Effects of Splenectomy on Antibody-Independent Immunity to
Plasmodium chabaudi adami Malaria

J. L. GRUN, C. A. LONG, AND W. P. WEIDANZ*

Malaria Research Group, Department of Microbiology and Immunology, Hahnemann University School of Medicine, Philadelphia, Pennsylvania 19102-1192

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Splenectomy of B-cell-deficient mice and immunologically intact mice before infection with Plasmodium chabaudi adami led to the development of significant parasitemias which eventually resolved in the latter mice. Whereas both eusplenic B-cell-deficient mice and immunologically intact mice resolved their acute P. chabaudi adami infection, only B-cell-deficient mice subsequently developed chronic low-grade malaria. Splenectomy of B-cell-deficient mice with chronic malaria led to recrudescing infections, suggesting that the expression of antibody-independent immunity to reinfection was spleen dependent. When dispersed spleen cells were injected into splenectomized mice before challenge with P. chabaudi adami, the kinetics of the resulting infection resembled that seen in splenectomized mice which had not been grafted with normal spleen cells. This finding indicates that immunity to P. chabaudi adami requires the presence of an architecturally intact spleen.

Accumulating evidence suggests that antibody-independent mechanisms play an important role in acquired immunity to malaria. Mice injected with Mycobacterium bovis BCG or Propionibacterium acnes were protected against subsequent challenge with various hemoprotozoa (5, 6). Other immunomodulating agents, including Salmonella spp. and zymosan, were found to have similar effects (4). More recently, a number of agents causing oxidant stress in erythrocytes have been reported to suppress malarial infections both in vivo and in vitro (1, 2, 7). Similar parasite-suppressive effects have been attributed to tumor necrosis factor and the newly described crisis-forming factor (8, 12, 16). The latter factor was detected in the sera of certain Sudanese living in malaria-endemic areas and proved capable of inhibiting normal parasite development in erythrocytes infected with Plasmodium falciparum.

Using a different approach, we have reported that B-cell-deficient mice and chickens protected against otherwise lethal malaria by chemotherapy subsequently developed chronic low-grade malaria and resisted challenge infection with homologous parasites (14, 15). More recently, B-cell-deficient mice infected with Plasmodium chabaudi adami were observed to suppress acute malaria in the same manner as immunologically intact mice in the absence of therapeutic intervention (10). To date, our findings suggest that antibody-independent immune mechanisms play a significant role in protecting the host against acute infections caused by certain hemoprotozoa but are essential for "premunition" or immunity to reinfection malaria, regardless of etiology (11). It is interesting to note that B-cell-deficient hosts with chronic malaria fail to sterilize their infections, suggesting that the total elimination of parasites from the blood requires antibody or other B-cell products. In contrast to B-cell-deficient animals, athymic hosts failed to develop antibody-independent immunity to malaria, indicating that T cells play an essential role in the induction and maintenance of this form of immunity (17).

Since it is known that the spleen is intimately involved in the expression of immunity to plasmodia (18), we have investigated the effects of splenectomy in B-cell-deficient mice infected with P. chabaudi adami to determine whether the expression of antibody-independent immunity to this parasite is indeed dependent upon the presence of an intact spleen in the infected B-cell-deficient host.

Two- to three-month-old BALB/c and (BALB/c × C57BL/10)F1 mice of both sexes bred in our animal facility and P. chabaudi adami 556KA strain were used in all experiments. In vivo suppression of B-cell development was carried out by lifelong injections of goat anti-mouse immunoglobulin M as described previously (15). P. chabaudi adami infections were initiated by the intravenous injection of 10⁵ parasitized erythrocytes. Subsequent parasitemias were determined by microscopic examination of thin blood films prepared from tail blood and stained with Giemsa stain. Splenectomy was performed before or after infection with P. chabaudi adami as indicated below, using the procedure described by Eling (9) and mice anesthetized with ketamine hydrochloride (160 to 200 mg/kg of body weight) dissolved in 0.4% acepromazine maleate. Cellular reconstitution of splenectomized mice was achieved by injecting one splenic equivalent of spleen cells suspended in cold Eagle basal medium intravenously or intraperitoneally as indicated.

The kinetics of P. chabaudi adami infection were compared in sham-operated immunologically intact mice and in mice which had been splenectomized 8 days before infection. Whereas acute infections in asplenic mice persisted with significant parasitemias for a period lasting several months postinfection before resolution, acute infections in sham-operated mice cleared within 3 weeks (Fig. 1). Similar results had been reported by Oster et al. (13), except their P. chabaudi adami infections were more severe in splenectomized mice than we observed. Different mouse strains were used in the two investigations. When the same experiment was performed with B-cell-deficient mice, the results were similar in that splenectomy 8 days before infection led to persistent high-grade infections (Fig. 2). In contrast to the eventual termination of infections in splenectomized immunologically intact mice, significant parasitemias were evident in asplenic B-cell-deficient mice throughout the 115-day observation period. As reported previously, eusplenic B-cell-
FIG. 1. Effects of splenectomy on the kinetics of *P. chabaudi* adami infections in 7-week-old male (BALB/c × C57BL/10)F1 mice. Immunologically intact mice were splenectomized or sham splenectomized 8 days before intravenous infection with $10^5$ parasitized erythrocytes. Each point represents the mean parasitemia in four or five mice.
FIG. 2. Failure of splenectomized B-cell-deficient (BALB/c × C57BL/10)F₁ mice to terminate acute *P. chabaudi adami* malaria. Eight 7-week-old male B-cell-deficient mice were splenectomized and five were sham splenectomized 8 days before intravenous infection with $10^3$ parasitized erythrocytes.
I U;

POSTINFECTION AFTER SURGERY

0 4 14 24 34 44 64

DAYS

FIG. 3. Recrudescent *P. chabaudi adami* malaria after splenectomy of B-cell-deficient BALB/c mice during the postcrisis chronic phase of infection. Seven deficient mice were splenectomized and five were sham splenectomized on the day 46 after initial intravenous infection with 10^5 parasitized erythrocytes. Each point represents the mean parasitemia in five to seven mice.

deficient mice developed chronic low-grade malaria with parasitemia of 0.2% after the resolution of their acute infections (10).

To determine whether the presence of the spleen was essential for the expression of reinfection immunity or premunition, B-cell-deficient mice were infected with 10^5 *P. chabaudi adami*, allowed to resolve their acute infections, and subsequently allowed to develop chronic low-grade malarial infections (Fig. 2). At 46 days postinfection, the mice were divided into two groups. B-cell-deficient mice in one group were splenectomized; mice in the second group were subjected to surgery, but their spleens were not removed. Splenectomized B-cell-deficient mice developed recrudescent malarial infections with kinetics similar to those observed in asplenic B-cell-deficient mice (Fig. 3). While parasitemias in sham-operated B-cell-deficient mice remained below 0.2%, mean parasitemias in splenectomized B-cell-deficient mice stabilized at between 1 and 10% for the duration of the experiment. *P. chabaudi adami* infections failed to recrudesce in immunologically intact mice splenectomized 46 days postinfection, indicating that these mice had sterilized their infections by that time.

To determine whether immunity to *P. chabaudi adami* was dependent upon the presence of an architecturally intact spleen or simply required the cells present in this organ, we splenectomized immunologically intact mice 2 to 4 days before test mice were grafted with a spleen equivalent of dispersed splenocytes. Control mice were either sham operated or splenectomized but not injected with splenocytes. All mice were infected with 10^5 *P. chabaudi adami* 6 days
Fifteen- to 17-week-old immunologically intact (BALB/c × C57BL/10)F1 mice were splenectomized or sham splenectomized between days 10 and 8 before intravenous infection with 10⁵ P. chabaudi adami. Some of the splenectomized animals were reconstituted either intravenously (U) or intraperitoneally (A) with a monodispersed spleen cell equivalent 6 days before infection. Each point represents the mean parasitemia in five to seven mice. After the time of splenocyte transfer, and the course of the resulting infection was the same in splenectomized mice whether or not they had received splenocytes from normal mice (Fig. 4). These data indicate that in addition to containing essential cell types, the spleen must be architecturally intact for immunity to P. chabaudi adami infection to be expressed, thus confirming the earlier observations of Oster et al. (13) that the intact spleen is critical for the development of protective immunity against this parasite.

Together, these findings suggest that antibody-independent immunity to acute P. chabaudi adami infections as well as to reinfection with this parasite is T-cell dependent and requires the presence of an intact spleen. How the spleen functions to decrease the level of parasites in the blood remains to be determined. Allison and Eugui (3) have suggested that parasitized erythrocytes entering the red pulp of the spleen must leave arterioles in areas where macrophages are concentrated in order to enter sinuses. They propose that mononuclear cells which possess a high capacity for a respiratory burst and which are concentrated in the postarteriole region can bombard parasitized erythrocytes with O₂⁻ or other reactive molecules as they pass through this region in close apposition to these effector cells. Although such cells have not yet been identified in the spleens of malarious mice, in collaboration with Leon Weiss we have recently observed the activation of splenic reticular cells in mice infected with Plasmodium yoelii (L. Weiss and W. P. Weidanz, Annu. Meet. Am. Soc. Trop. Med. Hyg., 33rd, Baltimore, Md., abstr. no. 1984). These cells, with their characteristic morphology and increased cytoplasmic processes, form an extensive, tightly meshed cellular labyrinth which filters parasitized erythrocytes as well as other cells from the blood, including monocytes and lymphocytes. It is conceivable that these activated reticular cells or certain of the cells which they entrap may participate in the destruction of intracellular plasmodia by one or more of the anti-
body-independent killing mechanisms which have been proposed recently or by other mechanisms which have not yet been defined.

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LITERATURE CITED