PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 317-320 (1978)

Depressed Splenic T Lymphocyte Numbers and Thymocyte Migratory Patterns in Murine Malaria (40340)

WILLIAM H. BRISSETTE,[1] ROBERT M. COLEMAN, AND NICHOLAS J. RENCRICCA

Department of Biological Sciences, University of Lowell, Lowell, Massachusetts 01854

Mice infected with the malarial parasite *Plasmodium berghei* develop a fulminating parasitemia, a concomitant severe anemia and usually succumb within several weeks (1, 2). A number of studies (3–5) have demonstrated that a significant degree of nonspecific immunosuppression is associated with malaria infection. *In vitro* studies of the T-cell mitotic response to phytohemagglutinin (PHA) have shown transient depression in mice with resolving *P. berghei yoelii* infections and permanent depression in mice with fatal *P.berghei*-infections (6). The cause of the observed nonspecific immunosuppression is unclear, although a number of possibilities have been proposed (7–10).

Alterations in lymphoid populations during infection and recovery may account, in part, for the observed changes in immune reactivity. Depressions in B- and T-cell populations in the thymus and lymph nodes of infected mice have been reported (10). The presence of adequate numbers of splenic T cells, although not measured in the previous study (10), are considered critical to the immune response in view of the role of the spleen in malaria. In this regard, alterations in the cellularity and compartmentalization of the spleen resulting from *P. berghei yoelii* infections have been observed (9). Contrasting splenic T- and B-cell populations have been reported for adult rats with resolving *P. berghei* infections and in immature young rats in which the infection was fatal (11). It is not certain, however, whether these differences between young and old rats may be attributed to an age-related altered responsiveness of T- and B-lymphocyte populations in immunologically immature and mature rats, or to some other factor.

[1] Submitted in partial fulfillment of the requirements for the M. S. degree in Biological Sciences, University of Lowell.

Different subpopulations of lymphoid cells have been shown to migrate to different sites (12). It has been shown that thymocytes, in contrast to lymph node cells, migrate predominantly to the spleen and liver. Labeled syngeneic lymphocytes subjected to heating or freeze thawing, prior to transfer, are taken up almost exclusively by the liver (13, 14). The relative increase in hepatic uptake of radioactive label has been suggested as a sensitive index of diminished cell viability (13, 14). Furthermore, administration of antilymphocytic serum directly to recipients of labeled lymphocytes caused a reduced uptake into lymphoid tissue with a striking increase in the radioactivity recovered from the liver (15).

The following study was undertaken to quantitate the absolute numbers of T lymphocytes in the spleen during the course of virulent malaria in mature mice and to determine where normal thymocytes migrate in the diseased host.

Materials and methods. Twelve to fifteen-week-old BALB/c mice (Charles River Labs) were injected intraperitoneally with 2.0×10^4 erythrocytes parasitized with *Plasmodium berghei* (NK/65 strain). At designated intervals, groups of four to six control and infected mice were monitored for circulating erythrocyte, parasitemia, and splenic T-lymphocyte levels.

Erythrocytes were counted electronically (Coulter Electronics, Inc.), and the percentage parasitemias scored from blood smears stained with Giemsa. Monocellular suspensions of dispersed spleen cells were prepared in TC medium 199 (Difco Laboratories, Inc.) containing 5% fetal calf serum, by sequential passage through 19- to 23-gauge needles (16). Counts of nucleated cells were performed by hemocytometer following red cell lysis with 3% acetic acid; total numbers of lymphocytes/spleen were determined from differen-

0037-9727/78/1592-0317$01.00/0

tial smears. Lymphocytes were harvested by layering 10-ml suspensions (representing 1 spleen) on 3 ml of Ficoll/Isopaque (Litton Bionetics, Inc.) and centrifuging at 900*g* for 30 min at 20° (17). Interface cells, containing 80 to 90% lymphocytes were washed and labeled with $Na^{51}CrO_4$ (50μCi) (New England Nuclear). Lymphocytes were washed several times, enumerated by hemocytometer and diluted to 2.0×10^6 cells/ml. Mouse anti-Thy-1.2 serum (AKR, Litton Bionetics, Inc.) was employed at a dilution of 1:4 and rabbit anti-mouse lymphocytic serum (Microbiological Assoc.) at a dilution of 1:8. Serum from syngeneic donors served as the control (1:8 dilution). Guinea pig complement (Cappel Labs.) was absorbed with mouse liver and spleen cells and diluted 1:4.

The numbers of θ-bearing cells were determined by a ^{51}Cr release cytotoxic assay (18). In the assay, 0.1 ml of diluted serum (normal, anti-θ, or antilymphocyte), and 0.1 ml of ^{51}Cr-labeled splenic lymphocytes (2×10^5 cells) from normal or infected mice were incubated in duplicate 3-ml tubes at 4° for 10 min. Following the addition of 0.1 ml of complement, tubes were reincubated for 45 min at 37° in a 7% CO_2 atmosphere. Thereafter, 0.5 ml of cold TC medium was added to each tube and following centrifugation, the supernatant material was assayed in a Bio-Gamma scintillation counter (Beckman Instruments). The percentage θ-bearing splenic lymphocytes were determined in the conventional manner.

The efficiency of normal thymocytes to seed the spleen of control and infected mice was determined as follows: Normal thymus suspensions were washed with 0.83% NH_4Cl–Tris buffer (19) and labeled by incubation with $Na^{51}CrO_4$ (200 μCi). Following several washings, suspensions were examined for viability with trypan blue and diluted to contain 1×10^8 cells/ml with a viability of at least 90%. At designated times during infection, groups of control and parasitized mice received an intravenous inoculum of 2×10^7 cells. Twenty-four hours later, splenic ^{51}Cr was determined and expressed as a percentage of injected standard. T-cell splenic seeding was determined in additional mice receiving 5×10^5, 5×10^6, or 2×10^7 ^{51}Cr-labeled thymocytes on Day 10 of infection and assayed 24 hr later.

All data reported herein is expressed as the group mean ±1 SE. Based on Student's *t* test, $P < 0.05$ was considered to be a significant difference.

Results. Plasmodium berghei infection in mice is characterized by a progressive parasitemia and concomitant anemia (Fig. 1) and is fatal within several weeks. During the course of infection, the numbers of nucleated splenic cells steadily increased severalfold (Fig. 2A), which easily accounted for the elevation in numbers of total lymphocytes (Fig. 2C). In distinction, the percentage (Fig. 2D) and absolute numbers (Fig. 2E) of splenic θ^+ cells (T lymphocytes) steadily declined to approximately 20% of control levels ($P < 0.001$) by Day 20. The efficiency of transplanted normal ^{51}Cr-labeled thymus cells to seed into the infected spleen was significantly ($P < 0.001$) reduced to approximately 45% of control levels by Day 5 and gradually declined further to 30% of control values on Day 20 (Fig. 3). The seeding efficiency was relatively constant over an inoculum range of 5×10^5 to 2×10^7 thymus cells, when transplanted on Day 10 of infection.

Discussion. Plasmodium berghei-infected mice succumb to the effects of high parasit-

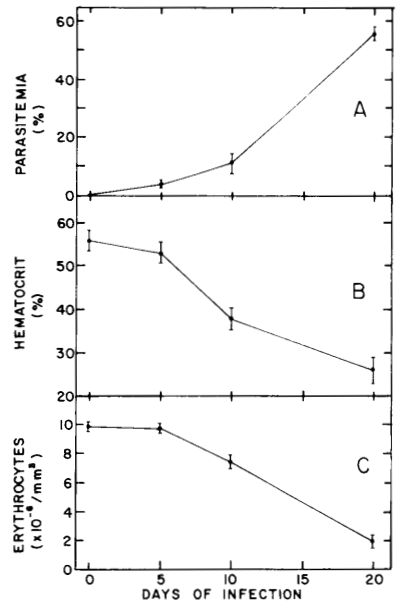

FIG. 1. Course of parasitemia and anemia following intraperitoneal injection of 2×10^4 *P. berghei*-infected erythrocytes.

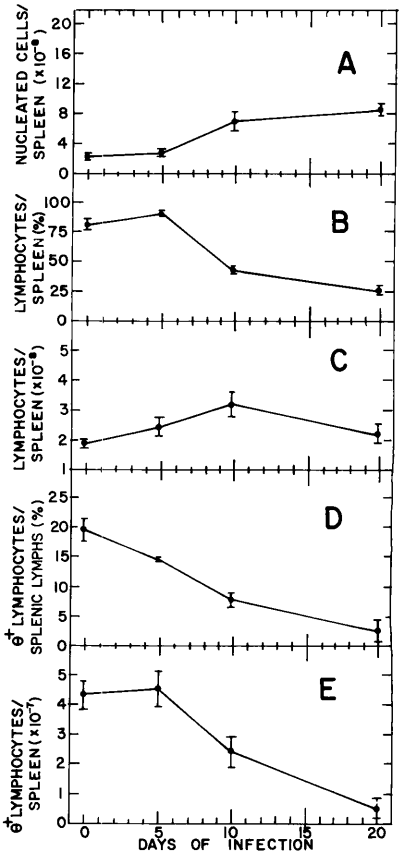

FIG. 2. Numbers of splenic nucleated cells, total lymphocytes and T lymphocytes following the intraperitoneal injection of 2×10^4 erythrocytes parasitized with *P. berghei*.

emia and severe anemia by the third week. In response to the progressive anemia (Fig. 1), an increase in erythroid precursors (20) and macrophages (21) may have accounted in part for the elevation in numbers of splenic nucleated cells in parasitized mice (Fig. 2A). The proportion of lymphocytes to total nucleated cells decreased dramatically to approximately 50% of normal on Day 10 (Fig. 2B). Nevertheless, the lymphocyte population contributed to the increased nucleated cellularity as reflected by an almost twofold rise in absolute numbers on Day 10 (Fig. 2C). In contrast to the lymphocyte population as a whole, the absolute numbers of T cells decreased on Day 10 of the infection (Fig. 2E) and the proportion of lymphocytes bearing θ antigen was depressed to about 40% of normal controls on this day (Fig. 2D). These results compare favorably with depressions in percentage of T cells observed in the spleens of *P. berghei*-infected young rats which succumb with high parasitemia and anemia similar to mice (11). It is possible, however, that in the latter study the absolute numbers of splenic T cells may not have been depressed, since spleen weights showed a greater than sixfold increase during the course of the infection. The decline in numbers of T cells in the spleen associated with the reported involution and depressed T-cell populations of the thymus and lymph nodes (10) suggest a general reduction in the entire pool. The observed progressive depression in T-cell numbers as well as the reported decrease in the volume of thymus-dependent areas of the spleen (9) could reflect a decline in available space as a result of the expanded erythropoietic activity (20) or could result from an overall decrease in numbers of thymus-derived cells seeding the spleen. Furthermore, adverse environmental effects in the diseased host may affect the ability of the spleen to accept T-cells or may affect the viability and/or survival of T lymphocyte populations. The thymocyte seeding study was initiated to examine this question.

The distribution patterns for thymocytes at 24 hr in normal BALB/c mice were similar to those reported for CBA mice (14). The percentage of ^{51}Cr-labeled thymocytes entering the spleen markedly decreased during the course of the infection (Fig. 3). At the same time increasingly higher uptake of radioactivity was observed in the liver. Our findings that proportional distribution of thymocytes remained unchanged for the dose range (5×10^5 to 2×10^7) in both normal (21%) and infected (8%) spleens argues against possible decreased available T-cell sites in the infected

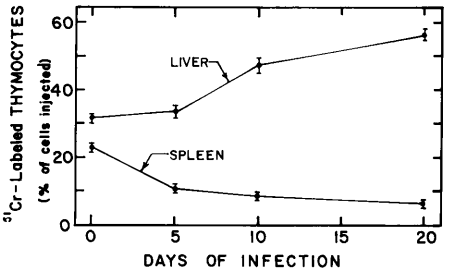

FIG. 3. Percentage of radiolabelled thymocytes in liver and spleen of infected hosts 24 hr after the intravenous transplantation of 2×10^7 cells.

spleen, at least at these inoculum levels. Others have shown that labeled lymph node cells in normal mice do not differ in distribution characteristics at 24 hr following intravenous injection using a four log dose range (10^5 to 10^8 cells) (12). Treatment of aliquots of labeled lymphocyte *in vitro* with cytotoxic materials including anti-lymphocyte serum and thymocytotoxic autoantibody (NZB mice) results in a dramatic increase in the uptake of radioactive label by the liver of recipients suggesting loss of cell viability (13–15, 22, 23).

Administration of anti-lymphocytes serum directly to recipients of labeled lymphocytes also caused increased liver uptake (15). Shirai *et al.* (22) state that the increase of the liver-localizing population after treatment of lymphocyte with thymocytotoxic autoantibody is consistent with the suggestions that T-cell depletion with aging of NZB mice is mediated by a continuous process of autosensitization which causes phagocytosis. It would appear possible that the viability of the thymocytes we inoculated into infected mice was adversely affected by the parasitized host environment.

Summary. The results obtained in this study show that a progressive depression in the splenic T-cell population occurs in *P. berghei*-infected mice and that T-cell migration is abnormal also. Since the thymus and lymph nodes involute in *P. berghei*-infected mice (10), it is likely that the total T-cell pool is depleted in the infected mouse. The decreased ability of transplanted thymus cells to seed into the infected spleen and the decreased T-cell population may indicate that infected mice have an environment hostile to T-cell viability.

1. Mercado, T. I., and Coatney, R. G., J. Parasitol. **37,** 479 (1951).
2. Hejna, J. M., Rencricca, N. J., and Coleman, R. M., Proc. Soc. Exp. Biol. Med. **146,** 462 (1974).
3. Greenwood, B. H., Brown, J. C., DeJesus, D. G., and Holborow, E. J., Clin. Exp. Immunol. **9,** 345 (1971).
4. Warren, H. S., and Weidanz, W. P., Eur. J. Immunol. **6,** 816 (1976).
5. Voller, A., Gall, D., and Manawadu, B. R., Z. Tropenmed. Parasitol. **23,** 152 (1972).
6. Jayawardena, A., Targett, G., Leuchars, E., Carter, R., Doenhoff, M., and Davies, A., Nature (London) **258,** 149 (1975).
7. McGregor, I. A., Turner, M. W., Williams, K., and Hall, P., Lancet **1,** 881 (1958).
8. Soni, J. L., and Cox, H. W., J. Trop. Med. Hyg. **23,** 577 (1974).
9. Moran, C. J., DeRivera, V. S., and Turk, J. L., Clin. Exp. Immunol. **13,** 457 (1973).
10. Krettli, A. V., and Nussenzweig, R., Cell. Immunol. **13,** 440 (1974).
11. Gravely, S. M., Hamburger, J., and Kreier, J. P., Infect. Immunol. **14,** 178 (1976).
12. Lance, E., and Taub, R., Nature (London) **221,** 841 (1969).
13. Bainbridge, D., Brent, L., and Gowland, G., Transplantation **4,** 138 (1966).
14. Zatz, M., and Lance, E., Cell. Immunology **1,** 3 (1970).
15. Taub, R., and Lance, E., Immunology **15,** 633 (1968).
16. Coleman, R. M., Rencricca, N. J., Stout, J. P., Brissette, W. H., and Smith, D. M., Immunology **29,** 49 (1975).
17. Parish, C. R., Transplant. Rev. **25,** 98 (1975).
18. Stobo, J. D., Rosenthal, A. S., and Paul, W. E., J. Exp. Med. **138,** 71 (1973).
19. Boyle, W., Transplantation **6,** 761 (1968).
20. Singer, I., J. Infect. Dis. **94,** 241 (1954).
21. Roberts, D. W., and Weidanz, W. P., Infect. Immunol. **20,** 728 (1978).
22. Shirai, T., Yoshiki, T., and Mellors, R., J. Immunol. **110,** 517 (1973).
23. Sprent, J., *in* "The Lymphocyte: Structure and Function" (J. J. Marchalonis, ed.). Marcel Dekker, New York (1977).

Received June 12, 1978. P.S.E.B.M. 1978, Vol. 159.