Host Defenses in Murine Malaria: Failure of Vaccination with Formolized Blood Parasites to Protect Athymic Mice from Plasmodium berghei

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The thymus dependency of immunity to erythrocytic Plasmodium berghei (NYU-2) infection generated in response to injections of Formalin-killed mixed blood parasites was shown by the demonstration that the vaccine protected immunologically intact nu/+ mice, but not their athymic nu/nu littermates.

Recent studies showed that mice can be protected against various species of erythrocytic murine plasmodia by vaccination with Formalin-killed blood parasites (14, 15). Evaluation of the mechanism of action of these vaccines revealed evidence that they primed recipients for the production of a protective humoral factor (12, 15), possibly antibody. However, it is also known that some successful killed vaccines engender a hypersensitivity measurable as delayed swelling reactions at sites of subcutaneous injections of malarial antigens (5) and cause the generation of a sensitized population of spleen cells which upon adoptive transfer to naive recipients cause alterations in migration patterns of bone marrow and lymph node cells (16). It appears, therefore, that killed vaccines may sensitize both B- and T-cell arms of the murine immunological defense mechanism. Hence, the data might be viewed as supporting the suggestion that successful vaccination is dependent on both B- and T-cell functions. Support for this suggestion has been provided by recent studies which show that both B and T cells are required for immunity to erythrocytic infection with avirulent Plasmodium yoelii in mice (9, 19, 20, 26). Furthermore, immunity to Plasmodium berghei infection of rats appears to be dependent on the integrity of both cellular and humoral arms of the immune response (1, 2, 6–8, 21–23, 27). However, at times, resistance to murine malarial parasites can be expressed in the absence of demonstrable antibody (17, 20). A full explanation of why immunity requires both B- and T-cell function at some times but not at others is not available presently.

Direct evidence for requirements for B-cell or T-cell functions in the acquisition of immunity to malaria in response to injections of killed antigens is also not available. The present study was undertaken to determine whether or not murine malaria immunity induced by a killed vaccine is thymus dependent. Congenitally athymic (nu/nu) mice, which are deficient in both specific T-cell-mediated immunity (24) and thymus-dependent B-cell function (18), were used to answer the question.

Male nu/nu and nu/+ mice of the BALB/c strain were obtained at 8 weeks of age from the breeding facility of the Trudeau Institute. They were maintained on a standard diet (4RF, Charles River formula; Agway, Waverly, N.Y.) and provided water ad libitum. P. berghei NYU-2 was used. The source and procedures used for maintenance of the parasites have been described (13, 14). Infections were initiated by the intravenous injection of 104 parasitized erythrocytes. A Formalin-killed, mixed blood-parasite antigen, made free of intact erythrocytes by lysis in 0.155 M NH4Cl, was prepared and standardized as described previously (13, 14). Mice received five intravenous injections of 105 Formalin-killed parasites at 3-day intervals. P. berghei infections were initiated on day 14 after the last antigen injection. Thin blood smears were prepared from tail blood and stained with Giemsa. If the level of parasitemia was 1% or greater, the number of parasitized erythrocytes in 200 erythrocytes was determined. For parasitemia less than 1%, the number of parasitized erythrocytes in a number of microscopic fields containing 2 × 105 erythrocytes was determined. Parasitemia is expressed as median parasitized erythrocytes per 105 erythrocytes in log10 units for each group of mice.

Figure 1 shows the course of P. berghei infections in nu/+ mice. It can be seen that infections developed at the same rate in vaccinated mice as in controls. However, vaccinated mice subsequently cleared the parasites and survived, whereas infections progressed until death in all controls. Although all previously vaccinated mice survived, the quality of the immunity pro-
vided through the immunizing regimen differed. This was evidenced by differences in the duration of infection. The immunized mouse with the most effective defense cleared its overt infection by day 15 after challenge, but it required 33 days for 5 of the 10 mice to clear parasites, and one mouse showed patent infection through day 81.

Figure 2 shows the course of *P. berghei* infections in nu/nu mice. The infections progressed rapidly, and all mice died, whether previously immunized against *P. berghei* or not.

The preceding experiment was repeated, and similar results were obtained.

This report shows that nu/nu mice are not protected from virulent *P. berghei* by injections of Formalin-killed blood stage parasites, whereas their nu/+ littermates are uniformly protected by the same immunizing regimen. Because nu/nu mice have defective thymus-dependent immunity whereas nu/+ mice are competent in this regard, these results support the view that a thymus is required for the vaccination regimen to effectively protect against lethal *P. berghei* infection. However, direct proof of a thymus requirement for vaccine-associated immunity is not provided by the present study. This would require the demonstration of thymus graft reconstitution of the vaccine-associated anti-*P. berghei* capacity of nu/nu mice.

The demonstration in this study, that immunity to *P. berghei* induced by killed vaccine in mice is thymus dependent, is consistent with the previous demonstration that immunity to this parasite in rats (1, 2, 8, 23) and immunity to another species of *Plasmodium, P. yoelii* (19, 26), in mice are both thymus dependent. Although the universality of the thymus dependency of specific acquired immunity to murine malaria is supported by observations from numerous laboratories, the precise nature of the immunological defense(s) compromised by the lack of a thymus has not been identified, and it is not known whether the state of immunity generated in response to injections of killed vaccine is identical with that generated in response to attenuated infections. Furthermore, the precise roles of T cells and B cells in the acquisition
and expression phases of the antimalarial immune response remain to be defined.

The demonstration that immunity to murine malaria is in part mediated by immunoglobulin G antibodies (6) supports the view that nu/nu mice might be deficient, through a lack of helper cells, in the capacity to produce protective immunoglobulin G antibodies. This suggestion is supported by the demonstration that immunoglobulin G types 1 and 2, as well as specific antimalarial antibodies (as measured by an indirect hemagglutination test), are depressed in nu/nu mice (26). However, the relationship between antibodies measured in the indirect hemagglutination test and those which protect from malaria in vivo remains to be established. Further, it is not clear whether immunoglobulin G antibodies are the sole thymus-dependent defense to murine malaria. For example, recent studies have shown that resistance to Plasmodium vinckei, P. yoelii, and Plasmodium chabaudi infections of mice can be raised through injections of Mycobacterium bovis (BCG) (3) or Corynebacterium parvum (4). The possibility that this defense, whose mechanism has yet to be identified, is thymus dependent should be considered in view of the marked potentiation of thymus-independent immunity which is at times caused by BCG (10, 11) or C. parvum (25).

In the present study it is notable that the course of P. berghei infections in vaccinated-protected nu/+ mice varied markedly. Because the mice were genetically identical, this variability suggests that factors which are not directly controlled by the host genome affect the capacity of vaccinated mice to respond to live P. berghei. The reason(s) for this variation is not known.

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LITERATURE CITED

