and may be an important mechanism whereby blood pressure can be lowered. However, the renin–angiotensin system can only be the cause of high blood pressure if the level of circulating angiotensin II is above normal, relative to the sodium balance, or there is an increased sensitivity to the actions of angiotensin II, compared with that in normotensive subjects.

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Active immunization and passive transfer of resistance against sporozoite-induced malaria in infant mice

Augustine U. Orjih, Alan H. Cochrane & Ruth S. Nussenzweig

Division of Parasitology, Department of Microbiology, New York University School of Medicine, 550 First Avenue, New York 10016, USA

Immunization attempts using a variety of plasmodial preparations have identified several developmental stages and vaccination procedures which can protect against malaria[1–3]. These indicate that immunoprophylaxis against malaria may also be possible in man. The immune response to these vaccines has only been investigated in adult animals, but in countries where malaria is prevalent, children are most at risk of developing lethal infections. Thus we must ask whether vaccination against malaria would be effective when applied early in life. Here we report the use of intramuscular (i.m.) immunization of very young mice with radiation-attenuated sporozoites of *Plasmodium berghei* as a vaccination procedure, and that these animals can be effectively protected against sporozoite challenge. This approach avoids the intravenous (i.v.) immunization, which although highly effective in adult animals, is barely acceptable for use in man. Another important aspect of the host–parasite relationship in endemic areas is the role of immunity transmitted congenitally from mother to infants. We therefore investigated whether sporozoite-immunized adult female mice could transfer this immunity to their litters. It was found that the offspring acquired antisporozoite antibodies from their mothers through the milk. Furthermore, most of these offspring resisted challenge by infective sporozoites.

All experiments were performed in A/J mice using the NK65 strain of *P. berghei*. Infant mice were bred in our animal facility and the adults were obtained commercially (Jackson Laboratories, Bar Harbor). Sporozoites, collected from the salivary glands of laboratory-bred *Anopheles stephensi*, were γ-irradiated at 15,000 rad before being used in immunization[4].

Young mice, 2–24 days old, and adult mice, 8–10 weeks old, were inoculated i.m. with 7×10^4 irradiated sporozoites suspended in 0.1 ml of tissue culture medium (TC199). These animals received 1–3 additional immunizing doses of 3.5×10^4 irradiated sporozoites, each given by the same route and at intervals of 1–2 weeks. One to two weeks after the last immunizing dose, the immunized animals and age-matched, non-immunized controls were challenged with 1×10^4 infective sporozoites i.v., to test for protection. At the time of challenge, the young mice were 5–7 weeks old. Animals which failed to show parasitized erythrocytes in Giemsa-stained blood smears, 2 weeks after challenge, were considered to be protected.

The results of six experiments, involving the immunization and challenge of 74 young and 24 adult animals, are summarized in Table 1. A high percentage of the immunized young mice developed resistance to sporozoite challenge. The level of protection among the young animals varied from 71 to 100%. The highest per cent protection occurred in those animals which had received the first immunizing dose of sporozoites at the age of 7–14 days. Adult immunized mice had a significantly lower per cent protection. In each of the experiments, the control animals developed parasitaemia within 5 days after sporozoite challenge.

In addition to the demonstration that, in mice, sporozoite vaccination can be effectively administered early in life, our data suggest that the effectiveness of this vaccination varies with the age of the recipient animals. Adult mice are highly protected by i.v. immunization with sporozoites, but when the immunizing sporozoites are given i.m., the per cent protection against challenge decreases considerably[5]. In our experiments, only 33.3% of the adult animals were protected after i.m. immunization (Table 1).

Young animals did not produce detectable serum antibody levels in response to a single dose of irradiated sporozoites. However, after two and three immunizing doses, the young and adult mice had comparable levels of antibodies, as measured by the indirect immunofluorescent antibody test (Table 2).

Further experiments were done to determine whether sporozoite-immunized mice could transfer protection to their offspring; 70 mice corresponding to 13 litters born and nursed by sporozoite-immunized mothers, were challenged with infective sporozoites when the pups were between 18 and 25 days old (Table 3). The challenge had to be delayed until this age because a large proportion of mice <2 weeks old born to and nursed by non-immunized mothers, were found to be resistant to sporozoite-induced *P. berghei* infection (our unpublished

Table 1 Protection against *P. berghei* sporozoite-induced infection of very young and adult mice immunized i.m. with γ-irradiated sporozoites

Age of mice at the start of immunization	Challenged	Protected	Percentage of protection
Infants			
2–6 days	20	16	80.0
7–14 days	44	41	93.2
16–24 days	10	8	80
Adults	24	8	33.3
Non-immunized controls			
Infants	21	0	0
Adults	10	0	0

The data represent the results of six experiments. Irradiated sporozoites were administered 2–4 times, with intervals of 1–2 weeks, each animal receiving a total of $1.05–1.75 \times 10^5$ irradiated parasites during the immunization period. Non-immunized controls were age-matched with the immunized animals.

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Table 2 Serum antibody levels in young and adult animals after i.m. injection of γ-irradiated sporozoites

No. of immunizing doses of sporozoites	$\log_2$ anti-sporozoite antibody titre	
	Pups	Adult mice
1	<4	5.0±0.6
2	7.0±0.5	7.0±0.3
3	9.2±0.2	9.0±0.3

Antibody response as measured by indirect immunofluorescence of young and adult mice to either 1, 2 or 3 i.m. inoculations with irradiated *P. berghei* sporozoites. The primary immunizing dose consisted of 7×10^4 sporozoites; the second and third doses, given at weekly intervals, each consisted of 3.5×10^4 parasites. Serum was collected 2 weeks after completion of each immunization. Anti-sporozoite antibody titres are expressed as mean values ± s.e.m. for each group of 5 mice. The lowest titre considered as parasite-specific was 4. The experiment was repeated on three different occasions with similar results.

data). Such non-immunological resistance is possibly the result of an exclusive milk diet[6,7].

Of the pups born to and nursed by sporozoite-immunized mothers, 60% resisted the infective sporozoite challenge (Table 3). In contrast, age-matched mice born to and nursed by non-immunized mothers were almost uniformly (95%) susceptible to sporozoite challenge. The percentage of resistant offspring of immunized mothers varied from litter to litter. In one experiment, in which we simultaneously challenged four litters using the same sporozoite preparation, the range of protection varied from 28.5 to 100%.

Mice born to and nursed by sporozoite-immunized mothers showed minimal levels of antibodies at birth, which increased gradually with suckling time, reaching maximal levels at 2 weeks old. This progressive increase in antibody level with time suggests that mice acquire antibodies from their mothers largely through the milk. This was further supported by experiments in which mice born to non-immunized mothers were transferred to immune foster mothers. After 12 days of nursing by the latter, the pups had acquired an antibody level similar to that found in age-matched pups born to and nursed by sporozoite-immunized mice. Indirect immunofluorescence antibody titres, obtained at 13 and 21 days of age, were determined for individual serum samples and expressed as a mean value ± s.e.m. for each group of 5 mice. Titres of the immune mothers were usually a twofold higher dilution than those of the pups. Pups born to non-immunized mothers then transferred to immune foster mothers had $\log_2$ anti-sporozoite antibody titres of 8.6±0.4 and 9.0±0.5 at 13 and 21 days old, respectively, compared with titres of 9.0±0.2 and 9.0±0.3 for pups nursed by immunized mothers. The experiment was repeated three times, with similar results. Protection against sporozoite-induced infection in these animals also seems to be largely transferred through the milk; 55% of the animals born to non-immunized mothers and nursed by sporozoite-immunized mice resisted a sporozoite challenge, whereas all the offspring of immune mice, foster-nursed from their first day onwards by non-immunized mothers were susceptible to sporozoite infection (data not shown).

Table 3 Protection against *P. berghei* sporozoite-induced infection of pups born to and nursed by sporozoite-immunized mice

Mothers	Pups			
			Percentage of protection	
No.	No. challenged†	No. protected	Mean	Range
13 Immunized*	70	42	60	28.5–100
7 Non-immunized	22	1	4.5	0–20

The data represent the results of five experiments which included 13 litters of immunized mothers and 7 litters of non-immunized mothers.

*Mice were immunized with a total of 1.1×10^5 γ-irradiated sporozoites i.v., given in five doses before onset of pregnancy.

†Pups were 18–25 days of age when challenged i.m. with 2.1×10^4 infective sporozoites. This inoculum consistently resulted in patent infection in control animals.

The passively acquired antisporozoite antibodies largely belong to the IgG class. In contrast, mice actively immunized with sporozoites possess high levels of both IgG and IgM, as detected by immunofluorescence. Whereas the passive transfer of anti-sporozoite antibodies through the milk has been clearly documented in this system, our experiments do not rule out the concomitant transfer of immune cells, which could also contribute to the functional immunity detected in the offspring[8,9].

Regarding the role of antibodies in anti-sporozoite-mediated resistance to malaria infection, it has been recently demonstrated that monoclonal antibodies to *P. berghei* sporozoite surface antigen confer complete protection on recipient mice[10]. However, our experiments constitute the first instance in which the passive transfer of sporozoite-induced immunity was observed in natural conditions.

In view of these findings in rodents, it is certainly worth investigating whether passive transfer of sporozoite-induced immunity occurs in infant humans living in areas in which malaria is endemic.

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Inducer T lymphocytes synthesize a factor that stimulates proliferation of cloned mast cells

Gary Nabel, Stephen J. Galli, Ann M. Dvorak, Harold F. Dvorak & Harvey Cantor

Departments of Cell Biology and Pathology, Sidney Farber Cancer Institute, Departments of Pathology, Beth Israel Hospital and Harvard Medical School, and the Charles A. Dana Research Institute, Beth Israel Hospital, Boston, Massachussetts 02215, USA

Inducer T lymphocytes activate other cells to divide and express new function. Known target cells include other lymphocytes and haematopoietic stem cells[1]. We now provide evidence that the inducer T cell acts on another important target population: mast cells. Mast cells have a central role in the expression of immediate hypersensitivity and are also prominent in T-cell mediated reactions of the delayed type[2–7]. Because the proliferation of differentiated cells is often regulated by soluble growth factors, we examined an inducer T-cell clone for its ability to stimulate mast cell proliferation. We report here that cloned $Ly1^+2^-$ inducer T cells produce a factor that selectively induces morphologically and karyotypically normal mouse mast cell clones to proliferate. We therefore suggest that inducer T cells may regulate mast cell numbers by releasing a soluble growth factor that stimulates them to divide. Because mast cell products also affect certain T-cell functions[8–10], mast cell–T cell interactions may comprise part of an immunoregulatory circuit.

Mast cells proliferate in the thymus of NZB mice[11] and elsewhere in association with T cells[2–7]. To explain these findings, Burnet proposed that certain mast cell populations are

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Malaria vaccine protects offspring

from F.E.G. Cox

THERE can be no doubt that an antimalarial vaccine is one of the major requirements of tropical medicine and a number of laboratories are attempting to develop one. There is equally no doubt that vaccination against malaria is possible and has been achieved using a wide range of experimental model systems, but the protection produced varies in efficacy and all the methods so far described have serious drawbacks.

In the life cycle of the malaria parasite in the human host, the infective stages injected by the mosquito are the sporozoites, which circulate in the body for about half an hour before entering the liver where a massive phase of multiplication occurs. This results in the production of thousands of merozoites which invade red blood cells where another multiplication phase, erythrocytic schizogony, takes place. The products of this phase are further merozoites which invade fresh red blood cells in which erythrocytic schizogony again occurs and this process is repeated until the host dies or recovers. Eventually sexual stages are produced and these are taken up by and infect the mosquito vector[1].

The various stages in the life cycle differ antigenically from one another; thus as the immunity produced is stage specific, a blockbuster approach to immunization is not feasible. At present the main targets for vaccines are the sporozoites, the merozoites, the erythrocytic stages and the gametocytes[2]. The use of irradiated sporozoites as a vaccine has been largely confined to rodent models but with considerable success. Adult mice can be protected by the intravenous injection of irradiated sporozoites[3]. The protective antigen is a surface antigen that can be demonstrated with the use of monoclonal antibodies[4] and monoclonal antibodies against this antigen protect mice against challenge with the homologous species[5]. On page 331 of this issue of *Nature* Orjih and colleagues report that these findings have been extended to young mice and the offspring of immunized mothers.

Briefly, adult mice given one to four immunizing doses of irradiated sporozoites intramuscularly were poorly protected against challenge with live sporozoites several weeks later. Young mice 2–14 days old, on the other hand, were well protected. The fact that adult mice can be protected by the intravenous route but not the intramuscular one is interesting but what these experiments do show is that young animals can be immunized in an acceptable way. Of even greater importance is the fact that when adult female mice were immunized intravenously with irradiated sporozoites, over half the offspring were protected against challenge and similar levels of protection were found in mice born to normal mothers but fostered onto immunized ones.

These results are particularly important because, in many parts of the world, malaria is essentially a disease of children. In endemic areas, babies acquire immunity passively from their mothers and are then protected during the critical first few months of life. Control schemes, however, upset this balance and it is in such situations that a vaccine that protects young children is likely to be of most use. Another important aspect of these results is that similar findings may apply to other vaccines such as the merozoite one being developed at Guy's Hospital in London[2], and recently described improvements in the *in vitro* cultivation of human malaria parasites[6,7] and adjuvants[8] have moved such a vaccine a further step forwards. The merozoite vaccine is known to protect monkeys but for obvious reasons the possibility of transfer of immunity to their offspring has not yet been investigated.

Those who have advanced the cause of a malaria vaccine have never suggested that a totally effective vaccine would ever be possible but have argued that a vaccine could produce better protection than a natural infection and could ameliorate the course of the disease. These experiments with *Plasmodium berghei* appear to vindicate this cautious approach. However, before too much euphoria sets in it must be remembered that the passage of immunity from mothers to offspring differs markedly from species to species and what happens in a mouse or monkey need not necessarily happen in man.

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F.E.G. Cox is Professor of Zoology at King's College London.

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