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Splenomegaly, Enhanced Phagocytosis, and Anemia Are Thymus-Dependent Responses to Malaria

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Immunologically competent mice and mice with defined immunological deficiencies were infected with *Plasmodium yoelii*. Splenomegaly, enhanced phagocytosis, and anemia were most marked in infected mice having intact thymic tissue. Whereas the spleens of infected nude mice increased minimally in size, the relative blood hemoglobin levels and the rates of carbon clearance in these mice were similar to those of noninfected, immunologically intact mice. Thymus-reconstituted nude mice and B-cell-deficient mice responded to infection in a manner similar to that of infected immunocompetent mice. These data demonstrate that the hallmarks of malaria, i.e., splenomegaly, enhanced phagocytosis, and anemia, are thymus-dependent responses to infection.

Splenomegaly in association with macrophage hyperplasia is a prominent feature of malaria (13). Evidence suggesting that these host responses to disease may be thymus dependent include: (i) the observation that mice depleted of T-cells developed less splenomegaly when infected with *Plasmodium yoelii* (6) than did immunologically intact animals, and (ii) the finding that macrophages did not accumulate within the spleens of nude mice infected with *Plasmodium berghei* (16).

Other characteristics of malarial infection include enhanced phagocytosis and anemia (1, 17). To determine if these host responses to malaria are also thymus dependent, carbon clearance, anemia, and splenomegaly have been investigated in athymic "nude" mice, B-cell-deficient mice, and immunologically intact mice infected with *P. yoelii*. The data show that enhanced phagocytosis and anemia, in addition to splenomegaly, are indeed thymus-dependent host responses to malaria infection.

MATERIALS AND METHODS

Experimental animals. nu/nu and nu/+ mice of both sexes having a BALB/c background were obtained from our closed colony reared under conventional conditions.

Thymic reconstitution of nu/nu mice and the preparation of B-cell-deficient mice, as well as the assessment of their immunological competence, were accomplished by methods described previously (11).

Serum immunoglobulin M (<0.6 mg/100 ml) was not detected in three mice treated with anti-μ serum. Other mice in our laboratory treated in the same manner circulated goat anti-μ in their sera for prolonged periods of time, failed to make detectable immunoglobulin M, and did not produce plaque-forming cells in response to immunization with sheep erythrocytes. Furthermore, their spleen cells were responsive to stimulation with concanavalin A but not with lipopolysaccharide (J. L. Grun, unpublished data).

Experimental infection. Mice 2 to 5 months of age were infected intraperitoneally with 10^6 erythrocytes parasitized with the 17X strain of *P. yoelii*. Parasitemias were determined by examination of blood films prepared from tail blood and stained with Giemsa stain.

Where indicated, parasitemias were arrested by chemotherapy with clindamycin HCl, 74 mg/kg of body weight daily per os for 5 consecutive days as described previously (11). This was done before conducting tests of carbon clearance to insure that phagocytosis was measured in the absence of patent parasitemia. Uninfected control mice also were treated with clindamycin.

Carbon clearance. Pelican India ink (Gunther Wagner, Germany) was dialyzed against phosphate-buffered saline (pH 7.2) to remove toxic components. The suspension containing 3.3 mg of carbon per ml was then sterilized by autoclaving and stored at 4°C. The suspension of carbon (0.05 to 0.1 ml) was injected into the left retro-orbital plexus. At appropriate time intervals, blood samples were taken from the right retro-orbital plexus with heparinized 75-mm capillary tubes having a volume of 0.08 ml (A. H. Thomas Co., Philadelphia, Pa.) and placed in tubes containing 3 ml of 0.1% Na_2CO_3 in distilled water. The optical density of each sample was determined spectrophotometrically at 675 nm. A blood sample taken from the same mouse before the injection of carbon was used as a blank. The rate of carbon clearance (K) in each mouse was equal to the slope of a linear regression line fitted to the data by the method of least squares, wherein the log of optical density of a given blood sample was

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plotted against the time of sampling in minutes. For convenience, the absolute value of K was multiplied by 10^3.

At the termination of the experiment, the mice were sacrificed and their spleens were weighed.

Determination of hemoglobin levels. Relative hemoglobin levels were determined spectrophotometrically by diluting the blood sample obtained from each mouse before the injection of carbon, 1:250 in 0.1% Na_2CO_3, and measuring the absorption of this solution at 550 nm.

RESULTS

When splenomegaly, phagocytosis, and anemia were assessed in athymic mice and thymus-bearing mice infected 13 days before with 10^6 parasitized erythrocytes, enhanced phagocytosis, as evidenced by the accelerated clearance of carbon, and anemia, which was indicated by decreased hemoglobin levels, were observed only in those mice with functional thymic tissue (Table 1). The rate of carbon clearance in infected nu/nu mice was not significantly different from that seen in noninfected nu/+ mice or nu/nu mice. Similarly, infected nu/nu mice failed to demonstrate significant anemia when tested at this time.

Whereas the spleens of infected nu/nu mice showed a slight increase in weight as compared with the spleens of noninfected nu/nu mice, splenomegaly was most evident in infected thymus-bearing nu/+ mice, which demonstrated a sevenfold increase in relative spleen weight (Table 1). The increase in the relative spleen weight of infected nu/nu mice which had been thymic grafted was fivefold. Thymus-grafted animals possessed T-cell competence, as demonstrated by their ability to develop allergic contact dermatitis upon sensitization and challenge with *p*-nitroso-*N,N*-dimethylaniline.

To determine if these responses to infection required B-cell participation, splenomegaly, phagocytosis, and anemia were assessed in nu/+ mice which had been treated from birth with anti-μ-chain serum and infected with 10^6 parasitized erythrocytes 12 days before testing. Such B-cell-deficient mice developed splenomegaly in response to infection with *P. yoelii* and cleared carbon in an accelerated manner (Table 2).

TABLE 1. *Increased carbon clearance, splenomegaly, and anemia in thymus-bearing mice infected with P. yoelii*

Genotype (no.)	Infection[a]	Relative spleen size[b]	Carbon clearance (K) $\times 10^{3}$[c]	Relative hemoglobin concn (%)[d]
nu/nu (5)	+	0.94 ± 0.16	50.3 ± 8.7	89.6 ± 14.6
nu/nu (5)	−	0.42 ± 0.11	42.6 ± 16.0	95.8 ± 4.2
nu/+ (6)	+	3.06 ± 0.95	125.8 ± 37.0	64.6 ± 10.2
nu/+ (6)	−	0.40 ± 0.9	50.2 ± 8.2	100.0
nu/nu-thymus grafted[e] (5)	+	2.20 ± 1.13	96.4 ± 25.2	72.9 ± 4.2

[a] Mice were infected with 10^6 *P. yoelii*-infected erythrocytes. Both infected and noninfected mice were treated with clindamycin HCl, 74 mg/kg, for 5 consecutive days beginning on day 8 after infection.

[b] Relative spleen size equals (mean spleen weight/body weight) × 100 ± standard deviation.

[c] Mean slope ± standard deviation of linear regression lines fitted by method of least squares to individual carbon clearance plots. Mean correlation coefficients ranged from 0.95 to 0.98.

[d] Relative hemoglobin values are given as percentage of the mean value obtained for uninfected nu/+ controls.

[e] nu/nu mice were thymus grafted at 3 weeks of age and infected with *P. yoelii* at 5 months of age.

TABLE 2. *Increased carbon clearance, splenomegaly, and anemia in B-cell deficient mice infected with P. yoelli*

Treatment[a] (No.)	Infection[b]	Relative spleen size[c]	Carbon clearance (K) $\times 10^{3}$[d]	Relative hemaglobin concn (%)[e]
None (6)	−	0.45 ± 0.05	25.7 ± 3.5	100
None (6)	+	3.09 ± 1.05	66.0 ± 24.8	70.8 ± 10.4
Anti-μ serum (6)	+	3.35 ± 1.03	118.1 ± 46.1	64.6 ± 8.3
Normal goat serum (4)	+	4.17 ± 0.06	148.0 ± 20.4	60.4 ± 10.4

[a] Nu/+ mice were injected intraperitoneally with either goat anti-mouse μ-chain or normal goat serum three times per week starting on the day of birth.

[b] Mice were infected with 10^6 *P. yoelii*-infected erythrocytes. Both infected and noninfected mice were treated with clindamycin HCl, 74 mg/kg, for 5 consecutive days beginning on day 7 after infection.

[c] Relative spleen size equals (mean spleen weight/body weight) × 100 ± standard deviation.

[d] Mean slope ± standard deviation of linear regression lines fitted by method of least squares to individual carbon clearance plots. Mean correlation coefficients range from 0.95 to 0.98.

[e] Relative hemoglobin values are given as percentage of the mean value obtained for uninfected nu/+ controls ± standard deviation.

In addition, anemia, as reflected by decreased levels of hemoglobin, was observed in all infected T-cell competent mice regardless of their B-cell deficiency. Spleen size, carbon clearance, and hemoglobin level were similar in both anti-μ-treated nu/+ mice and untreated nu/+ mice which had not been infected with *P. yoelii*.

DISCUSSION

These data demonstrate that certain characteristic features of malaria are thymus-dependent host responses to infection. Thus, after infection with *P. yoelii*, marked splenomegaly, enhanced phagocytosis, and anemia were observed in B-cell-deficient mice, thymus-reconstituted nu/nu mice, and nu/+ mice which were immunologically intact. In contrast, infected nu/nu mice displayed minimal splenomegaly, failed to develop anemia, and did not clear carbon in an accelerated manner, in contrast to infected mice having functional thymic tissue.

The observation regarding the lack of marked splenomegaly in infected nu/nu mice is consistent with the finding of Jayawardena et al. (6), who noted that splenomegaly was minimal in thymectomized, irradiated mice infected with *P. yoelii*. Since these authors observed that the blood monocyte response was depressed in infected T-cell-depleted mice, it is quite possible that the activation of T-cells during infection may enhance the differentiation of stem cells into monocytes, which are then recruited by the spleen. Lymphocytes activated by mitogen have been shown to produce factors which stimulated clonal growth of bone marrow cells (12), and Wyler and Gallin (16) have demonstrated the presence of a monocyte-chemotactic factor in the spleens of immunologically intact mice infected with *P. berghei*. Assuming that enhanced phagocytosis within both the spleen and the liver during infection depends in part upon this recruitment of blood monocytes, infected T-cell-deficient animals might be expected to have difficulty clearing particles from the blood. Indeed, the data demonstrate that this is so. In addition, the finding that carbon clearance was increased in B-cell-deficient animals is consonant with other data from our laboratory which show that the rate of carbon clearance in B-cell-deficient chickens infected with *P. gallinaceum* was similar to that seen in infected chickens possessing normal immunological capability (R. G. Rank and W. P. Weidanz, unpublished data). Both splenomegaly and phagocytosis were most marked in infected mice which had received normal goat serum throughout life. This may have been due to repeated immunization with goat serum proteins.

The fact that anemia occurred in B-cell-deficient mice but not in nu/nu mice infected with *P. yoelii* may also be related to enhanced phagocytosis by an activated mononuclear phagocytic system. Thus, the stimulation of erythrophagocytosis in infected animals (18) may depend upon macrophage activation via T-lymphocytes, which have been shown to be activated by the mitogenic effects of malarial infection (7, 14) or by mediators released from phagocytizing mononuclear cells within the spleen itself, as has been suggested (16) to account for the generation of a nonspecific chemotactic factor early during infection.

Do these thymus-dependent host responses to infection contribute to immunity in malaria? In a previous report (10) we demonstrated that B-cell-deficient chickens cured of acute malaria by means of drug therapy possessed a potent antibody-independent mechanism which protected them against residual endogenous parasites as well as against exogenous challenge. Preliminary evidence (D. W. Roberts and W. P. Weidanz, unpublished observations) suggests that the same mechanism is functional in B-cell-deficient mice. Whereas the naive murine host needs both T- and B-lymphocyte systems to survive acute infection with the relatively avirulent *P. yoelii* (11, 15), B-cell-deficient mice resisted challenge infection after drug therapy of their acute disease. In contrast, malaria recurred in T-cell-deficient mice which had been similarly treated (11). It seems probable that the recruitment of monocytes to the spleen by factors produced by T-cells in response to infection as well as the maturation and activation of splenic macrophages aid in the destruction of plasmodia. This could be achieved by the phagocytosis of free merozoites as well as by the stimulation of erythrophagocytosis, which have been shown to occur in malaria (17, 18). In addition, activated macrophages may produce factors which cause the intracellular death of plasmodia, as has been suggested by Clark et al. (3, 4) to account for the mechanism by which hosts stimulated with BCG or *Corynebacterium parvum* are able to resist infection by assorted hemoprotozoa. Thus, the regulation of the mononuclear phagocytic system by T-cells responding to malarial infection, as reflected by increased splenic cellularity and enhanced phagocytosis, may be a prerequisite for the development of immunity in malaria. This concept does not diminish the role of humoral immunity in malaria; the synergistic action of cells and specific antibody in immunity to malaria is well established (5). Rather, it suggests that T-cells play a significant role in the development of immunity to malaria in addition to their function as helper cells in the

production of protective antibodies.

Finally, it should be noted that the T-cell-dependent responses characteristic of malaria are not unique to this disease. Splenomegaly and enhanced phagocytosis in both schistosomiasis and trypanosomiasis have been shown to be thymus dependent (9; R. G. Rank and J. P. Robinett, personal communication). It is quite possible that these responses contribute to an important resistance mechanism which is activated during the early stages of certain infectious diseases before the initiation of classical immune responses.

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