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Acute malaria prolongs susceptibility of mice to *Plasmodium berghei* sporozoite infection*

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SUMMARY

The fate of immune response against sporozoite stage in malaria infection was investigated. Two groups (A and B) of mice were inoculated twice with infective sporozoites of *Plasmodium berghei*. The mice in group A were maintained on chloroquine prophylaxis to prevent the sporozoite infection from causing malaria. Group B animals on the other hand were allowed to develop acute malaria from the infection which was subsequently cured with chloroquine. Upon examination for stage specific immune responses, it was found that the animals in group A produced high antibody titres against sporozoites and none against erythrocytic stages. The mice in group B produced little anti-sporozoite antibodies but had high antibody titres against blood forms. Challenge infection with *P. berghei* sporozoites showed that group A animals had become resistant against sporozoite-induced parasitaemia, whereas the mice in group B remained susceptible. The possible significance of suppression of protective immunity by malaria in host–parasite relationship is discussed.

Keywords malaria anti-sporozoite protection

INTRODUCTION

Malaria suppresses immune response to a variety of antigens and is believed to contribute to the high incidence of bacteria infections among children in tropical countries (reviewed by Wyler, 1983; Weidanz, 1982). It has been suggested that malaria predisposes humans to Burkitt's lymphoma (Burkitt, 1968). However, the significance of malaria-induced immunosuppression in the survival of the parasite itself is not clear. Antibodies against blood form of the parasite are often detectable at high titres even during severe malaria. Cellular immunity appears not to be affected either, for Wyler & Brown (1977) found that acute falciparum malaria in children did not cause hyporesponsiveness of their peripheral blood T cells to *Plasmodium falciparum* antigens in an *in vitro* blastogenesis assay. In any case, it is important to point out that the malaria parasite expresses several antigens at the erythrocytic stage in its life cycle (reviewed by Nussenzweig, 1982; McBride, Walliker & Morgan, 1982; Deans *et al.*, 1982). Suppression could affect immune response to one or more of these antigens but might not be detected in *in vitro* tests. It has been shown with non-plasmodial antigens that malaria-induced immunosuppression does not affect all immunogens (Barker, 1971).

Several years ago, Orjih & Nussenzweig (1979) reported that humoral immune response to attenuated sporozoite vaccine was severely suppressed in mice suffering from acute *P. berghei*

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infection. Data in the present report show that this suppression also affects protective immunity. In this study both immunity and parasitaemia were induced with infective sporozoites (Orjih, Cochrane & Nussenzweig, 1982).

MATERIALS AND METHODS

Animals. Adult female A/J mice (Jackson Memorial Laboratories, Bar Harbor, Maine, USA) were used in all the experiments.

Malaria parasite. Strain NK 65 of *P. berghei* was used throughout the studies. The laboratory maintenance of this parasite including its cyclical transmission and the characteristics of the infection in rodents have been described by Vanderberg, Nussenzweig & Most (1968).

The sporozoites used in the present experiments were harvested from salivary glands of female *Anopheles stephensi* mosquitoes which had fed on *P. berghei* infected hamsters 18 days previously. The methodology for extraction and handling of sporozoites has already been described (Orjih & Nussenzweig, 1980). Sporozoites used for infection of animals were not purified, whereas those used as antigen in serological tests were purified by passing the salivary gland extract through a DEAE–cellulose column (Moser *et al.*, 1978).

Experimental design. Two groups, A and B, of 10 mice each, were inoculated i.v. with 10,000 infective sporozoites per mouse, on day 0 and also on day 10. Starting from day 1, the mice in group A were treated daily with 0·4 mg of chloroquine base, administered i.p. until day 20. The purpose of this chemoprophylaxis was to prevent the animals in this group from developing parasitaemia from the sporozoite infections.

The animals in group B did not receive any drug treatment until day 20. By this time the animals had developed acute malaria from the sporozoite inoculations. The mice were started on curative chemotherapy from day 21. Each mouse received a daily i.p. injection of 0·4 mg chloroquine base for the next 10 days.

Serological tests. The indirect immunofluorescent antibody test (IFAT) for detection of anti-sporozoite and anti-blood form antibodies was performed as described by Orjih & Nussenzweig (1979), except that absortion procedure to remove cross-reacting anti-blood form antibodies in anti-sporozoite test was omitted. Test sera were collected from animals 17 days after the second dose of sporozoites. The lowest serum dilution tested in IFAT was 1 : 16. This is because at lower dilutions, negative control serum may give some fluorescence with the test antigens.

Circumsporozoite precipitin (CSP) reaction was carried out as described by Vanderberg *et al.* (1972). Only undiluted serum samples were tested for CSP reaction.

Challenge infection. To test for anti-sporozoite-mediated protective immunity against malaria, the animals in groups A and B were challenged i.v. with 10,000 infective sporozoites of *P. berghei.* The challenge was administered on day 44 of the experiment, i.e. 24 and 14 days after the completion of chloroquine treatments for groups A and B, respectively.

Controls. Two main control systems were set up. One was designed to show the effect of previous chloroquine treatment on sporozoite challenge infection as administered in this study. For this purpose 10 mice were treated with chloroquine together with the animals in group A above, but the control mice did not receive inoculations of sporozoites. These control animals received 10,000 sporozoites during challenge experiment.

Another control system was designed to determine whether recrudescence in *P. berghei* infection can occur after the curative chloroquine treatment given to group B mice above. Eight mice received two inoculations of infective sporozoites exactly as the animals in groups A and B. They were allowed to develop parasitaemia as those in group B and were subsequently cured with chloroquine together with group B mice. After the cure the control mice did not receive challenge infection like groups A and B animals did, but they were however, monitored for parasitaemia periodically until conclusion of the experiment.

Also to account for recrudescence, a total of 12 mice infected with sporozoites exactly as those in the experimental groups and cured with chloroquine, were splenectomised 15 days after the completion of chemotherapy. Subsequently, they were monitored every other day, for at least 14 days for parasitaemia.

Examination for protection. Starting from the fourth day after the sporozoite challenge all the animals were monitored daily for parasitaemia in Giemsa stained blood smears obtained from tail cut. Mice which failed to show patency 14 days after the challenge were considered protected.

RESULTS

Effect of chloroquine prophylaxis and therapy

Animals in group A which received daily injections of chloroquine from day 1 to day 20 were monitored for parasitaemia every two days starting from day 4. None of the animals developed detectable parasitaemia until after the challenge experiment. Some of the animals were tested serologically for antibodies against sporozoites and blood forms. Results of this test are shown in Table 1. Group A mice produced high titres of anti-sporozoite antibodies, as shown in IFAT, but no detectable anti-blood form antibodies (titre less than 16). This group of mice also gave positive CSP reaction. CSP reaction is strictly specific for sporozoites.

Before the mice in group B were started on drug therapy they had high parasitaemia with a mean of 40%. They were also very anaemic and sick. At the completion of the chloroquine treatment the animals appeared healthy and had no more parasitaemia until after challenge experiment. Splenectomy experiment showed that malaria parasites in the mice were indeed completely cleared by the chloroquine therapy.

In IFAT (Table 1) group B mice showed low anti-sporozoite antibody titres, some of them probably due to cross-reaction with anti-blood form antibodies (Orjih & Nussenzweig, 1979). However, some of the mice certainly produced anti-sporozoite antibodies because 30% of them were CSP positive. All the group B animals produced high titres of anti-blood form antibodies.

Table 1. Antibody responses to *P. berghei* sporozoite infections which did not cause malaria and to those which caused acute malaria (groups A and B mice, respectively)

	Fluorescent antibody titre			
	Anti-sporozoite		Anti-blood form	
Mice	Mean*	Range	Mean	Range
Group A	819·2	256–1,024	negative	
Group B	59·2	16–256	281·6	256–512

* Ten mice were tested individually in each group.

Challenge-induced parasitaemia

Starting from the fourth day after sporozoite challenge all animals were monitored daily for parasitaemia. As shown in Table 2, 96% of the animals in group A, i.e. the animals which did not develop malaria parasitaemia from the previous two inoculations, were completely protected against a challenge infection. Since all the drug treated control animals promptly developed parasitaemia after the challenge, the protection of the animals in group A was not due to the previous chloroquine treatment; rather it was due to the anti-sporozoite immunity which they developed in response to the previous sporozoite inoculations (Orjih *et al.*, 1982).

In contrast with group A, the animals in group B all became patent after the sporozoite challenge. The parasitaemia which appeared in the animals showed predictable characteristics, namely it appeared between 5 and 10 days following challenge, hardly exceeded 1%, and lasted for only about a week after which the parasitaemia was no longer detectable. It was determined that the early elimination of the parasitaemia was due to immunity against erythrocytic stage of the parasite. Thus animals which had been cured of acute malaria induced with blood forms, and subsequently

Table 2. Protection against sporozoite challenge of mice in which previous exposures to infective sporozoites of *P. berghei* either did not cause malaria (group A) or caused acute malaria (group B)

Mice	Challenged	Protected	Protection (%)
Group A	50	48	96
Group B	49*	0	0
Controls			
Effect of drug	50	0	0
Recrudescence	None in a total 40 intact and 12 splenectomized mice		

* The experiments were done five times with 10 mice per group each time. In one experiment one mouse in group B died of malaria before drug treatment.

challenged with parasitized erythrocytes also controlled the parasitaemia within 2–3 weeks (data not shown).

Forty mice which were treated exactly as those in group B, but were not challenged, were also monitored for parasitaemia together with the challenged ones. None of these control animals redeveloped parasitaemia. This, in addition to the splenectomy experiment above, suggests that the parasitaemia observed in group B mice after the sporozoite challenge was not a recrudence of the drug cured malaria.

DISCUSSION

The present study has demonstrated that acute malaria can suppress the ability of a vaccination procedure to induce protective immunity. Furthermore, the findings give support to the view that malaria-induced immunosuppression is one of the causes of recurrent malaria in endemic areas. It probably operates by interferring with the development of immunity against protective antigens of the parasite. In this way the host is kept continuously susceptible to infection.

From what is currently known about the immunology of malaria, the parasite appears to be particularly susceptible to host's immune system at the sporozoite stage. Like merozoites, the sporozoites are highly immunogenic; but unlike the merozoites they show little or no antigenic diversity (Nussenzweig, 1982). The parasite may therefore depend largely on immunosuppression for protection of this sensitive stage. Rapid internalization of sporozoites in hepatocytes could be considered another means of escape from host's immune system, but the finding that in nature humans produce antibodies against sporozoites (Nardin *et al.*, 1979), indicates that this defence mechanism is not very effective.

The mechanism of malaria-induced immunosuppression is still a subject of interest. Some workers feel that antibody response is more affected than cellular immune response (Greenwood, Playfair & Torrigiani, 1971). Recently, Harte, De Souze & Playfair (1982) found that passively acquired antibodies against malaria suppress development of activity immunity. In immunity against sporozoites both antibodies and cellular immunity seem to be capable of mediating protection. Monoclonal antibodies have prevented sporozoite infections in both mice and monkeys (Yoshida *et al.*, 1980; Cochrane *et al.*, 1982). Furthermore, immunized female mice can transfer protective immunity to their infants through the milk (Orjih, Cochrane & Nussenzweig, 1981). But there are some evidence that the presence of antibodies is not always necessary for the expression of anti-sporozoite-mediated resistance (reviewed by Cohen, 1979). The present study has shown that whatever mediates anti-sporozoite protection is also susceptible to malaria-induced immunosuppression.

This study has also shown that acute malaria is capable of preventing the expression of potentially protective immune response. An inoculation of infective sporozoites into mice rapidly sensitized the animals and antibodies were detected within three days, i.e. before the infection produced parasitaemia (Orjih *et al.*, 1982). The results of the present experiments suggest that suppression is not limited to inductive phase of an immune response. Apparently, a weak immune response can still be inhibited by malaria.

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