRAY: Immune serum from monkeys infected several times with sporozoites of *P. cynomolgi* has no effect on *P. cynomolgi* sporozoites and the animals are fully susceptible to sporozoites of *P. cynomolgi* in the liver. I have examined sporozoites of *P. falciparum*, *P. malariae*, and *P. ovale* in the sera of adult Liberians under constant malaria attacks from birth, and have seen no sign of agglutination or circumsporozoite precipitation. These sporozoites were fully infective to chimpanzee livers.

NUSSENZWEIG: Circumsporozoite precipitation is easily produced in rats but it takes many more sporozoites injected many more times to produce it in monkeys and even more in man.