Malaria vaccination with irradiated sporozoites: serological evaluation of the antigen and antibody responses*

M. P. Bawden,1 T. T. Palmer,2 M. F. Leef,3 & R. L. Beaudoin4

Vaccination against Plasmodium falciparum with attenuated sporozoites is the goal of the US Navy’s Malaria Vaccine Program. One requirement in the development of this vaccine is an immunological test to study the sporozoite antigen and immune responses it induces. Using an indirect fluorescent antibody test (IFAT) and P. berghei in the mouse or rat as a model, we have made significant progress toward this goal. Four antigens were detected in vaccine preparations: sporozoite-specific antigens, mosquito antigens, antigens on the sporozoite that are common to erythrocytic stages, and bovine serum albumin, an antigenic element of the isolation medium no longer employed. The IFAT was a reliable monitor of vaccination in a mouse and rat model in conjunction with protection to challenge. The test was a sensitive monitor of vaccine quality. Anamnestic responses to bites of infected mosquitoes were detected in mice previously immunized with irradiated sporozoites.

The primary objective of the US Navy’s Malaria Vaccine Program is to develop a vaccine effective against Plasmodium falciparum. At present, this vaccine is based upon the attenuated sporozoite as the immunizing antigen. Such an agent will be used to prevent infection in relatively small groups of non-immunes who enter endemic areas and remain for defined periods of time, and will be especially beneficial where chloroquine resistance is present. Immunization with irradiated sporozoites of P. falciparum tentatively meets this objective (1, 2, 3), but the preparations require refinement before they can be practically administered to any sizable number of people. A requisite tool for development of this vaccine is an immunological test that can be used to study the antigen and immune responses it elicits. This paper will briefly review immunological tests that have been used to study the immune response to sporozoite vaccines and will describe an indirect fluorescent antibody test (IFAT) developed in our laboratory for this purpose (4). Emphasis will be placed upon information we have gathered since 1974 from the IFAT about the irradiated sporozoite as the immunizing antigen and the antibody responses that it induces.

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1 Head, Serology Branch of the Malaria Division Immunoparasitology Department, Naval Medical Research Institute, Bethesda, MD 20014, USA.
2 Assistant to the Officer-in-Charge, US Naval Medical Research and Training Unit, Canal Zone, Panama, and Adjunct Associate of the Gorgas Memorial Laboratory, Balboa Heights, Canal Zone.
3 Head, Serology Branch of the Malaria Division, Immunoparasitology Department, Naval Medical Research Institute, Bethesda, MD 20014, USA.
4 Head, Malaria Division, Immunoparasitology Department, Naval Medical Research Institute, Bethesda, MD 20014, USA.

REVIEW OF IMMUNOLOGICAL METHODS FOR THE STUDY OF SPOROZOITE ANTIGEN

Protection against challenge

Historically, the first immunological method used to study sporozoite immunity was protection against challenge with infective sporozoites (5). This method is the ultimate standard with which all others must be compared, since it is a direct measure of vaccine-induced immunity. In the laboratory, it can be applied by injecting infective sporozoites (5) or by allowing infected mosquitoes to inoculate the sporozoites (2, 3). In addition, one can test the species, strain and stage specificity of the immunity by selecting the challenge inoculum: from erythrocytic stages or sporozoites of different strains and species.
Agglutination

This method, which has theoretical potential because one can observe the reaction and discern its specificity, was successfully applied by Mulligan et al. (7). They demonstrated a correlation between high titres of agglutinin and the quality of immunity induced by vaccination of birds with ultraviolet-attenuated sporozoites of P. gallinaceum. Of equal importance was the observation that immunization of birds by blood-stage infection (which includes only erythrocytic and exoerythrocytic stages) also produced agglutinin reactive with freshly isolated, viable sporozoites (8). This is, in our opinion, a clear demonstration of stage-common antigen on viable sporozoites freshly dissected from mosquitoes in contrast with the results presented by Nardin & Nussenzweig (9).

We tried the agglutination technique for studying the immune response in mice vaccinated with irradiated sporozoites of P. berghei (Jarvinen & Bawden, unpublished results). In our hands, this approach proved impractical for technical reasons; the main objection was the need for excessive numbers of sporozoites to titrate the agglutinin in each serum. We have therefore abandoned further study of this serological reaction.

Passive transfer and sporozoite-neutralizing activity (SNA)

Passive transfer of immune serum is closely allied, both historically and in practice, with protection against challenge. However, it is practical only in animal models. Nussenzweig et al. (10) made an extensive study of this technique and the related phenomenon of sporozoite neutralization by immune serum from mice vaccinated with irradiated sporozoites of P. berghei. They demonstrated neutralizing antibody by reducing infectivity of sporozoites, in vitro, with immune serum. In addition, they showed increased blood clearance of sporozoites from mice pretreated with immune serum. However, these techniques have limited application in studying malarial immunity in man.

Circumsporozoite precipitin reaction (CSP)

Vanderberg et al. (11) found that the serum of mice vaccinated with sporozoites of P. berghei contained a specific antibody that precipitated around viable sporozoites in vitro, hence the name circumsporozoite precipitin reaction. This test was used extensively to evaluate the immune response of vaccinated mice and rats (12, 13). It was also used to study the antigenic character of rodent, primate, and human malarial sporozoites (14, 15).

Fluorescent antibody tests

From the small amount of data available, it appears that the CSP reaction has limited usefulness in monitoring experimental immunization, since the appearance of CSP antibody does not consistently correlate with the onset of protective immunity in man (16, 17). However, further experiments should be done to determine the appearance of CSP antibody in vaccinated persons. In animal models, CSP antibody production did not correlate with protection (12, 18, 19). Its most effective and extensive use was to study antigenic relationships between sporozoites of different strains and species.

Hypersensitivity

From a practical point of view, a simple skin test would be desirable for assaying sporozoite immunity in man. With this in mind, we examined hypersensitivity reactions by footpad swelling in vaccinated mice. After extensive study (Smrkovski & Wood, unpublished data), we concluded that if there was a sporozoite specific response it was masked by a strong response to antigens of mosquito origin, which contaminate all vaccine preparations. When purer sporozoite preparations are available, these studies should be repeated to find out whether sporozoite-specific hypersensitivity responses can be used as monitors of immunization.

Serological tests that employ fluorescent stains attached to specific antibody have added significantly to our understanding of malarial immunity (20). This type of assay has a distinct advantage; the specificity of the antigen-antibody reaction can be visualized. Also, the test requires relatively small quantities of reagents and can be used to study the immune response of man and animals. Employing fluorescent antibody techniques, various investigators demonstrated that sporozoites are antigenic (21, 22) and that they possess antigens that are common to the erythrocytic stages of the same species (23, 24, 25). Common antigens were also demonstrated between exoerythrocytic stages and erythrocytic stages of the same species (23). It is this feature of the IFAT, that is, its potential to differentiate reactions with all three major stages of the malarial parasite, that prompted us to choose it to study sporozoite antigens and immune responses. In our opinion, the IFAT offers the best means of investigating these complex specificities because it can be modified to meet the needs of the system and the operator can actually localize the reaction at its site of action. This feature is invaluable, and in this paper we shall summarize the advances we have made in understanding sporozoite immunology. We shall not, however, include all details of methods or results; these
SPORozoite VACCINE: ANTIGEN AND ANTIBODY RESPONSES

will follow in later publications.

The IFAT we use is briefly outlined by Palmer (26) and Bawden et al. (4), and has been discussed in detail at several recent scientific meetings. All of this work was done with the ANKA strain of P. berghei maintained in Anopheles stephensi and the NMRI mouse (6). Antigen employed in the IFAT was dried and frozen before use. The conjugated antisera used were reactive with IgG, IgM and IgA.

REVIEW OF ADVANCES MADE AT THE NAVAL MEDICAL RESEARCH INSTITUTE WITH AN IFAT TEST

Antigens in sporozoite preparations

We have clearly demonstrated 4 distinct antigens in the irradiated sporozoite preparations used for vaccination (4, 27). The first group comprises sporozoite-specific antigens. The second group comprises sporozoite antigens that contaminate all preparations of sporozoites produced by current methods (28, 29, 30). The third group comprises antigens on the sporozoite that are common to the erythrocytic (and probably exerythrocytic) stages of the malaria parasite. Last is material employed in the isolation of sporozoites from infected mosquitoes. For example, bovine serum albumin was used extensively for density gradient isolation of sporozoites (31, 32). This protein suppressed the immune response to sporozoite vaccination and predisposed the recipients to anaphylaxis upon booster or challenge injection (33). This type of antigen can be avoided by substituting homologous serum (28) as a component of the isolation medium.

Sporozoite-specific and stage-common antigens. Our criterion for defining sporozoite-specific antigens is that they induce antibody that reacts only with sporozoites in the IFAT. Thus, these antisera do not induce antibody that is detectably reactive with erythrocytic stages. (Exoerythrocytic stages must also be included as having potentially common antigens but we have not yet tested any of our sera for this reaction. This will be done when these stages are readily available.) The occurrence of sporozoite-specific antibody in immunized mice, rats, or rabbits was dependent upon 3 factors. The first was the route of inoculation. When 2 rabbits were immunized with about 16 million viable sporozoites in 5 intravenous doses, both developed antibody that reacted with erythrocytic stage-common antigens. They also produced antibody reactive with sporozoites. When 6 rabbits were immunized by allowing infected mosquitoes to inoculate the sporozoites by bite, none ever produced antibody to common antigens. They all produced high-titre antibody (about 1:2000 average) that was strictly sporozoite-specific. The route of inoculation accounted for this difference in response.

The second factor that affected the specificity of the immune response to sporozoite vaccination was the species of animal used. Of 350 mice injected intravenously with 1–3 doses of 30 000 irradiated sporozoites, only 10 (about 3%) had detectable antibody to common antigen. The time of appearance of this antibody was irregular. In contrast, 40 (93%) of 43 rats injected intravenously with 7 weekly doses of 25 000 irradiated sporozoites, and challenged with viable sporozoites, produced antibody reactive with common antigens. The third factor that appeared to affect the production of antibody to common antigen was the number of doses administered. In rats, this antibody was detected only after the 7th vaccination, but in mice it occurred in an irregular way after one or more doses.

Mosquito-specific antigen. The current methods of isolating sporozoites from infected mosquitoes do not remove all the antigenic debris that is produced when mosquitoes are disrupted to release sporozoites (29, 30). Also, one could postulate that sporozoites would have mosquito antigens attached to their surface since they are in constant contact with mosquito tissues. We were unable to detect the latter by using mosquito-specific antisera or by absorbing antisporozoite sera with mosquito antigen. However, mosquito antigens were present in vaccine inocula as discrete antigens. In mice vaccinated with one or more doses of irradiated sporozoites, the antibody response to this debris was equal to the response to the sporozoites (27). In the rat, the response to the sporozoite was stronger than the response to mosquito debris, even though the doses were the same as those used in the mouse. Improved isolation techniques reduced the quantity of this debris (29), but mosquito-specific antibody responses were still detectable.

IFAT as a monitor of vaccination

In conjunction with protection-against-challenge, the IFAT was a successful monitor of vaccination. For example, in the mouse a single dose of 30 000 irradiated sporozoites induced immunity in 100% of the vaccinated individuals (34). The maximum protection was demonstrated about 10 days after the vaccination. Prior to this, all immune animals had a sporozoite-specific antibody response that became detectable on the third day, reached its peak on the fourth and fifth days, and thereafter declined rapidly. (These results are an extension of those previously reported (4)). Thus, all immune mice had a sporozoite-specific response prior to the onset of solid immunity. The relationship in vaccinated rats was somewhat different. To induce 90–100% immunity in a
group of rats, it required 7 weekly doses of 25 000 ir-
radiated sporozoites. Sporozoite-specific antibody
was not detected in all rats until after the second vac-
cinating dose when the average titre was 1:128. Sub-
sequent doses caused a slight increase in antisporo-
zoite antibody, so that the average titre was 1:512.
This high level of antibody was sustained throughout
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IFAT as a monitor of vaccine quality

The IFAT is a powerful tool when applied as a
monitor of vaccine quality. There are two types of
quality that can be considered. The first is the purity
of the vaccine, i.e., its freedom from unwanted antige
ts. This is illustrated in the paper by Wood et al. (29).
Particulate debris in a sporozoite vaccine prepared by filtration
was reduced by an average of 97%, but 2 immunizing doses of
this cleaner antigen caused a substantial antibody response to mosquito
contaminants in 8 of 10 vaccinated mice.

The IFAT can also be used to study the antigenic
quality of vaccine preparations or variations pro-
duced by an alternate route of immunization. In this
case, comparison is always made to the antibody re-
response observed with intravenous doses of 30 000
irradiated sporozoites in mice. Using this approach,
we found that other methods of attenuation such as
formalin treatment, freezing, and lyophilization are
less effective in producing immunity and the anti-
genicity of the product is correspondingly poorer.
The efficacy of these preparations as booster doses is
also under study.

Quality of natural booster by infected mosquitoes

We have employed the IFAT in recent experiments
to study the effectiveness of bites by infected mos-
quitos as booster inoculations in immune mice. These
studies are not yet complete but the preliminary re-
results indicate that the bite of 1–4 infected mosquitoes
causd a significant increase in the average titre of
sporozoite-specific antibody in mice vaccinated 5–7
weeks before being bitten.

CONCLUSION

The IFAT test is an important research tool. It has
made possible significant advances in understanding
the antigenic properties of sporozoite vaccine prep-
ations and the various immune responses they in-
duce. Methods previously applied to the study of
sporozoite vaccines have drawbacks. The most im-
portant disadvantage is that these methods are too restric-
tive. They focus only on responses to the sporozoite
itself and cannot be used to characterize the unwanted
antigens present in candidate sporozoite vaccine pre-
parations. With the IFAT, we have characterized the
major classes of antigens of vaccine preparations. We
are confident that this approach will also be useful in
evaluating the effectiveness of P. falciparum spor-
zoite vaccines in malaria-free humans.

On the other hand, the ability to detect exposure to
sporozoites in an endemic area would have great sero-
epidemiological significance as well as importance in
evaluating the immune status of a resident population
in which immunization trials are to be conducted. Be-
cause people in these areas may have antibody cross-
reactive with common antigens, a different approach
is required for detecting their sporozoite experience.
In this case, a sporozoite-specific test is needed and we
are currently developing such a method (27).
VACCINATION ANTIPALUDIQUE AU MOYEN DE SPOROZOITES IRRADIÉS: ÉVALUATION SÉROLOGIQUE DE L’ANTIGÈNE ET DES RÉPONSES IMMUNITAIRES


REFERENCES