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SPOROZOITE-INDUCED IMMUNITY IN MAMMALIAN MALARIA:

A REVIEW OF RECENT WORK

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SPOROZOITE-INDUCED IMMUNITY IN MAMMALIAN MALARIA: A REVIEW OF RECENT WORK*

During the past few years we have been investigating a number of aspects of sporozoite-induced immunity in rodent malaria (2,3,4,5,7,12,13), and more recently in simian malaria also (6). This work was presented at the Inter-American Malaria Research Symposium at San Salvador in November 1971 and is being published in the American Journal of Tropical Medicine and Hygiene (8). This report covers progress made since then in the various areas encompassed by our research.

1. Studies of the immune response to sporozoite-induced simian malaria

The main purpose of these studies is to test the general validity of the finding that immunization with X-irradiated sporozoites induces total protection in rodents against an otherwise 100 per cent lethal infection (4,10). The development of the immune response in the monkeys immunized with X-irradiated sporozoites is being followed by detection of antisporozoite antibodies and other manifestations of protective immunity.

a. Anti-sporozoite (CSP) antibodies in simian malaria

We are investigating antibodies produced against sporozoites of three simian malarial species, *Plasmodium brasilianum*, *P. simium*, and *P. cynomolgi*. All serum samples collected before immunization gave a negative CSP reaction, except some sera of squirrel monkeys that reacted with sporozoites of *P. brasilianum*. The squirrel monkeys had been imported from South America, from an area where there is a known high incidence of *P. brasilianum* infections. These animals have therefore presumably been repeatedly exposed to infected mosquitoes. Laboratory-born squirrel monkeys always gave a negative CSP reaction. None of the positive sera cross-reacted with sporozoites of *P. simium* or *P. cynomolgi*.

*Prepared by Dr. Ruth S. Nussenzweig, Department of Preventive Medicine, New York University Medical Center, New York, New York.

Because of the much greater sporozoite yield, the very consistent pattern of mosquito infections, and the fact that all the imported rhesus have been malaria-free, the Macaca mulatta-P. cynomolgi bastianelli-Anopheles stephensi system has become our experimental model of choice.

Our data indicate that an irradiation dose of 10 Krads renders the sporozoites of these three simian malarias uninfective. Even when injected in considerable amounts, as in the case of rhesus immunized with 2×10^6 irradiated sporozoites, which is more than 1000-fold the minimal infective dose, these irradiated sporozoites never produced detectable parasitemias.

Rhesus were immunized with two intravenous injections, each consisting of 2×10^6 X-irradiated sporozoites. The inocula were separated by a period of three weeks during which no CSP antibody was detectable. Soon after the second injection, CSP antibody was demonstrable in the monkeys. The immunizing dose (2×10^6) of sporozoites corresponds to the number of parasites obtained from a salivary gland dissection of 50-150 mosquitoes.

When half this immunizing dose was given (1×10^6 irradiated sporozoites per injection), a period of seven to eight weeks had elapsed and three to four boosters had been given before CSP antibodies became detectable. Subsequent boosters always produced a rise in antibody titers, which usually were detectable only at low serum dilutions. Anti-P. cynomolgi antisera did not cross-react with sporozoites of P. simium.

Squirrel monkeys repeatedly immunized with X-irradiated sporozoites of P. brasilianum also acquired CSP antibodies that reacted with sporozoites of the homologous system.

b. Protective immunity

Positive anti-P. cynomolgi sporozoite antisera are being tested for sporozoite neutralizing activity by incubating viable sporozoites with immune serum and complement. These sporozoites are then injected into "normal" monkeys, and their infectivity is compared to that of sporozoites of the same batch, preincubated with "normal" (unimmunized) monkey serum. Should these "immune sera" inactivate the sporozoites, it

would then be a good indication of protective immunity of the donor animals and a sign for us to challenge these immunized animals.

Concurrently the minimal sporozoite dose that consistently produces infection is being determined, and will be used as challenging inoculum. It is quite low for P. cynomolgi bastianelli, but considerable higher for P. brasilianum, of which about 50,000 or 100,000 sporozoites have to be intravenously injected into squirrel monkeys, resulting in a prepatent period of over four weeks.

The evaluation of the correlation among CSP antibody titers, neutralizing antibodies, and protective immunity will have to await the results of the challenge of these and other groups of animals.

c. Antigenic analysis of sporozoites of simian and possibly also human malaria

Rats were found to be very good CSP antibody producers in the P. berghei system. A single small dose of either irradiated or viable sporozoites (10,000 or 75,000 sporozoites i.v.) initiates CSP antibody production. In fact, CSP antibodies become detectable one week after the immunization of rats, whereas in mice a sevenfold larger immunizing dose takes three weeks to produce CSP antibodies which were detectable only in a certain proportion of animals (11).

We have now found that sporozoites of simian malaria also induce a very rapid and good antibody response in rats. A single dose of 400,000 X-irradiated or viable sporozoites of P. cynomolgi consistently produces detectable CSP antibodies one week after intravenous administration to rats. These antisera are being used to compare the antigenicity of various sporozoite preparations of P. cynomolgi, which differ in their age (time of development in the mosquito) and degree of maturation. This comparison becomes important because of observations in the P. berghei system, indicating that antisera produced against salivary gland sporozoites did not cross-react with oocyst sporozoites and that immunization with oocyst sporozoites failed to produce protective immunity (14).

The possibility of immunizing rats and obtaining CSP antibody against other mammalian malaria parasites would be particularly fruitful if antibodies against the sporozoites of the malaria parasites of humans could also be obtained. Should this be the case, i.e., if CSP antibodies against human malaria parasites could easily be induced in rats, that would then provide an extremely simple technique for determining the antigenic relationship of geographically different malaria strains. It would also permit us to evaluate the antigenic relationship of the sporozoites of certain apparently closely related simian and human malaria species (i.e., P. brasilianum and P. malariae).

At least in rodent malaria, the cross-reactivity of the CSP reaction between sporozoites of the different species corresponded to a very extensive cross-protection upon immunization with attenuated sporozoites (5). It might therefore be hoped that the occurrence of common cross-reacting antigens, as indicated by CSP cross-reactions, could provide us with some clues regarding general immunologic similarities between different malarial species.

2. Further studies on the immune mechanism in rodent malaria

Recent data on the effect of sporozoite dose on the degree of protective immunity produced by a single sporozoite injection have raised a number of interesting questions regarding the role of contaminating mosquito tissue in the immunizing inoculum. It was found that raising the dose above a certain level decreased significantly its capacity to induce protection. Whereas a single dose of 7.5×10^4 sporozoites resulted in a number of totally protected mice, a fourfold increase of the inoculum (3.0×10^5) resulted in no protected animals in repeated experiments (11). Since a fourfold larger immunizing dose signifies also a fourfold increase of mosquito debris, this could possibly produce a saturation of the RES of these mice, or interference with the development of their protective immune response. Whatever mechanism might be involved, it does not seem to interfere with CSP antibody formation, which occurs very rapidly after this single large immunizing dose.

The problem of the effect of mosquito debris is now being investigated by adding normal mosquito tissue to an otherwise optimal immunizing antigen dose, through purification of sporozoite antigen by density gradient centrifugation, and through immunization by the bite of X-irradiated mosquitoes, which introduces only a relatively small amount of mosquito tissue.

Controversial results of studies on the effect of repeated injection of normal mosquito tissue on the susceptibility of mice to sporozoite challenge have led us to reexamine this question. We used various routes of immunization, comparing the effects of repeated injection of sporozoite-infected and normal mosquito salivary glands into mice, and challenging part of the animals intravenously and others by the intraperitoneal route. The conclusion was that injection of normal mosquito tissue did not result in protection of the animals under any of these experimental conditions (10).

3. Sporozoite purification through density gradient centrifugation

This is being attempted by using a continuous Renografin/BSA gradient, according to the techniques described by Chen and Schneider (1) and Schneider and Chen (9) for the purification of sporozoites of avian malaria.

The purification of sporozoites of both *P. berghei* and *P. cynomolgi* is being worked out, since both of these preparations would be of great usefulness in further immunologic and other biologic research.

The immunogenicity of purified *P. berghei* and *P. cynomolgi* is being investigated by immunizing rats with these preparations and comparing the rate of CSP antibody formation with that induced by control sporozoites. In both instances it has been shown that purified preparations are fully immunogenic, at least as far as induction of CSP antibody is concerned.

Mice are also being immunized by repeated injections of gradient-purified sporozoites of *P. berghei* to determine whether their capacity to induce protective immunity has not been impaired. In the case of purified sporozoites of *P. berghei*, it has been shown that their infectivity has remained unaltered.

The antigenic components of purified salivary gland sporozoites obtained from mosquito thoraxes, and oocyst sporozoites from mosquito abdomens, will be compared by gel-diffusion technique.

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