INFECTION AND IMMUNITY, June 1986, p. 637–643
0019-9567/86/060637-07$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 52, No. 3

T-Cell Immunity in Murine Malaria: Adoptive Transfer of Resistance to *Plasmodium chabaudi adami* in Nude Mice with Splenic T Cells

LISA A. CAVACINI, CAROLE A. LONG, AND WILLIAM P. WEIDANZ*

Malaria Research Group, Department of Microbiology and Immunology, Hahnemann University, Philadelphia, Pennsylvania 19102

Received 11 December 1985/Accepted 19 February 1986

Acute infections caused by the murine malarial parasite *Plasmodium chabaudi adami* are resolved by antibody-independent mechanisms of immunity. The fact that athymic nude mice developed high-grade unrelenting malaria and died when infected with this parasite suggested a significant role for T lymphocytes. Using adoptive transfer techniques, we demonstrated that spleen cells from either nonimmune or immune donor BALB/c mice eventually suppressed *P. chabaudi adami* infections in histocompatible recipient nude mice in a dose-dependent manner. Infections in recipients of "immune" spleen cells were less severe, demonstrating a depressed peak parasitemia and a shortened duration of patent infection, than was observed in recipients of normal spleen cells. Also, when sufficient numbers of immune spleen cells were transferred, the second wave of parasitemia (characteristic of this infection in nonimmune mice) failed to occur. T lymphocytes mediated protection in recipient mice, since T-cell-enriched, but not B-cell-enriched, spleen cell fractions suppressed *P. chabaudi adami* infections in nude mice. Protection was best achieved with T cells that bore the L3T4 phenotype. Patent parasitemias developed in all recipient mice, suggesting that the grafted cells did not limit parasite growth directly but achieved this end by activating other as yet unidentified inhibiting cell systems.

Acquired immunity to clinical malaria requires the activation of immune mechanisms sufficient to limit or inhibit the growth of asexual blood-stage plasmodia (9, 10). While the exact nature of the underlying mechanisms remains to be determined, many researchers agree that antibodies play a major role. This opinion stems from the observation that protection against certain malarial species has been achieved by the passive transfer of hyperimmune serum (5, 11, 13) as well as by T-dependent isotypes of polyclonal (33) or monoclonal antibodies specific for merozoite antigens (19, 26). Also, B-cell-deficient hosts often die when infected with ordinarily avirulent parasites (28, 30, 35, 36). Even so, no single measurement of antibody activity has correlated unfailingly with resistance, and several reports show that immunity to human as well as to experimental malaria can be achieved by nonantibody mechanism(s) (14, 23, 28). The injection of agents to induce oxidant stress suppressed parasitemias in infected mice (1, 3, 7, 8, 27). Moreover, plasmodia were killed or inhibited by several cytokines such as tumor necrosis factor (17, 31, 32) and crisis-forming factor (23, 24). Together, these findings suggest a possible role for cell-mediated immunity in malaria. For the most part, this possibility has stimulated little interest, as most current efforts to develop malaria vaccines continue to focus on protective antibody responses induced by parasite antigens.

Previously, we described a novel murine malaria model in which the resolution of acute infection was achieved by antibody-independent means requiring an intact T lymphocyte system (14). Thus, B-cell-deficient as well as immunologically intact mice resolved acute *Plasmodium chabaudi adami* infections with similar kinetics, whereas athymic nude mice developed high-grade chronic malaria and eventually died. The expression of this immunity was dependent upon the presence of an architecturally intact spleen (15), was suppressed by treatment with anti-thymocyte serum (J. L. Grun, Ph.D. dissertation, Hahnemann University, Philadelphia, 1982), and could not be achieved by the passive transfer of hyperimmune serum prepared against *P. chabaudi adami* (D. Russo, personal communication). Resistance to this parasite by B-cell-deficient mice displayed a memory component and specificity, the hallmarks of classical acquired immunity, and appeared to be effected by some as yet undefined functions of cell-mediated immunity (16). It seemed reasonable to us that this experimental model could prove useful in delineating the role of cell-mediated immunity in malaria and eventually in defining the mechanisms involved. Using adoptive transfer procedures, we began to characterize lymphocyte populations responsible for the induction and expression of immunity to *P. chabaudi adami* in mice.

MATERIALS AND METHODS

Mice. Histocompatible *nu/nu* and *nu/+* BALB/c mice of both sexes ranging in age from 10 to 20 weeks were obtained from Norman Reed and Walter Frost, Montana State University, Bozeman. Age- and sex-matched animals were used in all experiments.

Malarial parasites. *P. chabaudi adami* 556KA and *Plasmodium yoelii* 17X strains were originally obtained from David Wyler and John Finerty, respectively, both at the National Institutes of Health. Parasites were cloned as described previously by Hoffmann et al. (18) and maintained as frozen stabilates in the vapor phase of a liquid nitrogen freezer. Parasite material was tested for, and found to be free of, lactate dehydrogenase elevating agent and mouse hepatitis virus. All experimental infections were initiated with parasitized erythrocytes obtained from donor mice infected with stabilate material. Resulting parasitemias were estimated by enumerating parasitized erythrocytes on Giemsa-stained films of tail blood, as described previously (18).

Immunization of donor mice against *P. chabaudi adami*. BALB/c *nu/+* mice were infected intraperitoneally with 10^6

* Corresponding author.

erythrocytes parasitized with *P. chabaudi adami*. Six to eight weeks after the initial infection, at a time when parasites were no longer detected in the blood, the animals were again injected intraperitoneally with 10^6 parasitized erythrocytes. The resulting low-grade parasitemias were suppressed to subpatent levels within 7 to 10 days, at which time the spleens were removed and dissociated into single-cell suspensions in RPMI 1640 medium, before use in adoptive transfer studies. Spleen cell suspensions from nonimmune *nu*/+ mice were prepared in an identical manner.

Fractionation of splenic lymphocytes. Spleen cells from immunized and nonimmune *nu*/+ mice were enriched for T cells by passage through nylon wool columns as described by Julius et al. (25). Prior to passage, spleen cells were depleted of erythrocytes by hypotonic shock. Approximately 75% of the effluent cells were T lymphocytes, as determined by immunofluorescence, using a fluorescein-labeled monoclonal antibody specific for the Thy-1.2 alloantigen (New England Nuclear Corp., Boston, Mass.). B-cell-enriched populations of spleen cells were recovered from nylon wool columns with the method described by Trizio and Cudkowicz (34). The B-cell-enriched population was further depleted of T cells by treatment with anti-Thy-1.2 antiserum (New England Nuclear) and selected guinea pig complement (GIBCO Laboratories, Grand Island, N.Y.). The B-cell-enriched population lacked T cells, as determined by immunofluorescence. Approximately 79% of these cells displayed membrane-bound immunoglobulin.

Preparation of Lyt T-cell subsets. Nylon-wool-enriched splenic T cells (10^8) were treated with anti-Lyt-2.2 antiserum diluted 1:100 in phosphate-buffered saline (New England Nuclear) for 60 min at 4°C. This procedure was followed by treatment with low-toxin rabbit complement (Accurate Scientific, Hicksville, N.Y.) for 60 min at 37°C. Prior to use, the rabbit complement (diluted 1:7 in phosphate-buffered saline) was adsorbed for 1 to 2 h at 4°C with normal mouse spleen cells to a final concentration of 5%.

Splenic T cells were enriched for Lyt-2^+ cells by treatment with monoclonal antibody GK1.5, in the form of ascites fluid, kindly provided by T. M. Pellas, University of Pennsylvania, Philadelphia. Nylon-wool-enriched T cells (10^8) were incubated with GK1.5 (final dilution, 1:200 in phosphate-buffered saline) for 30 min on ice, followed by incubation with absorbed low-tox rabbit complement for 45 min at 37°C.

Adoptive-cell transfer. Histocompatible *nu*/*nu* recipient mice, three to five animals per group, were injected intravenously with known numbers of viable splenic lymphocytes. Within 2 h, all recipient mice, as well as the uninjected *nu*/*nu* control mice, were infected intraperitoneally with 10^5 parasitized erythrocytes.

Preparation of hyperimmune serum. Successfully reconstituted nude mice, i.e., animals that had cleared their *P. chabaudi adami* infections, were challenged three times with 10^6 *P. chabaudi adami*-parasitized erythrocytes on days 42, 82, and 102 postinfection. Eight days after the final challenge, serum was harvested and pooled before use in passive protection tests.

RESULTS

Adoptive transfer of immunity to *P. chabaudi adami*. Spleen cells from immunized euthymic *nu*/+ mice were compared with spleen cells from nonimmune mice for their ability to protect recipient athymic *nu*/*nu* mice against infection with *P. chabaudi adami*. Groups of nude mice received various numbers of spleen cells ranging from 10^5 to 10^7 cells. Nongrafted nude mice served as controls. All mice were challenged with 10^5 *P. chabaudi adami*-parasitized erythrocytes.

As shown in Fig. 1A, control nude mice which had not received spleen cells developed acute malarial infections characterized by rapidly ascending parasitemias, which reached an initial peak value of approximately 30%. Subsequently, parasitemias increased gradually, and the animals displayed high-grade malarial infections until they died. Recipients of normal spleen cells, regardless of dose, developed ascending parasitemia curves that had slopes similar to those of nongrafted control mice (Fig. 1A). However, in marked contrast to control mice, recipients of normal spleen cells subsequently suppressed their infections in a dose-dependent fashion; i.e., the duration of infection was inversely proportional to the number of spleen cells injected (Fig. 1A), with a minimum of 10^6 spleen cells necessary for uniform protection. Two of four recipients of 10^5 normal spleen cells eventually resolved their acute infections on days 45 and 63, respectively. Although a second wave of parasitemia was typically seen in all mice after the resolution of their acute malaria, the time when it occurred in individual hosts showed considerable variation (data not shown).

Spleen cells from immunized *nu*/+ mice also transferred immunity to *nu*/*nu* mice. Once again, grafted mice developed rapidly ascending parasitemias with slopes resembling those seen in control mice. Parasitemias were then suppressed in a dose-dependent manner similar to that observed in recipients of nonimmune spleen cells (Fig. 1B). However, the duration of acute malaria in recipients of immune spleen cells was shortened in comparison with that of recipients of nonimmune spleen cells. This was especially evident in the recipients of 10^7 spleen cells; resolution of acute disease occurred 2 weeks earlier in these mice than it did in recipients of nonimmune spleen cells. Also, it should be noted that a second wave of parasitemia was not seen in these mice, but did occur in two of three mice that had received 10^6 immune spleen cells. What appear to be second and third peaks of parasitemia displayed by recipients of 10^6 immune spleen cells in Fig. 1B are in fact part of a second wave of parasitemia that did not occur synchronously in all animals. Although not evident in the parasitemia curves shown in Fig. 1B, three out of four recipients of 10^5 spleen cells suppressed their acute infections to subpatent levels within 45 to 55 days and subsequently developed second waves of parasitemia.

Adoptive transfer of immunity to *P. chabaudi adami* with fractionated splenic lymphocytes. Having determined that the adoptive transfer of spleen cells, whether from nonimmune or immune donors, to nude mice conferred protection upon the recipients, we separated spleen cells into T- and B-cell-enriched populations, as described above.

BALB/c *nu*/*nu* animals were injected intravenously with either 7×10^6 normal spleen cells enriched for T cells, or 7×10^6 normal spleen cells enriched for B cells. Control *nu*/*nu* mice did not receive cells. On the same day, all animals were infected intraperitoneally with 10^5 *P. chabaudi adami*-parasitized erythrocytes. Only those animals receiving T-cell-enriched normal spleen cells were able to control their infection (Fig. 2A). They did so within 36 days and thus resembled animals receiving a similar dose of unselected normal spleen cells (Fig. 1A). Recipients of normal spleen cells enriched for B cells failed to suppress their infections, which resembled those seen in nongrafted control mice. Subsequently, the successfully reconstituted animals re-

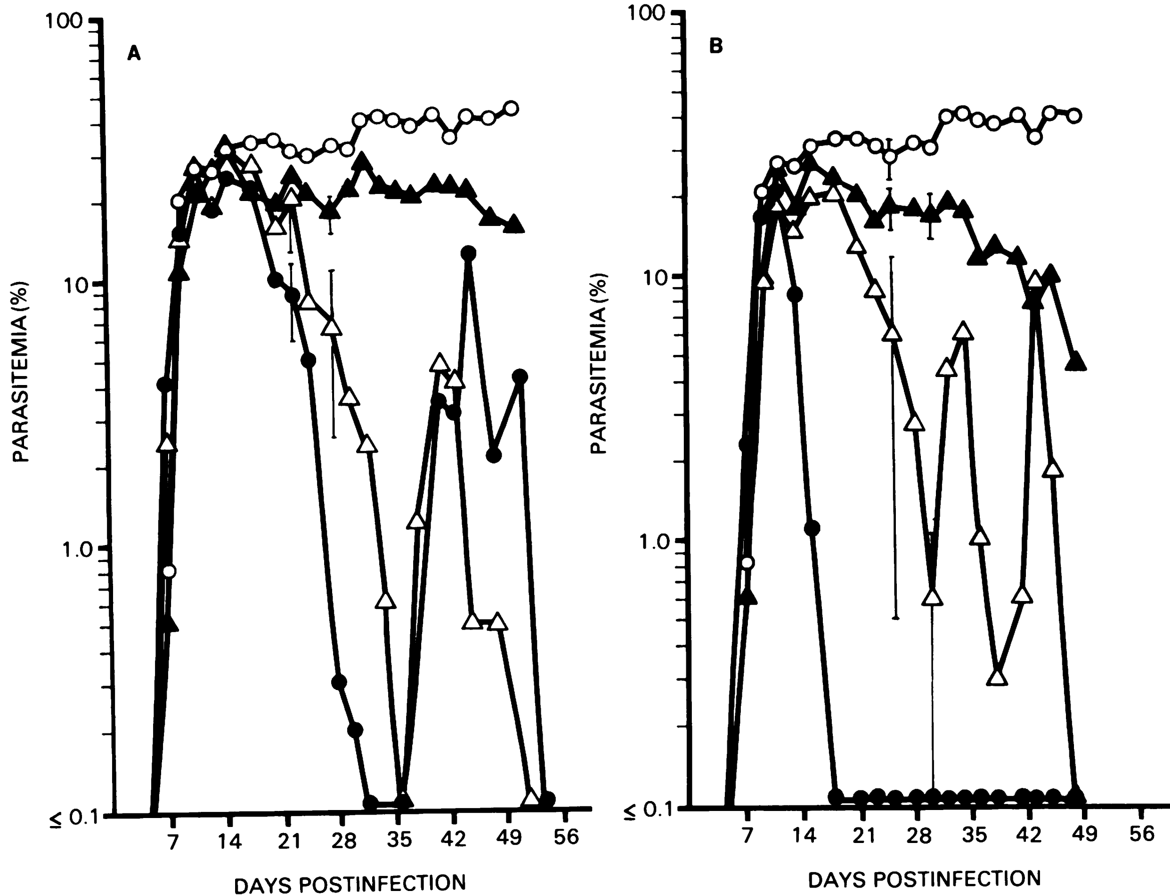

FIG. 1. Protection against *P. chabaudi adami* infection in nude mice reconstituted with spleen cells from nonimmune (A) and immunized (B) *nu*/+ congenic mice. Recipient mice (four per group) received 10^7 (●), 10^6 (△), or 10^5 (▲) spleen cells. Control nude mice were left untreated (○). All mice were challenged intraperitoneally with 10^5 *P. chabaudi adami* on the day of transfer. D, Death. In this and subsequent figures, the points on the graphs represent the mean parasitemia for each experimental group plus or minus one standard deviation from the mean.

sisted challenge infections with homologous parasite administered on three separate occasions (days 42, 82, and 102) (data not shown).

Similar results were seen when spleen cells from immune donors were fractionated into T- and B-cell-enriched populations. In this experiment, either 10^7 immune spleen cells enriched for T cells or 10^7 immune spleen cells enriched for B cells were injected intravenously. On the same day, recipient animals as well as nongrafted control *nu*/*nu* mice were infected with 10^5 *P. chabaudi adami*-parasitized erythrocytes. Parasitemias in recipients of immune T cells peaked at day 10 at levels less than 12% and were resolved by day 17 (Fig. 2B). On the other hand, B-cell recipients developed high-grade malaria similar to infections seen in untreated control mice and eventually died.

Enhanced suppression of *P. chabaudi adami* infection by reconstitution with L3T4$^+$ T cells. Nylon-wool-enriched T cells derived from immunized euthymic mice were further separated according to their Lyt phenotype by selective lysis with monoclonal antibodies specific for the L3T4 or Lyt-2 T-cell antigens and complement. BALB/c *nu*/*nu* mice received 10^7 unselected T cells, 10^7 L3T4 enriched T cells, or 10^7 Lyt-2$^+$ selected T cells. On the same day, *P. chabaudi adami* infection was initiated in these mice, as well as in untreated control mice, by the intraperitoneal injection of 10^5 parasitized erythrocytes. As shown in Fig. 3, recipients of both enriched T-cell populations resolved their acute infections; however, the recipients of the L3T4 enriched T cells resolved their infections 10 days earlier than mice that received Lyt-2$^+$ enriched T cells. The infection profile seen in mice given unselected T cells was intermediate between the groups given selected T-cell populations (data not shown). Untreated control mice developed typical chronic high-grade malarial infections and died. We cannot explain why a second wave of parasitemia was seen in the recipients of the L3T4 or Lyt-2$^+$ fractions of donor spleen cells; nevertheless, a second peak of parasitemia did occur in animals injected with 10^7 unselected T cells.

On day 105 postinfection, approximately 20% of the splenic T lymphocytes of recipients of L3T4 enriched T cells bore the Lyt-2$^+$ phenotype, whereas approximately 70% of the splenic lymphocytes of the recipients of Lyt-2$^+$ enriched T cells displayed this marker.

Passive transfer of hyperimmune serum. To determine whether the sera of reconstituted nude mice could protect nonimmune mice against infection with *P. chabaudi adami*, naive BALB/c *nu*/+ mice were treated intravenously with 0.5 ml of hyperimmune serum prepared as described above.

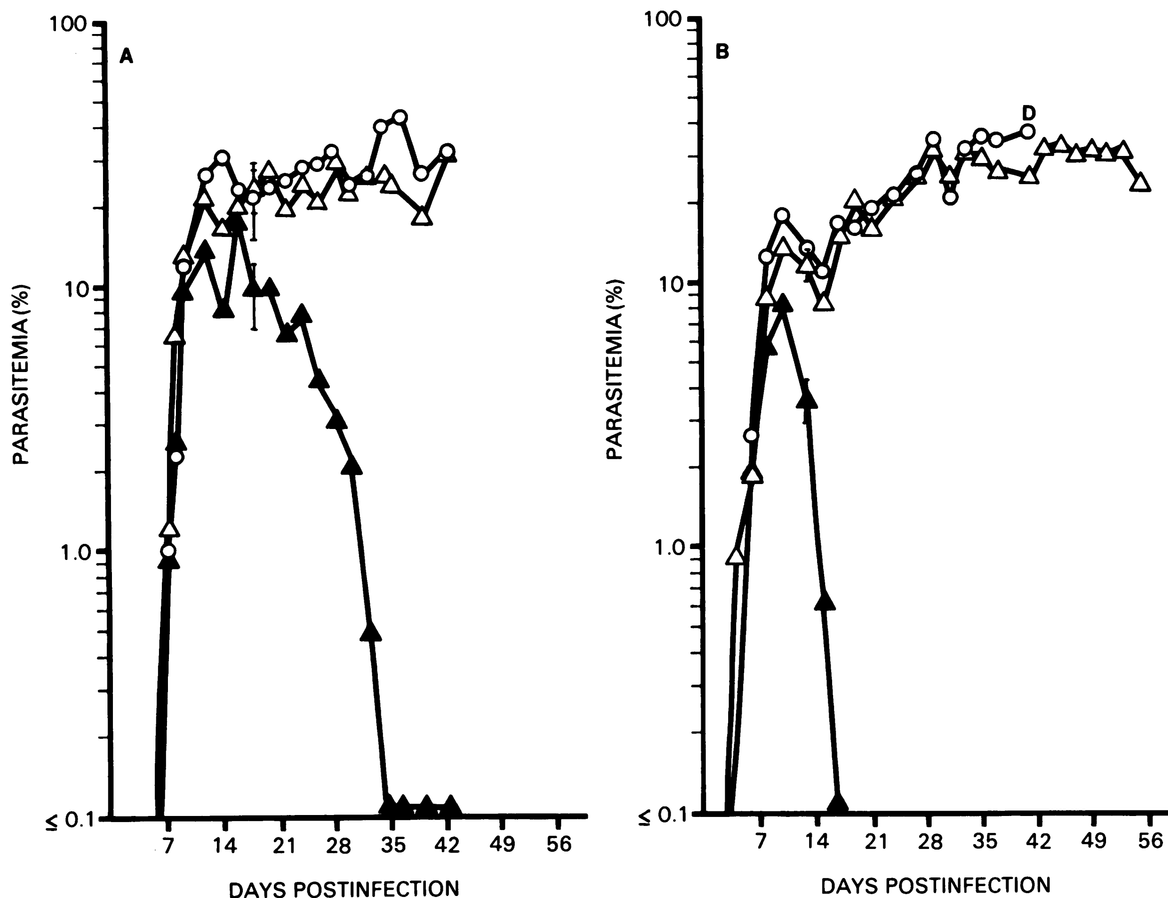

FIG. 2. Adoptive transfer of immunity to *P. chabaudi adami* with lymphocytes from normal (A) and immune (B) mice. Nude mice in panel A were reconstituted with 7×10^6 T-cell-enriched spleen cells (▲), with 7×10^6 B-cell-enriched spleen cells (△) from nonimmune *nu*/+ congenic mice, or were left untreated (○). Recipient nude mice in panel B received 10^7 T-cell-enriched spleen cells (▲), 10^7 B-cell-enriched spleen cells (△) from immune donor mice, or were left untreated (○). All mice were challenged intraperitoneally with 10^5 *P. chabaudi adami* on the day of cell transfer. Four animals were used per group. D, Death.

On the same day, these mice, as well as untreated control BALB/c *nu*/+ mice, were infected intravenously with 10^3 *P. chabaudi adami*-parasitized erythrocytes. Recipients of hyperimmune serum developed acute malaria with infection kinetics identical to that observed in untreated control mice (Fig. 4).

DISCUSSION

Previous attempts to study cell-mediated immunity in malaria have been complicated by the use of model systems in which antibodies are known to play an essential role in resolving acute infection. For example, the passive administration of hyperimmune serum or monoclonal antibodies protected mice against acute infection with *P. yoelii* (20), and B-cell-deficient mice died when infected with the 17X strain of this parasite (29, 36). Thus, whereas Jayawardena et al. (21) reported that T cells bearing the Lyt-1^+2^- phenotype mediated resistance to *P. yoelii*, a finding recently confirmed by Brinkmann et al. (4), it is quite possible that they were measuring the helper role of T lymphocytes in this antibody-dependent model system.

Preliminary to elucidating the mechanisms that mediate immunity to *P. chabaudi adami* and identifying the plasmodial antigens involved, we used adoptive transfer procedures to identify the lymphoid cells that participate in antibody-independent immunity to the parasite. Initial studies showed that spleen cells from both immune and nonimmune donor mice were capable of protecting recipient nude mice from otherwise lethal *P. chabaudi adami* infections. In no instance did the adoptive transfer of spleen cells, regardless of source, suppress the ascending parasitemia which occurred in the early stage of disease. Instead, protection was indicated by the subsequent suppression of parasitemia and host survival. Protection occurred in a dose-dependent manner and was best effected by spleen cells derived from immunized donors, as indicated by a shortened duration of infection and the suppression of a second wave of parasitemia characteristically seen in immunologically intact mice infected with *P. chabaudi adami*. Reconstituted nude mice that had resolved their infections developed low-grade, short-lived parasitemias when challenged on day 42 with homologous parasites, indicating that their developing resistance to malaria had a memory component. Such animals were fully resistant when challenged a second time on day 82 postinfection. The fact that the transferred lymphoid cells, despite their origin and numbers, failed to alter the initial course of infection indicated that such grafts do not contain effector cells in sufficient numbers to directly kill parasites or suppress their replication but rather function indirectly by activating additional cell systems which accomplish this

mission. When nude mice that were immune to *P. chabaudi* were challenged with a heterologous murine malarial parasite, *P. yoelii* 17X, they developed significant parasitemias; and in fact five of nine animals reconstituted with less than 10^7 spleen cells died (data not shown), suggesting that immunity to *P. chabaudi adami* possessed T-cell-mediated specificity.

When T cells were compared with B cells for their ability to adoptively transfer protection to nude mice, T cells from immune or nonimmune donors protected recipient mice from otherwise lethal *P. chabaudi adami* infections. B-cell-enriched lymphocyte fractions, even when obtained from immune mice, failed to suppress *P. chabaudi adami* infections, which eventually killed the recipient mice. These results contrast with those of other researchers who have studied the *P. yoelii* model infection and have observed that the transfer of immune B cells protected recipients against challenge infection with this parasite (21, 26). Immunity to *P. chabaudi adami* was best achieved with T cells bearing the L3T4 marker indicative of the T helper/inducer subset (12). That Lyt-2^+ enriched spleen cells also protected recipient mice from infection could in part be explained by our inability to totally delete L3T4 cells from the Lyt-2^+ enriched fraction.

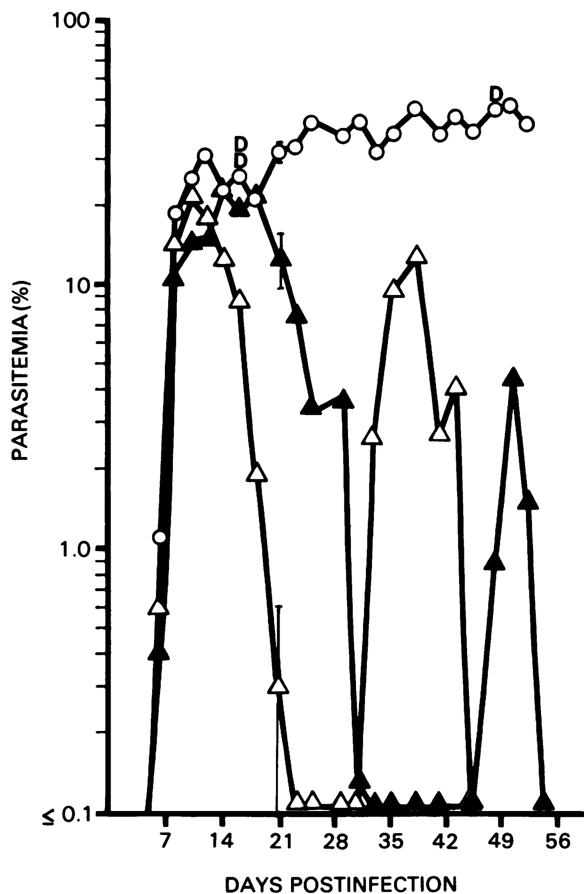

FIG. 3. Enhanced suppression of *P. chabaudi adami* infection in nude mice reconstituted with L3T4 cells from immune *nu*/+ congenic donor mice. Recipient nude mice received 10^7 L3T4$^+$ enriched spleen cells (△). Other nude recipients received 10^7 Lyt-2^+ enriched spleen cells (▲) or remained untreated (○). All mice (four per group) were challenged intraperitoneally with 10^5 *P. chabaudi adami* on the day of transfer. D, Death.

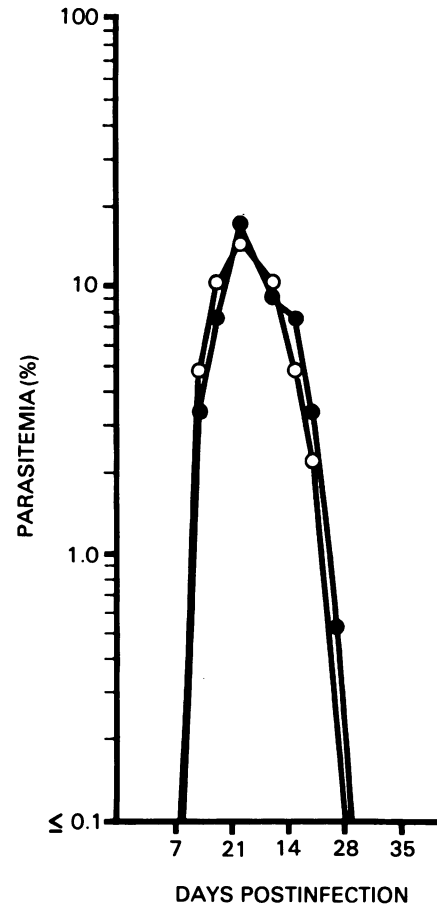

FIG. 4. Failure of hyperimmune serum to transfer protection against *P. chabaudi adami* infection. Naive BALB/c *nu*/+ mice received 0.5 ml of serum pooled from hyperimmunized reconstituted BALB/c *nu*/*nu* mice (●) or were left untreated (○). All mice (three per group) were challenged intravenously with 10^3 *P. chabaudi adami*-parasitized erythrocytes on the day of transfer.

Hyperimmune serum prepared by challenging T-cell-reconstituted nude mice with *P. chabaudi adami* on three subsequent occasions failed to alter the kinetics of *P. chabaudi adami* infections when passively transferred to immunologically intact mice prior to challenge. These findings demonstrate that the grafted T cells did not function as helper cells in the production of protective antibodies but probably functioned in a manner analogous to that observed in immunologically intact mice, where the passive transfer of hyperimmune serum was without effect despite the ready detection of parasite-specific antibodies by immunofluorescence and immunoprecipitation techniques (D. Russo, personal communication).

It is likely that the grafted T cells are activated by malarial antigens to secrete factors that in turn stimulate other cells or cell systems to exert an antiparasite effect (6, 22). Obvious candidates would be the cells of the mononuclear phagocytic system, which, when activated, has been shown to produce parasite inhibitory factors such as hydrogen peroxide and superoxide anion (2), which may damage parasites by the peroxidation of membrane lipids (1). Another candidate would be the splenic reticular cells, which become highly

activated and secretory during malaria (L. Weiss, U. Geduldig, and W. P. Weidanz, submitted for publication). Parasitized erythrocytes often can be found adhering to the surface of these cells, where they may be intimately exposed to toxic molecules.

What is unique about the present study is the finding that immunity to acute *P. chabaudi adami* malaria is antibody independent. The use of this model system allows the investigator to study the contribution of T cells to those cell-mediated immune responses that achieve protection exclusive of their helper role in antibody formation. Our results indicate that immunity to acute infection with *P. chabaudi adami* is mediated by the L3T4 subset of T lymphocytes. Future studies will focus on the identification and characterization of cell-mediated immune mechanisms which protect the host against infection with this parasite as well as on the definition of malarial antigens which activate these T cells. While immune mechanisms identified in the murine infection models may represent extremes of effector responses available in the host repertoire to resist malarial infection, the value of such models is that they serve as indicators of the range of mechanisms that may function separately or in an integrated fashion to protect humans against this disease. Thus, it is conceivable that once identified, certain of these mechanisms may provide new approaches for the prophylaxis of malaria.

ACKNOWLEDGMENTS

We thank Alice Smith for her excellent secretarial assistance.

This work was supported by Public Health Service grants AI12710 and AI21089 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Allison, A. C.** 1984. Cellular immunity to malaria and babesia parasites: a personal viewpoint. Contemp. Top. Immunobiol. **12**:463–490.
2. **Allison, A. C., and I. A. Clark.** 1977. Specific and nonspecific immunity to haemoprotozoa. Am. J. Trop. Med. Hyg. **26**: 216–222.
3. **Allison, A. C., and E. M. Eugui.** 1983. The role of cell mediated immune responses in resistance to malaria, with special reference to oxidant stress. Annu. Rev. Immunol. **1**:361–392.
4. **Brinkmann, V., S. H. E. Kaufmann, and M. M. Simon.** 1985. T-cell-mediated immune response in murine malaria: differential effects of antigen-specific Lyt T-cell subsets in recovery from *Plasmodium yoelii* infection in normal and T-cell-deficient mice. Infect. Immun. **47**:737–743.
5. **Brown, I. N., and R. S. Phillips.** 1974. Immunity to *Plasmodium berghei* in rats: passive serum transfer and role of the spleen. Infect. Immun. **10**:1213–1218.
6. **Clark, I. A., A. C. Allison, and F. E. G. Cox.** 1976. Protection of mice against Babesia and Plasmodium with BCG. Nature (London) **259**:309–311.
7. **Clark, I. A., W. B. Cowden, and G. A. Butcher.** 1983. Free oxygen radical generators as antimalarial drugs. Lancet **i**:234.
8. **Clark, I. A., and N. H. Hunt.** 1983. Evidence for reactive oxygen intermediates causing hemolysis and parasite death in malaria. Infect. Immun. **39**:1–6.
9. **Cohen, S.** 1979. Review lecture: immunity to malaria. Proc. R. Soc. Lond. B. Biol. Sci. **203**:323–345.
10. **Cohen, S., and P. H. Lambert.** 1982. Malaria, p. 422–477. *In* S. Cohen and K. S. Warren (ed.), Blackwell Scientific Publications, Ltd., Oxford.
11. **Cohen, S., I. A. McGregor, and S. Carrington.** 1961. Gamma globulin and acquired immunity to human malaria. Nature (London) **192**:733–737.
12. **Dialynas, D. P., Z. S. Quan, K. A. Wall, A. Pierres, J. Quintas, M. R. Loken, M. Pierres, and F. W. Fitch.** 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK15: similarity of L3T4 to the human Leu 3/T4 molecule. J. Immunol. **131**:2445–2451.
13. **Diggs, C. L., and A. G. Osler.** 1969. Humoral immunity in rodent malaria. II. Inhibition of parasitemia by serum antibody. J. Immunol. **102**:298–305.
14. **Grun, J. L., and W. P. Weidanz.** 1981. Immunity to *Plasmodium chabaudi adami* in the B cell deficient mouse. Nature (London) **290**:143–145.
15. **Grun, J. L., C. A. Long, and W. P. Weidanz.** 1985. Effects of splenectomy on antibody-independent immunity to *Plasmodium chabaudi adami* malaria. Infect. Immun. **48**:853–858.
16. **Grun, J. L., and W. P. Weidanz.** 1983. Antibody-independent immunity to reinfection malaria in B-cell-deficient mice. Infect. Immun. **41**:1197–1204.
17. **Haidaris, C. G., J. D. Haynes, M. S. Meltzer, and A. C. Allison.** 1983. Serum containing tumor necrosis factor is cytotoxic for the human malaria parasite *Plasmodium falciparum*. Infect. Immun. **42**:385–393.
18. **Hoffmann, E. J., W. P. Weidanz, and C. A. Long.** 1984. Susceptibility of CXB recombinant inbred mice to murine plasmodia. Infect. Immun. **43**:981–985.
19. **Holder, A. A., and R. R. Freeman.** 1981. Immunization against blood-stage rodent malaria using purified parasite antigens. Nature (London) **294**:361.
20. **Jayawardena, A. N.** 1981. Immune responses in malaria, p. 85–136. *In* J. M. Mansfield (ed.), Parasitic diseases, vol. 1. The immunology. Marcel Dekker, Inc., New York.
21. **Jayawardena, A. N., D. B. Murphy, C. A. Janeway, and R. K. Gershon.** 1982. T cell mediated immunity in malaria. I. The Ly phenotype of T cells mediating resistance to *Plasmodium yoelii*. J. Immunol. **129**:377–381.
22. **Jayawardena, A. N., G. A. T. Targett, E. Leuchars, R. L. Carter, M. J. Doenhoff, and A. J. S. Davies.** 1975. T cell activation in murine malaria. Nature (London) **258**:849–859.
23. **Jensen, J. B., M. T. Boland, J. S. Allan, J. M. Carlin, J. A. V. Waa, A. A. Divo, and M. A. S. Akood.** 1983. Association between human serum-induced crisis forms in cultured *Plasmodium falciparum* and clinical immunity to malaria in Sudan. Infect. Immun. **41**:1302–1311.
24. **Jensen, J. B., S. L. Hoffman, M. T. Boland, M. A. S. Akood, L. W. Laughlin, L. Kurniawan, and H. A. Marwoto.** 1984. Comparison of immunity to malaria in Sudan and Indonesia: crisis form versus merozoite-invasion inhibition. Proc. Natl. Acad. Sci. USA **81**:922–925.
25. **Julius, H. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus-derived murine lymphocyte. Eur. J. Immunol. **3**:645–649.
26. **Majarian, W. R., T. M. Daly, W. P. Weidanz, and C. A. Long.** 1984. Passive immunization against murine malaria with an IgG3 monoclonal antibody. J. Immunol. **132**:3131–3137.
27. **Ockenhouse, C. F., and H. L. Shear.** 1984. Oxidative killing of the intraerythrocytic malaria parasite *Plasmodium yoelii* by activated macrophages. J. Immunol. **132**:424–431.
28. **Rank, R. G., and W. P. Weidanz.** 1976. Nonsterilizing immunity in avian malaria, an antibody independent phenomenon. Proc. Soc. Exp. Biol. Med. **151**:257–259.
29. **Roberts, D. W., R. G. Rank, W. P. Weidanz, and J. F. Finerty.** 1977. Prevention of recrudescent malaria in nude mice by thymic grafting or by treatment with hyperimmune serum. Infect. Immun. **16**:821–826.
30. **Roberts, D. W., and W. P. Weidanz.** 1979. T cell immunity to malaria in the B cell deficient mouse. Am. J. Trop. Med. Hyg. **28**:1–3.
31. **Rzepczyk, C. M., and I. A. Clark.** 1982. Failure of bacterial lipopolysaccharide to elicit a cytostatic effect on *Plasmodium vinckei petteri* in C3H/HeJ mice. Infect. Immun. **35**:58–63.
32. **Taverne, J., H. M. Dockrell, and J. H. L. Playfair.** 1981. Endotoxin-induced serum factor kills malarial parasites in vitro. Infect. Immun. **33**:83–89.
33. **Taylor, D. W., C. T. Bever, F. M. Rollwagen, C. B. Evans, and R. Asofsky.** 1982. The rodent malaria parasite *Plasmodium*

yoelii lacks both types 1 and 2 T-independent antigens. J. Immunol. **128:**1854–1860.
34. **Trizio, D., and G. Cudkowicz.** 1974. Separation of T and B lymphocytes by nylon wool columns: evaluation of efficacy by functional assays *in vivo*. J. Immunol. **113:**1093–1097.
35. **Weidanz, W. P., and J. L. Grun.** 1983. Antibody independent mechanisms in the development of acquired immunity to malaria, p. 409–423. *In* T. K. Eisenstein, P. Actor, and H. Freedman (ed.), Host defenses to intracellular pathogens. Plenum Publishing Corp., New York.
36. **Weinbaum, F. I., E. B. Evans, and R. E. Tigelaar.** 1976. Immunity to *Plasmodium berghei yoelii* in mice. I. The course of infection in T cell and B cell deficient mice. J. Immunol. **117:**1999–2005.