INFECTION AND IMMUNITY, Feb. 1979, p. 270-275
0019-9567/79/02-0270/06$02.00/0

Vol. 23, No. 2

Splenomegaly in Murine Trypanosomiasis: T Cell-Dependent Phenomenon

JANE P. ROBINETT AND ROGER G. RANK*

Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72201

Received for publication 25 September 1978

Splenomegaly resulting from *Trypanosoma musculi* infection was found to be dependent upon a functioning T-lymphocyte system. When both humoral and cell-mediated immune systems were suppressed by treatment with cyclophosphamide 2 days after infection, splenomegaly was inhibited for about 6 days. However, when only humoral immunity was suppressed by treatment with cyclophosphamide 3 days before infection, splenomegaly still occurred. In addition, splenomegaly was absent in congenitally athymic nude mice. Nude mice and immunologically intact heterozygote mice were also sacrificed at varying times after infection, and spleens were examined histologically. A lymphocytic hyperplasia was observed in immunologically intact mice but not in nude mice. These data indicate that splenomegaly in *T. musculi* infections is T cell dependent and that splenomegaly is the result of a proliferation of B and/or T lymphocytes.

Splenomegaly has long been recognized as a characteristic feature of several protozoan diseases, including trypanosomiasis, malaria, and leishmaniasis. However, until recently there has been relatively little information pertaining to the actual mechanism of splenomegaly. Jayawardena et al. (4) and Roberts and Weidanz (7) have demonstrated that splenomegaly in murine malaria (*Plasmodium berghei yoelii*) is a T cell-dependent phenomenon. Moreover, Wyler and Gallin (12) have isolated a chemotactic factor for mononuclear cells from spleens of mice infected with *P. berghei yoelii*. They also found that this factor was T cell dependent and suggested that it may be responsible in part for the development of splenomegaly in malaria-infected animals. *Trypanosoma musculi* infections are also characterized by a marked splenomegaly which occurs concomitantly with the onset of patency, increasing about eightfold by 15 days after infection and subsiding shortly after the resolution of the infection. Although it has been reported that splenomegaly during *T. musculi* infections is largely the result of a lymphoid hyperplasia (1), little is known about whether this splenomegaly is dependent upon an intact B- and/or T-lymphocyte system. In this report we will demonstrate that splenomegaly in murine trypanosomiasis is a T cell-dependent phenomenon.

MATERIALS AND METHODS

Experimental animals. Mice utilized in the cyclophosphamide (Cy) and histopathology experiments were BALB/c Sp Crl NCTR females 6 to 8 weeks of age and were housed under conventional conditions in the university animal facility. Nude mice (nu/nu) and mice heterozygous for the nude trait (nu/+) with a BALB/c background were reared in our closed colony under separate but similar conventional conditions. Both male and female nu/nu and nu/+ mice of varying ages were included in these experiments and were distributed randomly among groups.

Experimental infection. The strain of *T. musculi* was originally obtained from the American Type Culture Collection (Rockville, Md.) and is passaged routinely in mice in our laboratory. Parasitemias were assessed by diluting heparinized blood drawn from the retroorbital plexus in saline containing 0.004% Formalin and counting the immobilized trypanosomes in a hemocytometer (11). For infection purposes, trypanosomes were suspended in phosphate-buffered saline, pH 7.4. Mice were routinely infected with 10^5 trypanosomes by the intraperitoneal route.

Assessment of splenomegaly. Immediately upon sacrifice of the mice, spleen weights were determined. Splenomegaly was expressed in terms of the relative spleen weight, which was calculated by dividing the spleen weight by the total body weight. This figure was multiplied by a factor of 100 to facilitate data presentation.

Cy administration. Mice were injected intraperitoneally either 3 days before or 2 days after infection with *T. musculi* with 300 mg of Cy (Mead-Johnson, Evansville, Ind.) per kg. The dose of Cy was routinely dissolved in phosphate-buffered saline.

RESULTS

Mice were infected with 10^5 trypanosomes, and relative spleen weights and parasitemias were determined on groups of four mice at var-

ious intervals after infection. Parasitemias became patent 3 days after infection and reached peak levels by day 11 (Fig. 1). Splenomegaly was evident 7 days after infection, and by day 15 the relative spleen weight had increased fivefold. Although the relative spleen weight decreased after day 15, it was still slightly elevated 10 days after the parasitemia had cleared. When this experiment was repeated with daily measurements, the relative spleen weight increased as early as 4 days after infection and doubled by 5 days after infection.

To determine whether splenomegaly was dependent upon an intact immune response, we treated mice with Cy 2 days after infection. It has been shown previously that acquired immunity to an antigen can be suppressed by Cy administered shortly after the antigen (9). Because we were primarily interested in whether Cy prevented or delayed the onset of splenomegaly, we restricted our observation period to the first 12 days of the infection. When mice were treated with Cy, splenomegaly was inhibited for about 6 days after infection (Fig. 2). The relative spleen weight in treated mice increased by day 9 but remained significantly less than that of the control mice. Treatment with Cy resulted in greatly increased levels of parasites during the observation period. In a separate experiment, a group of mice were treated with Cy but were not infected (data not shown). Relative spleen weights declined from 0.5 to 0.2 by 3 days after Cy treatment but increased to 0.8 by day 9 and remained at this level for as long as 21 days after treatment.

Because these data suggested that splenomegaly is dependent upon an intact immune response, we next wanted to determine the effect of selective depression of B cells on the development of splenomegaly during *T. musculi* infection. Mice were treated with Cy 3 days before infection because others have shown that this regimen will selectively depress humoral but not cell-mediated responses (8). Although the relative spleen weight of Cy-treated mice was lower than that of the untreated mice at the time of the infection, the spleens of Cy-treated mice increased in weight at the same rate as those of the controls and by 12 days after infection had surpassed the control values (Fig. 3). On the other hand, parasitemias in Cy-treated mice attained higher levels than in untreated mice by day 12. These data suggested that splenomegaly during *T. musculi* infection is a B cell-independent phenomenon.

To determine whether T cells are essential for splenomegaly to occur, we measured the relative spleen weights in groups of four nu/nu and four nu/+ mice at various intervals after infection. There was a significant difference between the relative spleen weights of nu/+ and nu/nu mice during the course of *T. musculi* infection (Fig. 4). Whereas the relative spleen weight in nu/+ mice increased fivefold by 15 days after infection, it only increased twofold in nu/nu mice. Moreover, the parasitemias in nu/nu mice continued to increase until the end of the observation period in contrast to parasitemias in nu/+ mice, which resolved by day 24.

Because splenomegaly was essentially absent in nu/nu mice, we next wanted to determine how the spleens of infected nu/nu and nu/+ mice differed histologically. Groups of three nu/nu and three nu/+ mice were sacrificed at

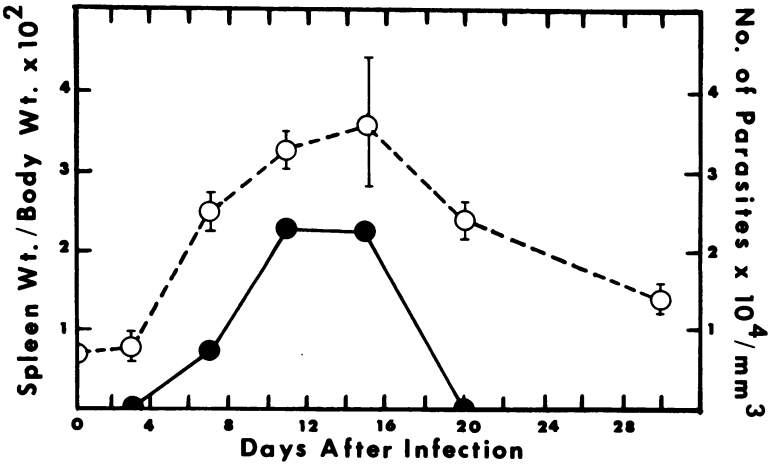

FIG. 1. *Splenomegaly resulting from infection with T. musculi. Each point is the mean of four mice. The standard deviation is indicated for the relative spleen weight. Symbols: ○, relative spleen weight; ●, parasitemia.*

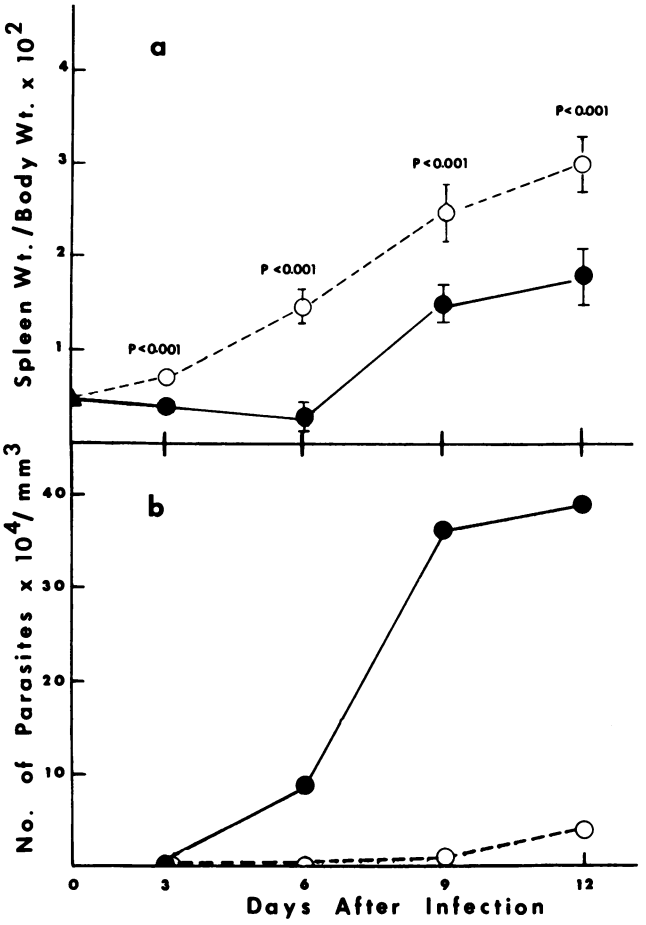

FIG. 2. *Inhibition of splenomegaly by Cy given 2 days after infection. Each point is the mean of four mice. (a) Relative spleen weights ± standard deviation. The level of significance, according to a one-tailed t test, is given for each pair of values. (b) Parasitemia. Symbols: ●, Cy-treated; ○, control.*

various intervals after infection with *T. musculi*. All spleen sections were stained with hematoxylin and eosin. At 3 days after infection, there was only slight evidence of stimulation in the spleens of nu/+ mice, with mitosis occurring in both germinal centers and the periarteriolar lymphatic sheath. Lymphocytic hyperplasia was apparent in the spleens of nu/+ mice beginning on day 6, with coalescence of lymphatic nodules observed on days 9 through 15. Lymphocytic hyperplasia was still obvious by day 30. In contrast, lymphocytic hyperplasia was not observed in the spleens of infected nu/nu mice, although there was an increase in the numbers of immature lymphoid cells in the periarteriolar lymphatic sheath. As expected, the T cell-dependent areas of the periarteriolar lymphatic sheath were depleted of lymphocytes. Increased extramedullary hematopoiesis was observed in both groups of mice beginning on day 6 and was still present on day 21 of the infection. Whereas an increase in macrophage numbers was not apparent in nu/+ mice, a slight increase in macrophage numbers was noted in the red pulp of nu/nu mice throughout the course of the infection.

DISCUSSION

In this investigation, we have demonstrated that splenomegaly resulting from infection with *T. musculi* is dependent upon the T-lymphocyte system. This was accomplished by observing the course of splenomegaly in mice with selective immune deficiencies which either were induced by Cy or were genetically determined (nu/nu).

Splenomegaly was inhibited in mice treated 2 days after infection with Cy but was not affected when mice were treated 3 days before infection. Cy has been shown to be an effective immuno-

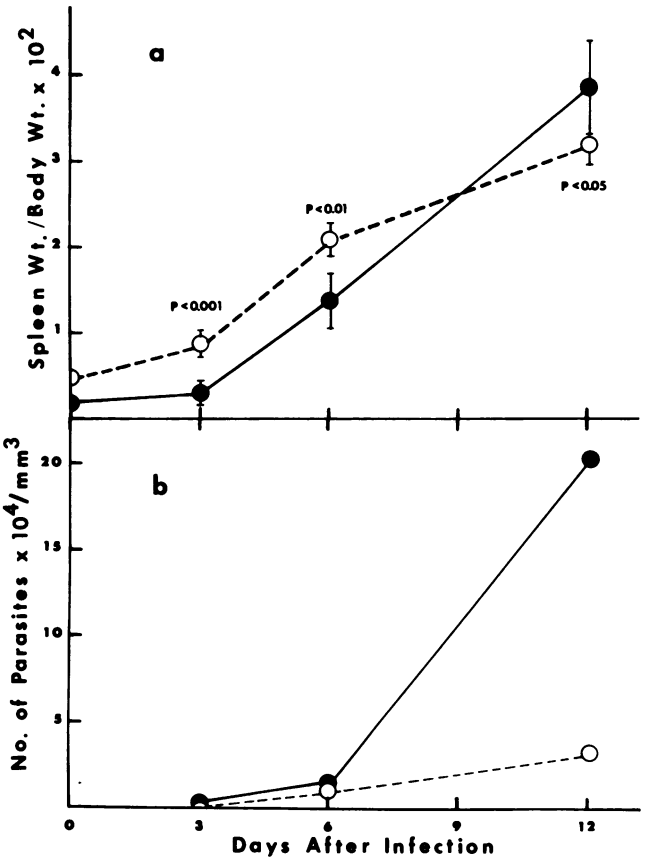

FIG. 3. *Cy treatment 3 days before infection does not inhibit splenomegaly. Each point is the mean of four mice. (a) Relative spleen weights ± standard deviation. The level of significance is given for each pair of values. (b) Parasitemia. Symbols: ●, Cy-treated; ○, control.*

suppressant of both humoral and cell-mediated immunity when administered shortly after immunization (9). However, it has also been demonstrated that when Cy is given 2 to 3 days before sensitization, B-cell and suppressor-cell function will be selectively depressed (8, 10, 13). T-cell function is also depressed but recovers by about 3 days after treatment (8). Thus, when Cy was injected into mice 2 days after infection, both humoral and cell-mediated responses to *T. musculi* were depressed. That immunosuppression occurred is evidenced by the greatly increased parasitemia. The inhibition of splenomegaly for the first 6 days of the infection could be attributed to a suppression of the immune response. It is interesting to note that splenomegaly occurred about the time that T-cell activity is reported to recover. However, recently Buhles and Shifrine (2) reported that blood monocyte levels are also depressed in mice for about 3 days after treatment with 300 mg of Cy per kg. It is possible that the depression of both splenic and circulating monocytes was responsible for the inhibition of splenomegaly. Kolb et al. (5) also observed that spleen weight is decreased for 6 days after treatment with 300 mg of Cy per kg. At 9 days, a slight splenomegaly occurred before the spleen returned to normal size at 12 days. Although we also observed this phenomenon, it is unlikely that it was a major factor in these experiments because the magnitude of this splenomegaly was not very great.

In contrast, splenomegaly was not inhibited when only B cells were depressed by Cy treatment before infection. The increased parasitemia again indicates that the Cy was indeed immunosuppressive even when administered before the infection. Because T cells and monocytes recovered shortly after Cy treatment, whereas B cells remain depressed for about 14 days, the data suggest that splenomegaly is B cell independent. Similarly, it has been reported that mice rendered B cell deficient by treatment with heterologous anti-immunoglobulin M se-

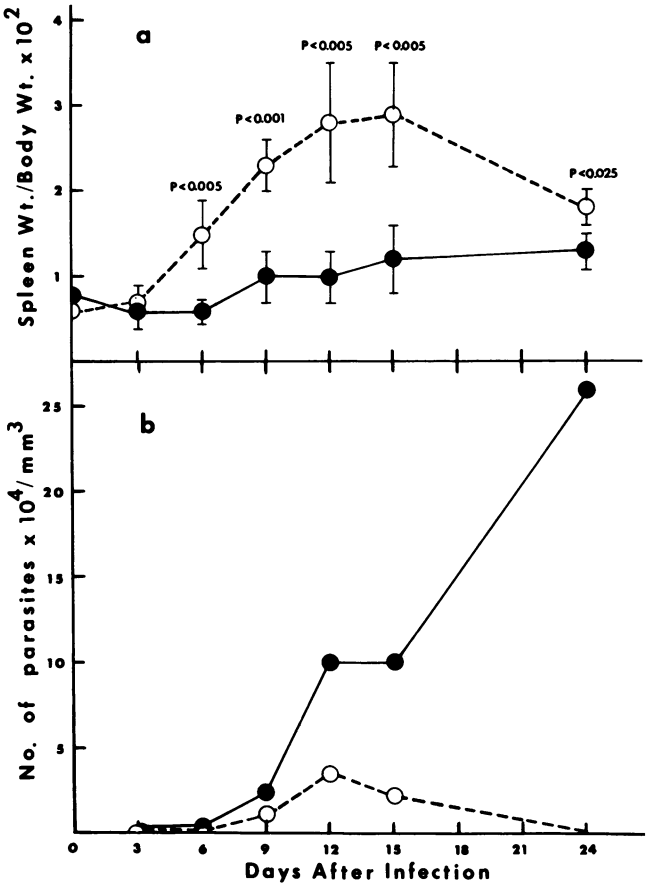

FIG. 4. *Absence of splenomegaly in nu/nu mice. Each point is the mean of four mice. (a) Relative spleen weights ± standard deviation. The level of significance is given for each pair of values. (b) Parasitemia. Symbols: ●, nu/nu; ○, nu/+.*

rum are capable of producing splenomegaly in response to malarial infection (7).

That splenomegaly is dependent upon the T-cell population was shown more definitively by the lack of splenomegaly in nu/nu mice infected with *T. musculi*. It has been shown previously that nu/nu mice are unable to resolve infections of *T. musculi* and remain infected for the lifetime of the animals (6). Thus, it appears that T cells are intimately involved in the resolution of *T. musculi* infections as well as in the development of splenomegaly. These results correspond to those of Roberts and Weidanz (7) and Jayawardena et al. (4), who reported that nude mice and T cell-deprived mice, respectively, did not develop splenomegaly upon infection with *P. berghei yoelii*.

The exact mechanism by which T cells cause splenomegaly remains open to speculation. Our observations of lymphocytic hyperplasia in nu/+ mice but not in nu/nu mice suggest that the splenomegaly is simply a result of an increase in the number of cells because of an immune response to the parasite. These observations support those of Albright et al. (1), who found that the number of T cells in the spleen increased about 10-fold whereas B cells increased only about 5-fold. The lack of splenomegaly in nude mice could thus be easily explained by the deficiency of functional T cells. B-cell proliferation would also be decreased in nude mice by the lack of helper T cells necessary for the production of T cell-dependent antibody. Although there was some evidence of lymphocyte stimulation in the spleens of nude mice, this was not unexpected because nude mice do mount a T cell-independent antibody response to *T. musculi* (6). Another possible mechanism of the splenomegaly is the recruitment of monocytes from the peripheral blood by chemotactic factors released from T cells, as has been proposed in murine malaria (12). Because the proportion of

splenic macrophages remained the same between control and infected mice, the total number of macrophages should have increased as the spleen enlarged. Thus, it is possible that the recruitment of macrophages may have contributed to the splenomegaly. Finally, splenic enlargement may, to some extent, be attributed to the increase in hemopoietic activity which was observed in both nu/+ and nu/nu mice. Jarvinen and Dalmasso (3) have reported that a slight anemia occurs during infection with *T. musculi*. The increase in hemopoietic activity is to be expected to compensate for the erythrocyte loss.

The significance of the splenomegaly is twofold. First, splenomegaly represents a massive immune response to the parasite as indicated by the lymphocytic hyperplasia. Because *T. musculi* is a blood parasite, the spleen plays a central role in the sensitization and proliferation of B and T cells and the resulting production of antibody. Splenic enlargement in this system is essentially an indication of the intensity of this response. Furthermore, splenomegaly is an indication of the T-cell response to the parasite. Second, splenomegaly provides for an increase in the number of macrophages available to eliminate parasites from the system. Whether the macrophages eliminate parasites by opsonization or are activated by T-cell lymphokines remains to be determined. Splenomegaly has also been correlated to immunosuppression to heterologous antigens (1). However, it must be emphasized that the host still responds intensely to the parasite. The immunosuppression to other antigens may be the host's means of concentrating its efforts against the massive antigen load placed on it by the parasite. The mechanism by which this occurs is extremely important in terms of host-parasite relationships and may be intimately connected to splenomegaly.

ACKNOWLEDGMENTS

This work was supported by Public Health Service Biomedical Research Support grant 5S07RR5350 from General Research Support Branch, Division of Research Resources and the Graduate Student Research Fund, University of Arkansas for Medical Sciences.

The BALB/c mice were supplied by Robert S. Speirs, National Center for Toxicological Research, Jefferson, Ark., in a cooperative study on the immunosuppressive effects of cyclophosphamide. We thank Gary Burger, Department of Pathology, University of Arkansas for Medical Sciences, for the interpretation of the histological data. We also thank Almen L. Barron, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, and Dean W. Roberts, National Center for Toxicological Research, for their advice in the preparation of this manuscript.

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