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Plasmodium chabaudi Malaria: Protective Immunization with Surface Membranes of Infected Erythrocytes

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***Plasmodium chabaudi*-susceptible NMRI and B10.A mice were vaccinated with host cell plasma membranes isolated from *P. chabaudi*-infected erythrocytes. Most of the mice were protected from the lethal consequences of challenge with the homologous parasite, although protection was unassociated with a reduction in the course or peak of parasitemia. Vaccination also induced the production of antibodies against Pc90, which is the immunodominant protein expressed by parasites in host cell plasma membranes.**

Malaria is caused by protozoans of the genus *Plasmodium*. Mosquitoes of the genus *Anopheles* transfer sporozoites, which develop in the liver into the antigenically different merozoites. These infect erythrocytes, causing the disease. It is known that repeated infections lead to a partial protective immunity (for a review, see references 1 and 7). This form of immunity cannot prevent parasitemia, but it helps patients to cope with the infection. The protective mechanisms are not directed against sporozoites (3), but rather, they are directed against the blood stages. Targets may not only be the free merozoites but also the infected erythrocytes. The fact that erythrocytes are targets is supported by the observation that parasites within erythrocytes can be killed by serum derived from immune people living in malaria-endemic areas (4, 5). Moreover, there is a significant number of reports on murine, simian, and human malaria demonstrating the vaccine potential of infected erythrocytes and their components (for reviews, see references 1, 2, and 7). There is considerable information available that intraerythrocytic parasites express specific proteins on surface membranes of infected erythrocytes (for a review, see reference 8). In this study we show that vaccination with surface membranes induces protection against blood stages of *Plasmodium chabaudi*, which causes malaria in rodents.

P. chabaudi was passaged weekly in NMRI mice (12). Parasitemia was evaluated from Giemsa-stained blood smears. Erythrocytes were counted in a Neubauer chamber. *P. chabaudi*-infected erythrocytes were isolated over a Percoll step gradient as described previously (11). Surface membranes, i.e., the host cell plasma membranes, were then isolated in the form of erythrocyte ghosts, which were purified by the procedure that we developed previously (11; cf. also reference 10). Plasma membranes from nonparasitized erythrocytes of noninfected mice were also isolated in the form of erythrocyte ghosts, as described in detail previously (11).

Vaccination experiments were performed with female NMRI and B10.A mice when they were about 10 weeks of age. NMRI mice were immunized twice with 10^7 ghosts on days 0 and 14, while B10.A mice were immunized only once with 10^6 ghosts. The ghosts were suspended in 100 μl of 0.14 M NaCl, mixed with an equal volume of Freund complete adjuvant, and subcutaneously injected at the base of the tail. Seven days after the last immunization, the mice were challenged intraperitoneally with 10^6 *P. chabaudi*-infected erythrocytes.

For the preparation of sera, blood was drawn from the aorta dorsalis, clotted at 37°C for 30 min and subsequently at 4°C for 2 h, and centrifuged at 2,500 × *g* for 10 min. Supernatant serum samples were inactivated at 56°C for 30 min before they were centrifuged at 13,000 × *g* for 20 min.

Lyophilized erythrocyte ghosts were solubilized in sodium dodecyl sulfate (SDS) buffer containing 60 mM Tris hydrochloride (pH 6.8), 4% SDS, 5% mercaptoethanol, 10% glycerol, and 0.5% bromphenol blue and were run in 7.5% SDS-slab gels (200 μg of protein per lane) by the method of Laemmli (6). Staining was done with Coomassie brilliant blue R. Western blot analysis was done essentially by the protocol of Towbin et al. (9), as described recently (10).

Surface membranes of *P. chabaudi*-infected erythrocytes contained seven new proteins with apparent molecular masses of about 154, 145, 90, 72, 67, 52, and 33 kilodaltons, as revealed previously by SDS-polyacrylamide gel electro-

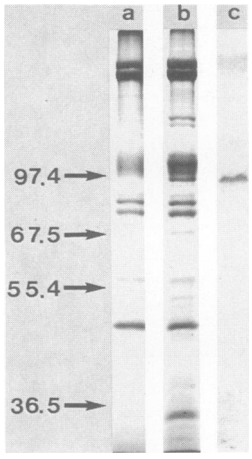

FIG. 1. Anti-Pc90 antibodies in serum of vaccinated NMRI mice. Lane a, Coomassie brilliant blue-stained SDS-polyacrylamide gels of ghosts isolated from noninfected erythrocytes; lane b, SDS-polyacrylamide gels of ghosts from *P. chabaudi*-infected erythrocytes; lane c, Western blot of infected erythrocyte ghosts by using pooled sera obtained from two NMRI mice on day 21 after two immunizations with 10^7 ghosts isolated from *P. chabaudi*-infected erythrocytes on days 0 and 14. Numbers on the left indicate molecular masses of standard proteins (in kilodaltons).

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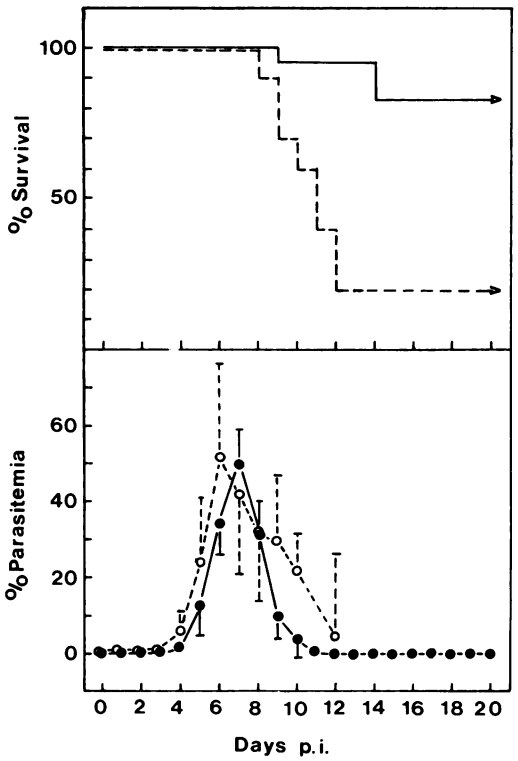

FIG. 2. Survival and parasitemia of NMRI mice after vaccination with erythrocyte surface membranes. As controls, 10 mice were injected with 10^7 ghosts isolated from noninfected erythrocytes on days 0 and 14 and challenged on day 21 with 10^6 *P. chabaudi*-infected erythrocytes (dotted lines). Correspondingly, 38 mice were immunized with 10^7 ghosts isolated from *P. chabaudi*-infected erythrocytes before they were challenged (solid lines). Parasitemia is shown only for the 8 mice in the control group that succumbed and the 33 mice in the immunized group that survived. Values of parasitemia are means ± standard deviations; only half bars of standard deviations are given, for clarity. p.i., Postinfection.

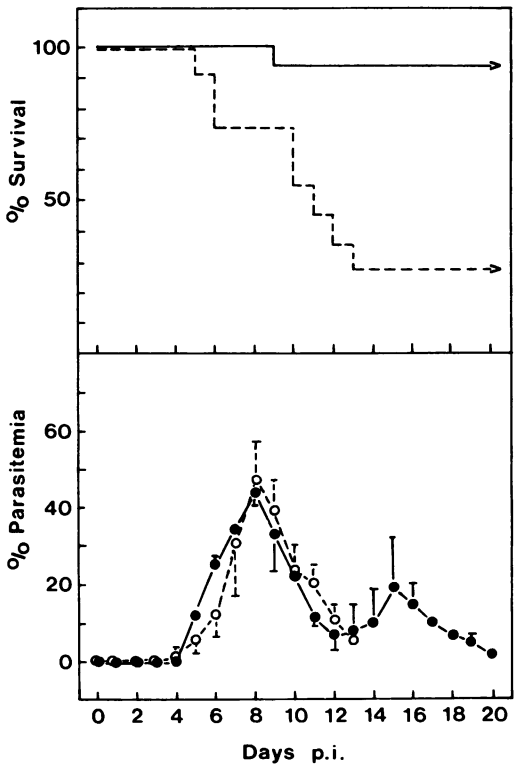

FIG. 3. Survival and parasitemia of vaccinated B10.A mice. A total of 19 mice were immunized with 10^6 ghosts isolated from *P. chabaudi*-infected erythrocytes (solid lines), and 11 mice were immunized with ghosts from noninfected erythrocytes (dotted lines); 7 days later, the mice were injected intraperitoneally with 10^6 *P. chabaudi*-infected erythrocytes. Parasitemia was evaluated as described in the legend to Fig. 2.

phoresis (11) (Fig. 1). The 90-kilodalton protein, designated Pc90, is synthesized by parasites and is transported to the erythrocyte plasma membrane, as shown previously by metabolic labeling (10). The Pc90 protein is the immunodominant parasite protein in host cell plasma membranes, since two immunizations of NMRI mice with 10^7 ghosts induced the emergence of antibodies directed predominantly against Pc90 (cf. the Western blot in Fig. 1). Such serum-converted mice also acquired the capability of surviving a blood-stage infection by *P. chabaudi* (Fig. 2). Indeed, about 90% of the serum-converted mice survived a challenge with 10^6 *P. chabaudi*-infected erythrocytes, which is in contrast to the 20% survival of control mice immunized with erythrocyte ghosts isolated from noninfected erythrocytes (Fig. 2). This protective immunization, however, did not prevent the appearance of parasitized erythrocytes in the peripheral blood. The prepatent period and the course and peak of parasitemia were about the same as those in control mice that succumbed to infection (Fig. 2).

In the genetically different B10.A mice, it was sufficient to vaccinate them only once with a lower dose, namely, 10^6 infected erythrocyte ghosts, in order to induce protection. Indeed, about 95% of the vaccinated mice survived, while 73% of the control mice succumbed to infection (Fig. 3). Again, parasitemia took about the same course in mice that were immunized with surface membranes from infected erythrocytes and in control mice that succumbed to infection (Fig. 3). Seven days after vaccination, i.e., at the time of challenge, anti-Pc90 antibodies could not be detected in Western blots of mouse sera (Fig. 4). However, antibodies appeared very early during the course of infection, i.e., at a parasitemia of about 5% (Fig. 4). In the challenged control mice, however, anti-Pc90 antibodies appeared significantly later. Anti-Pc90 antibodies could not be detected at a parasitemia of 24%, but were detected only as a very weak band in Western blots at the parasitemia maximum (Fig. 4).

Our data indicate that vaccination with host cell plasma membranes isolated from *P. chabaudi*-infected erythrocytes protects the *P. chabaudi*-susceptible NMRI and B10.A mice from the lethal consequences of challenge with the homologous parasite. Protection, however, did not affect the prepatent period, the course of the infection, or maximal parasitemia. Protection became apparent in a significant increase in mouse survival. The reason for protection remains unknown, although protection coincides with the production of antibodies against the parasite protein Pc90 in host cell plasma membranes (10, 11). However, this coincidence may be totally fortuitous and may have no relevance at all to the mechanism(s) of protection. Indeed, our data can exclude one plausible explanation for a role of anti-Pc90 antibodies in protection, namely, that the Pc90 protein in host cell plasma membranes is targeted by anti-Pc90 antibodies, thus causing lysis of the infected erythrocytes which, in turn, would ultimately entail the death of the intraerythrocytic parasites.

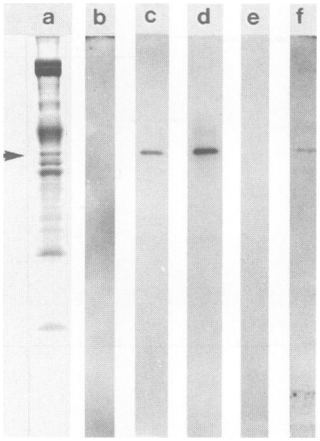

FIG. 4. Western blot analysis with sera of vaccinated B10.A mice. Mice were immunized either with 10^6 ghosts isolated from *P. chabaudi*-infected erythrocytes or with 10^6 ghosts from noninfected erythrocytes and challenged with 10^6 *P. chabaudi*-infected erythrocytes. Lane a, Coomassie brilliant blue-stained SDS-polyacrylamide gels of ghosts isolated from infected erythrocytes (the arrow points to Pc90). Lanes b to d, Western blots of infected ghosts with sera from immunized mice; sera from two mice were pooled just before challenge (lane b), from three mice at a parasitemia of approximately 5% (lane c), and from two mice at a parasitemia of approximately 45% (lane d). Lanes e and f, Corresponding Western blots of sera from control mice; sera were from two mice pooled at a parasitemia of approximately 24% (lane e) and approximately 44% (lane f). The Western blots shown here were done with one slab gel under identical conditions.

Whatever the function of the anti-Pc90 antibodies may be, our vaccination model using host cell plasma membranes as immunogens has an interesting potential for uncovering the mechanisms that are involved in the development of clinical tolerance of malaria.

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