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SPOROZOITE-INDUCED IMMUNITY IN MAMMALIAN MALARIA *

by

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The purpose of this report is to review the state of the art of immunization against malaria using the sporozoite stage, and to summarize some recent progress in this area, mainly in defining the specificity of the immune response to sporozoites of simian and human malaria. I will furthermore report the preliminary results we obtained by immunization of Rhesus monkeys with irradiated sporozoites of P. cynomolgi and attempt to correlate the findings with the results obtained in preliminary human trials (Clyde et al., 1973). Finally, I would like to point out a number of gaps in our knowledge of sporozoite-induced immunity, to which I am sure you will add other problems which remain to be investigated.

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 Plasmodia, in part because of the difficulties involved in working with infected mosquitoes, but also because of the quite deeply rooted, erroneous, concept that sporozoites are not immunogenic.

On the other hand, this really does not constitute a recently developed approach, since it was shown, quite convincingly, in the 1940's, that ultra-violet irradiated sporozoites of avian malaria induce anti-sporozoite anti-

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bodies and a partial, although very significant, protection in immunized birds (Mulligan et al., 1941).

The work on immunization with sporozoites of rodent malaria was started about seven years ago, when we observed that a single intravenous administration of irradiated sporozoites produced total protection, in a variable number of 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 considerably in different experiments, i.e., from 15 up to 100 %. It should be pointed out that two of the basic questions raised by these experiments, namely, the reasons for this considerable variation in the immunogenicity of different sporozoite preparations, and the related question of how to increase this protection so as to obtain resistance in 100 % of the animals, still remain to be solved. The closest we have gotten toward this aim has been to pre-treat the animals with Corynebacterium parvum, prior to the immunization. Corynebacterium parvum, a potent RES stimulant, was shown by us to increase considerably the non-specific resistance to sporozoite-induced infections (Nussenzweig, 1967). Combining the administration of this RES stimulant with the injection of a single dose of X-irradiated sporozoites, was now observed to increase considerably the percentage of protected animals. If this is simply an additive effect of non-specific plus specific resistance, or whether C. parvum acts as an adjuvant, amplifying the protective response induced by X-irradiated sporozoites, still remains to be clarified. Meanwhile C. parvum is being tried in the simian malaria system, to determine if it also enhances the immune response to sporozoites of P. cynomolgi.

If one increases the number of immunizing doses to 4 or 5 injections in mice, the results become very reproducible, close to 100 % protection being

observed in most of the experiments (Nussenzweig et al, 1969a). This experimental model has permitted us to determine a number of the characteristics of sporozoite-induced immunity in rodent malaria, which have been reviewed in a report to the Inter-American Malaria Research Symposium organized by the Pan-American Health Organization (Nussenzweig et al, 1972).

This has more recently led to investigations of the immune response to sporozoites of simian and human malaria.

Vaccination attempts of simian hosts, by the use of sporozoites attenuated by irradiation are at this point still in a somewhat preliminary phase. Results reported by others (Collins and Contacos, 1972) as well as our own unpublished observations, have provided some evidence of partial immunity in several of these attempts. However, optimal conditions of immunization in this system still remain to be established. Apart from the need of determining the optimal dosage, route and schedule of immunization, one, if not the most important, factor of success of these vaccination attempts will reside in 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, 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 were facilitated considerably by using rats as ant sporozoite antibody producers. This was based on the finding that injection of sporozoites

of simian malaria into this unnatural host leads to an arrest in parasite development and induces a very rapid and consistent circum-sporozoite antibody response (Nussenzweig et al, 1973). 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 be easily observed under a phase contrast microscope and is essentially similar to the circum-sporozoite (CSP) reaction previously described in rodent malaria (Vanderberg et al, 1969).

Currently we have observed that one or two intravenous injections of a total of $1-2 \times 10^5$ irradiated or non-irradiated sporozoites into rats result in CSP antibody formation, detectable in less than two weeks after the initial immunization. This pattern of antibody response has so far been consistent in all immunization attempts in rats with sporozoites of the various simian and human malarias. Furthermore, the intravenous immunization of Rhesus monkeys with irradiated sporozoites of Plasmodium cynomolgi has shown that these animals produce a similar, although delayed CSP antibody response.

ANTIGENIC MATURATION OF SPOROZOITES OF SIMIAN MALARIA

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

For this purpose infected Anopheles stephensi mosquitoes were sectioned, separating their thoraces from abdomens, collecting sporozoites from the abdominal and thoracic regions as well as from dissected salivary glands and midguts. This was done under conditions which would result 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 seven up to the twenty-fifth 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 haemocoel, or even to the salivary glands.

All sporozoite populations were analyzed by the following three criteria: (1) their infectivity for Rhesus monkeys, (2) their capacity to induce the formation of CSP antibodies and (3) 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 anti-sporozoite antisera. The basic antigenic characteristics of sporozoites from the abdominal region (haemocoel) were rather similar. They induced a minimal amount of antibodies, but very few produced a tail-like precipitate characteristic of the CSP reaction. Furthermore, most of these parasites were non-infective.

The results were quite different with sporozoites from the haemocoel of the thoracic region. These parasites were infective, although apparently less so than salivary gland sporozoites. They induced a considerable CSP antibody response, but relatively few individual sporozoites ($\leq 1\%$) 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 mosquitoes.

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 mosquitoes.

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 maximal 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 presently being completed.

SPECIES SPECIFICITY OF CSP ANTIBODIES OF SIMIAN MALARIA

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 using homologous sporozoites as antigen, to evaluate the presence of ant sporozoite antibodies. When positive, these sera were tested with sporozoites of other species and strains of simian and human malaria, to detect any possible cross-reactions.

The results obtained (Table 1) indicate that positive reactions occurred

only within the homologous system. Even in those instances in which 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. Antisera produced against sporozoites of simian malaria also did not 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 malaras 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, are presently being obtained.

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

These questions were approached through the use of antisera of different specificities produced by the intravenous immunization of rats with sporozoites of the 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 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 different geographical isolates reacted as strongly with homologous as with heterologous antisera.

These serological results have recently been confirmed in volunteers immunized by the bite of X-irradiated P. falciparum-infected mosquitoes (Clyde et al, 1973-a). One of these volunteers has been reported

to have developed resistance to repeated sporozoite challenge. He also had detectable ant sporozoite 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, 1973 b). 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.

These results are in close agreement with our earlier data (Nussenzweig et al, 1969 b) and Vanderberg et al, 1969) on the correlation between cross-reactivity of circumsporozoite antibodies and cross-protection in sporozoite-induced rodent malaria. Further determinations of the cross-reactivity among different simian and human malaria sporozoites might therefore help to predict the range of 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 in rodent malaria (Vanderberg et al, 1972). 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 which 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. The comparative antigenic analysis of these different sporozoite populations might hopefully lead to the characterization of the

"protective antigens."

It was recently demonstrated that sporozoites when concentrated and purified by gradient centrifugation retain their immunogenicity (Krettli et al., 1973). 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 attempts these parasites should therefore be harvested at the stage of infection when maximal maturation and migration to the haemocoel and salivary glands has occurred, which does not necessarily coincide with the time of maximal parasite yield.

SUMMARY

Previous work in rodent malaria has demonstrated that intravenous immunization 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 volunteers.

The purpose of the present experiments was to establish (1) some of the antigenic characteristics of various simian and human malaria sporozoites, (2) the stage specificity of the antigen(s) and (3) data on parasite infectivity. Rats were immunized with a number of preparations, providing antisera of different specificity. Rhesus monkeys were injected with different developmental stages of sporozoites to determine their infectivity. Only the sporozoites collected from the thoracic region (haemocoel and salivary glands) induced significant antibody formation and were consistently infective. An additional degree of maturation occurred in the salivary glands. These sporo-

zoites were the most infective and the best inducers of circumsporozoite antibody formation. This population also contained a higher percentage of parasites which produced precipitates detectable upon their incubation in immune serum. It was further established that antisporeozoite (CSP) antibodies to these simian and human malarias are strictly species specific, in so far as we observed no cross-reaction, even among malarial species believed to be closely related. Different geographical isolates, however, reacted intensively with antisera produced against any strain of the same species.

This knowledge should be valuable in determining the type of sporozoite preparation to be chosen for the development of a malaria vaccine.

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TABLE 1. SPECIFICITY OF THE CIRCUMSPOROZOITES (CSP) REACTIVITY OBSERVED IN THE SERA OF RATS IMMUNIZED WITH VARIOUS SIMIAN MALARIA SPOROZOITES.

Sporozoite Antigen		Antisera against sporozoites of:			
		<u>P. cynomolgi</u> ^a	<u>P. fieldi</u> ^b	<u>P. simiovale</u> ^b	<u>P. knowlesi</u> ^c
<u>Vivax-type</u>	<u>P. cynomolgi</u>	Pos.	Neg.	Neg.	Neg.
	<u>P. gonderi</u>	Neg.	Neg.	Neg.	Neg.
<u>Ovale-type</u>	<u>P. fieldi</u>	Neg.	Pos.	Neg.	Neg.
	<u>P. simiovale</u>	Neg.	Neg.	Pos.	Neg.
<u>Other types</u>	<u>P. knowlesi</u>	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.

TABLE 2. SPECIES SPECIFICITY AND STRAIN CROSS REACTIVITY OF ANTISPOROZOITE (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	<u>P. falciparum</u>	<u>P. vivax</u> (Sal II)
<u>P. falciparum</u>	Burma	Pos.	-
<u>P. falciparum</u>	Mark	Pos.	-
<u>P. coatneyi</u>		Neg.	-
<u>P. vivax</u>	Sal II	Neg.	Pos.
<u>P. cynomolgi</u>	B	Neg.	Neg.
<u>P. knowlesi</u>	H	Neg.	-

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