

Trypanosoma rhodesiense Infection in B-Cell-Deficient Mice

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B-cell-deficient C57Bl/6J mice (suppressed from birth with goat anti- μ) and controls (treated from birth with normal goat serum) were infected with *Trypanosoma rhodesiense*. There was a significant ($P < 0.01$) decrease in duration of survival of the μ -suppressed mice compared with that of controls. Whereas both μ -suppressed and control mice had an initial rise in parasitemia of similar magnitude, only the control mice exhibited a subsequent period during which the parasitemia fell to undetectable levels. In control mice, immunization with irradiated organisms prevented the development of detectable parasitemia after challenge with viable trypanosomes. However, immunization did not alter the course of infection in B-cell-deficient mice. These results indicate that immunity to *T. rhodesiense* infection in mice is dependent on B-cell immunocompetence.

Both antibody and cellular responses can be elicited by immunization or infection with African trypanosomes (12, 15). In mice, resistance to *Trypanosoma rhodesiense* and *T. gambiense* can be transferred to normal recipients with serum or B but not T cells from immune donors (2, 13, 14). Congenitally athymic (nude) mice are as resistant as littermate controls to infection with *T. rhodesiense* and can be effectively immunized (G. H. Campbell, K. M. Esser, and S. M. Phillips, submitted for publication). These observations suggest that B cells and their products are important in the development of resistance of mice to infection with trypanosomes. However, a contribution of T-lymphocyte effector mechanisms in these infections cannot be excluded. In mice, T-lymphocyte sensitization to trypanosome antigens has been demonstrated by assays for in vitro blastogenesis in response to trypanosome antigen (Campbell et al., submitted for publication) and delayed-type hypersensitivity (footpad swelling) (J. H. Finerty and E. Krehl, Abstr. Am. Soc. Parasitol. Meet., November 1975, New Orleans, La.).

To determine whether T cells would have an effector role in the absence of antibody during infection with trypanosomes, we used the model of antibody response suppression by injection of mice from birth with goat antiserum to mouse μ -chain (anti- μ) to examine the effect of B-cell "deprivation" on the course of *T. rhodesiense* infection. The treatment of mice from birth with goat antibody to mouse μ -chain (μ -suppression) selectively impairs B-cell development and sup-

presses antibody responses to challenging antigens (8). This treatment leaves intact allograft rejection (11), helper T-cell function (4), graft-versus-host reactivity (A. R. Lawton, R. Asofsky, R. Tigelaar, M. Hylton, and M. Cooper, Fed. Proc. 31:751, 1972), and in vitro T-cell mitogen and mixed-lymphocyte culture responses (17).

In the present experiments, B-cell-deficient and control mice were infected with *T. rhodesiense*, and the courses of their parasitemias were compared. Irradiated trypanosomes were also injected into both B-cell-deficient and control mice as immunizing antigen before challenge with live organisms. These experiments provide an assessment of the relative roles of B and T lymphocytes in protection against *T. rhodesiense* infection.

MATERIALS AND METHODS

Animals. Six- to eight-week-old male C57Bl/6J mice and pregnant C57Bl/6J female mice were obtained from Jackson Laboratories, Bar Harbor, Maine. Male ICR mice were obtained from the Walter Reed Animal Facility. Mice were exposed to 900 R of gamma irradiation from a ¹³⁷Ce source (Gammacell 40, Atomic Energy of Canada Limited).

Trypanosomes. The East African Trypanosomiasis Research Organization (EATRO) strain 1886 of *T. rhodesiense* was used for these experiments. EATRO 1886 was isolated from the blood of a human patient in 1971 and was subsequently maintained by rodent passage. Three days after infection of an ICR mouse, infected blood was obtained and diluted in heat-inactivated fetal calf serum (Microbiological Associates Inc., Bethesda, Md.) for the isolation of a single organism. A single organism was isolated in a hanging microdrop on a cover slip sealed over a moist chamber.

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The drop was aspirated into a syringe containing fetal calf serum and injected intraperitoneally (i.p.) into an ICR mouse. A clone of organisms was passed in ICR mice at 3-day intervals for a total of four passes before a stabilate was frozen at -70°C in M199 (Microbiological Associates) containing 10% glycerol.

C57Bl/6J mice were infected with this stabilate. Three days later the mice were exsanguinated. Trypanosomes were separated from cellular elements of the blood by DE52 (Whatman) column chromatography (7), washed in M199, and diluted to a concentration of 10^7 or 10^4 organisms per milliliter. Experimental infections of mice were initiated by the i.p. inoculation of 10^6 or 10^3 organisms.

To prepare antigen for immunization studies, organisms were obtained as described above and were irradiated with 60 krads, using a ^{60}Co gamma source (Gammacell 220, Atomic Energy of Canada Limited), before freezing at -70°C in M199 and 10% glycerol. Mice were immunized by i.p. injection of 10^7 irradiated organisms. In experiments examining the protection afforded by immunization, mice were challenged by i.p. injection of 10^3 organisms because immunization was variant specific (2; Campbell et al., submitted for publication) and larger numbers of organisms in the challenging inoculum might have contained heterologous variants.

Monitoring of parasitemia. Blood (4 to 10 μl) was collected daily from the tails of infected mice. Blood was diluted with 0.05% Nile blue A (Matheson, Coleman, and Bell) in sodium citrate buffer (pH 7.4). The trypanosomes were counted in a hemacytometer chamber. The parasitemia was defined as the number of trypanosomes per milliliter of peripheral blood.

Anti- μ suppression. Anti- μ was the generous gift of Richard Asofsky. The preparation of this antiserum was described previously (17). A crude globulin fraction of the goat anti- μ was prepared by repeated precipitation with half-saturated ammonium sulfate. It was dialyzed extensively against saline and passed through a 0.45- μm membrane filter (Millipore Corp., Bedford, Mass.). Normal goat serum (NGS) was treated in the same fashion for use as a control preparation. Pregnant C57Bl/6J mice were observed twice daily. Offspring less than 24 h old were injected daily with 0.05 ml of goat anti- μ for 7 days. They were then injected with 0.1 ml of goat anti- μ every other day for the remainder of the studies. Control groups were injected on the same schedule with NGS. Since escape from μ -suppression can occur only in the absence of circulating anti- μ (10), all mice were tested at 6 weeks of age for the presence of anti- μ . A small test blood sample (obtained by tail bleeding 48 h after the last antiserum injection and diluted 1:2) was examined by double diffusion in agar against the immunoglobulin M (IgM) myeloma protein McPc 471B (μ , k). All mice injected from birth with goat antiserum to mouse μ -chain (μ -suppressed) were found to be adequately suppressed by this criterion. Two μ -suppressed and two control mice were chosen at random in two experiments and were examined for the presence of splenic B cells by direct immunofluorescence with fluoresceinated specific anti- μ and polyvalent anti-Ig reagents, as described previously (17). In all cases, μ -suppressed mice had less than 1% Ig-positive spleen cells. Of the

spleen cells from control mice, 38 to 41% were positive for surface Ig (38% μ positive, 41% polyvalent Ig positive). In addition, in two separate experiments, pooled spleen cells from two μ -suppressed and two control mice were examined for their ability to respond to mitogens in vitro. As reported previously (3, 17), the T-cell mitogen (phytohemagglutinin and concanavalin A) responses of μ -suppressed mice were comparable to control levels, whereas the B-cell mitogen responses (lipopolysaccharide and polyinosinic acid) were nearly absent.

RESULTS

Course of infection in control or μ -suppressed mice. Five μ -suppressed male C57Bl/6J mice and 5 age- and sex-matched control mice, injected from birth with NGS (globulin fraction), were infected by the i.p. injection of 10^6 *T. rhodesiense* organisms. The results (Fig. 1) indicated a significant ($P < 0.01$, Student's *t* test) decrease in the duration of survival of μ -suppressed mice after infection with trypanosomes. The mean duration of survival of the μ -suppressed mice was 14.6 ± 1.6 (standard error) days compared with 31.8 ± 3.4 days for control mice. In addition, μ -suppressed mice failed to reduce the first peak of parasites and died after a persistently high (3.5×10^8 organisms per ml) parasitemia. In contrast, all control mice reduced the first peak of parasites to undetectable levels ($<10^6$ organisms per ml), but succumbed to a subsequent increase of parasitemia. There was a delay in the rise of parasitemias of μ -

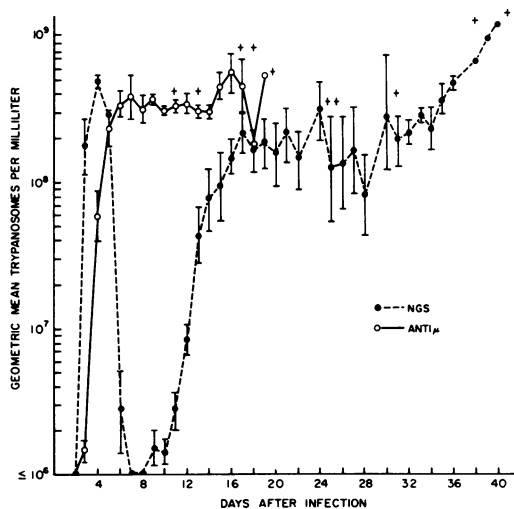


FIG. 1. Geometric mean parasitemia in μ -suppressed (anti- μ) or control (NGS-treated) mice after i.p. injection of 10^6 *T. rhodesiense* organisms. Each point represents the geometric mean parasitemia of 5 mice ± 1 (standard error). Deaths are indicated by + signs.

suppressed mice, resulting in substantially lower parasitemias compared with controls on day 3 and 4 after infection.

If μ -suppressed mice were completely deficient in immunity to trypanosomes, and the growth of the organisms was not limited by other factors, the parasites might have been expected to increase exponentially until the day of death without the plateau of parasitemia that occurred beginning on day 6 after infection. The plateau of parasites could possibly indicate a small degree of resistance conferred by T lymphocytes in the μ -suppressed mice. To test whether this plateau would be observed in mice made immunodeficient by lethal irradiation, C57Bl/6J mice were irradiated and then infected with *T. rhodesiense*.

Course of infection with *T. rhodesiense* in mice exposed to 900 R before infection. Control mice and mice subjected to 900 R of total body irradiation 24 h previously were injected i.p. with 10^6 *T. rhodesiense* organisms. The results (Fig. 2) again showed a plateau of organisms reached by day 4 after infection in the irradiated mice. By 7 days after irradiation, all irradiated mice began to show signs of irradiation sickness, whether infected with trypanosomes or not. The slight decline in parasitemia seen in irradiated mice starting 6 days after infection with *T. rhodesiense* was difficult to interpret because of the debilitated state of the hosts. Control mice had reduced their parasites to undetectable levels (10^6 organisms per ml) by day 6. The parasitemia began to increase again in control mice (not shown) by day 10 as in the previous experiment. Thus, even when immunocompetence of mice was reduced by lethal total body irradiation, the exponential rate of increase in parasite numbers did not continue beyond day 3 after infection. This suggests that the plateau of parasites was probably not due to a lymphocyte-mediated host response.

Effect of immunization on infection of control and μ -suppressed mice. Four μ -suppressed and 4 age- and sex-matched NGS-injected control mice were injected i.p. with 10^7 irradiated EATRO 1886 organisms 7 days before i.p. injection of 10^3 live trypanosomes. The results (Fig. 3) confirmed those results shown previously (Fig. 1), and indicated that prior immunization with irradiated organisms had no effect on the course of parasitemia in the μ -suppressed mice. Control mice that had been immunized with irradiated organisms did not develop detectable circulating parasites after challenge with live organisms.

DISCUSSION

These studies have demonstrated the necessity for B-cell effector mechanisms for resistance

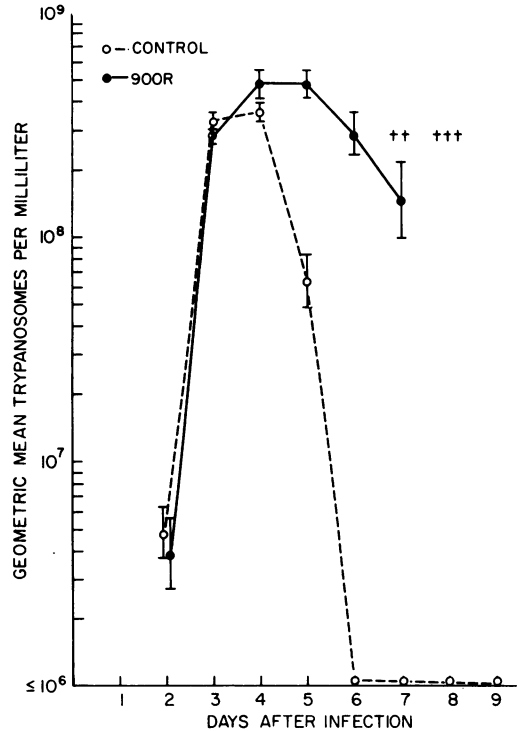


FIG. 2. Geometric mean parasitemia in normal (control) or irradiated (900 R) mice after i.p. injection of 10^6 *T. rhodesiense* organisms. Each point represents the geometric mean parasitemia of 5 mice \pm 1 (standard error). Mice were infected 24 h after irradiation.

against chronic infection with African trypanosomiasis. There was no evidence for any T-cell effector mechanisms in the absence of antibody. B-cell-deficient mice developed persistently high parasitemias and exhibited decreased survival time when compared with controls. These findings are in agreement with and extend the results of previous studies of chronic infection in athymic mice (6; Campbell et al., submitted for publication) as well as adoptive cell transfer studies (2, 13, 14) using more virulent strains of trypanosomes. Athymic (nude) mice were as capable of controlling parasitemias and surviving trypanosome infections as control mice (6; Campbell et al., submitted for publication) and have been immunized as effectively as controls (Campbell et al., submitted for publication). The adoptive transfer of immunity from immunized to unimmunized control mice was achieved by the transfer either of antibody or of purified B cells; adoptive transfer of T cells was ineffective.

Immunologically mediated resistance of immunocompetent mice to *T. rhodesiense* is reflected in the ability of mice to reduce the first peak of parasitemia. Thus, even in immunocom-

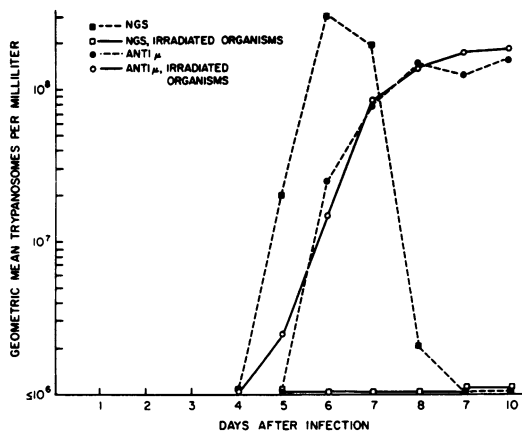


FIG. 3. Effect of pretreatment of μ -suppressed (anti- μ) or control (NGS) mice with 10^7 irradiated trypanosomes 7 days before challenge with 10^3 live *T. rhodesiense* organisms. Geometric mean parasitemias of four mice per group are shown for unimmunized mice (■-■; ●-●) or mice immunized with irradiated trypanosomes (□-□; ○-○). Immunized control mice did not develop detectable parasitemias during a period of observation extending to 21 days after challenge with viable organisms; all immunized μ -suppressed mice died within 14 days after infection. The course of parasitemia and times of death in unimmunized μ -suppressed and control mice were similar to those observed previously (Fig. 1).

petent animals, infection with *T. rhodesiense* invariably leads to death. Presumably, organisms survive through their ability to alter their surface coat antigens (antigenic variation) and can resist the effect of existing antibody. Immunization is variant specific (2; Campbell et al., submitted for publication) and appears to be T-cell independent since athymic mice can be effectively immunized (Campbell et al., submitted for publication). Our results do not exclude the possibility that T cells could have some effector role in the presence of antibody, such as antibody-dependent cellular cytotoxicity (17), although they are not required for immunity.

The unexplained delay of rise in parasitemia seen in μ -suppressed mice (Fig. 1 and 3) early after infection is difficult to interpret. This delay was not seen in NGS-treated mice or mice irradiated with 900 R. A direct effect of anti- μ serum on trypanosomes was not likely, because in vitro treatment of trypanosomes with either anti- μ or NGS did not alter the time of appearance of organisms in normal mice (unpublished data). Mice suppressed from birth with anti- μ should be deficient for antibodies that arise as result of exposure to environmental antigens. It is conceivable, although highly speculative, that such natural antibodies with low affinity for the try-

panosomes might stimulate their proliferation without mediating immune destruction. The basis of the phenomenon of reduced parasitemias early in infection of B-cell-deficient mice remains conjectural. It does not alter the important observation that μ -suppressed mice do not reduce parasitemia after the initial rise, as occurs in control mice.

The plateau of organisms reached in the μ -suppressed mice was an interesting phenomenon. This plateau effect has been observed previously during chronic *T. brucei* infections in mice immunosuppressed with 600 to 850 R or cortisone acetate (1, 9). We also observed the plateau effect in mice irradiated with 900 R of total body irradiation. Possible explanations for this effect include the presence of some internal control mechanism(s) of the organisms or the development of growth-rate-limiting metabolic factors in the infected host.

B-cell-deficient mice (μ -suppressed) have been useful for investigations into the role of B-cell immunity in a variety of infectious diseases. μ -Suppressed mice have a spectrum of susceptibilities. A blood-induced *Plasmodium berghei yoelii* infection that was self-limited in immunocompetent mice was invariably lethal in μ -suppressed mice (17). Similarly, whereas μ -suppressed mice were unable to control infection with *Heligmosomoides polygyrus*, a gastrointestinal nematode of mice, effective immunity was generated in immunocompetent controls (Weinbaum and Cypess, unpublished observations). In contrast to these results, μ -suppressed mice are comparable to control mice in their ability to be immunized with *P. berghei* sporozoites (3), to resist infection with Herpes simplex hominis (W. H. Burns and H. C. Morse, personal communication), and to expel *Nippostrongylus brasiliensis* (5).

In the present studies, the requirement for B-lymphocyte immunocompetence for immunity to *T. rhodesiense* infection has been established. No effector function could be ascribed to T lymphocytes.

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