

Chronic Infection with *Trypanosoma musculi* in Congenitally Athymic Nude Mice

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Trypanosoma musculi produces a chronic infection with a consistently elevated parasitemia in nude mice. Thymic reconstitution of nude mice restores immunity to the infection.

Trypanosoma musculi, a stercoarian trypanosome, is a natural parasite of mice (*Mus musculus*), in which it produces an avirulent infection lasting 20 to 25 days. Upon recovery, the host demonstrates lifelong immunity to exogenous challenge, although it continues to harbor the organism in the vasa recta vessels of the kidney (6). Immunity to *T. musculi* was first studied by Taliaferro (3, 4) and was found to be similar to that resulting from *Trypanosoma lewisi* infections in rats. More recently, Viens et al. (5), using T cell-deprived mice, have done extensive work on the immune response to *T. musculi*. They observed that the control of the infection is dependent upon the joint action of both a T cell-dependent ablastin and a T cell-independent trypanocidal antibody. In this paper, we report the use of the congenitally athymic nude mouse as a model system for the study of immunity to *T. musculi*. Furthermore, we will cite evidence that nude mice do produce an immune response to *T. musculi* but require T lymphocytes to effect recovery from the infection.

Nude mice used in this study were derived from BALB/c and C57 backgrounds and were reared in our conventional but closed colony. Mice were infected at 6 to 8 weeks of age unless otherwise indicated. The strain of *T. musculi* was originally obtained from the American Type Culture Collection (Rockville, Md.) and has been passaged in mice for 2 years in our laboratory. Parasite numbers were enumerated for infection purposes by diluting infected blood in 0.004% formal saline and counting the immobilized trypanosomes in a hemocytometer (5). Parasitemias were determined on Giemsa-stained peripheral blood smears according to the method of Taliaferro and Pavlinova (4). Antigen for footpad inoculations was prepared by separating trypanosomes from whole blood on an anion-exchange column (DE52, What-

man Chromedia) using phosphate-buffered glucose (pH 8.0) according to the procedure of Lanham and Godfrey (1). The antigen preparation was diluted so that each footpad challenge of 0.05 ml of antigen contained 10^7 trypanosomes and was then alternately frozen at -70°C and thawed at 37°C six times. Footpad swelling was measured with a Schnelltaster dial micrometer (H. C. Kroplin, Hessen, Germany).

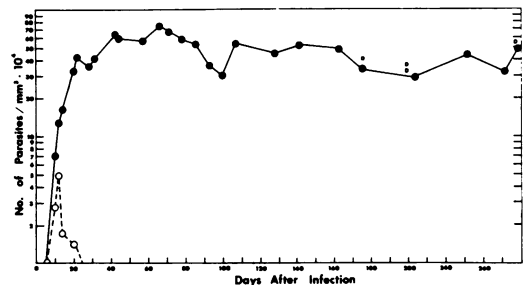


FIG. 1. Chronic infection with *T. musculi* in nude mice. Parasitemias represent the average of four nu/nu mice (●) and three nu/+ mice (○). D, Died.

To determine the course of *T. musculi* infection in nude mice, four nude mice (nu/nu) and three mice heterozygous for the nude trait (nu/+) were infected intraperitoneally with 10^4 trypanosomes. Parasitemias in nu/+ mice attained peak levels 12 days after infection and subsided by the 24th day after infection (Fig. 1). In contrast, parasitemias in nu/nu mice continued to increase until 42 days after infection and remained elevated thereafter until the animals died. Of 20 nu/nu mice infected with *T. musculi*, the average survival time was 152 days. These data indicate that the termination of *T. musculi* infection requires the presence of T cells in either a helper or effector capacity. In addition, dividing forms of the parasite are found throughout the course of the infection in

nu/nu mice, suggesting that ablastin is either absent or greatly depressed in nu/nu mice and is probably dependent upon T cells for its production.

To confirm that resolution of the parasitemia in nu/+ mice was dependent upon the presence of a functioning thymus, four nu/nu mice were grafted with a neonatal thymus according to the procedure of Rygaard (2). Ninety days later, five nu/nu mice, four nu/+ mice, and four thymus-grafted nu/nu (nu/nu_{thy}) mice were infected as before. Three of the four nu/nu_{thy} mice recovered from the infection, although their parasitemias were slightly higher and more prolonged than those observed in nu/+ animals (Fig. 2). These data indicate that the T lymphocyte system plays an essential role in the development of immunity to *T. musculi* infection.

That parasitemias became stabilized in nu/nu mice suggested that the animals were responding immunologically to the parasite. To determine if nu/nu mice could respond to trypanosomal antigen, groups of mice were challenged in the right hind footpad with freeze-thawed antigen 30 days after infection. All groups of infected mice responded with strong 3-h footpad swelling, which is indicative of an Arthus reaction (Fig. 3). Although nu/nu mice had significant reactions, the swelling was not as great as that seen in nu/+ or nu/nu_{thy} mice. In a previous experiment, 3-h footpad swelling in nu/nu mice was approximately half that observed in nu/+ mice. No 24-h or delayed-type hypersensitivity reactions were seen in any

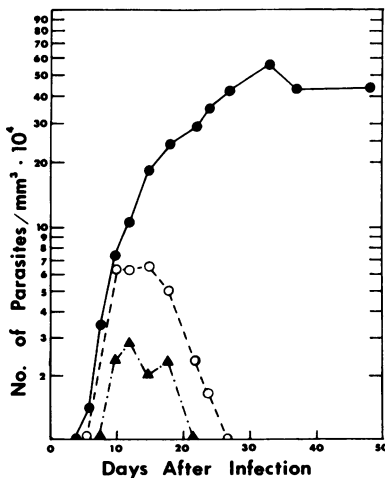


FIG. 2. Recovery of thymus-grafted nu/nu mice from *T. musculi* infection. Parasitemias represent the average of three nu/nu_{thy} (○), five nu/nu (●), and four nu/+ (▲) mice. The parasitemia of an unsuccessfully thymus-grafted mouse (not shown) was similar to that of the nu/nu group.

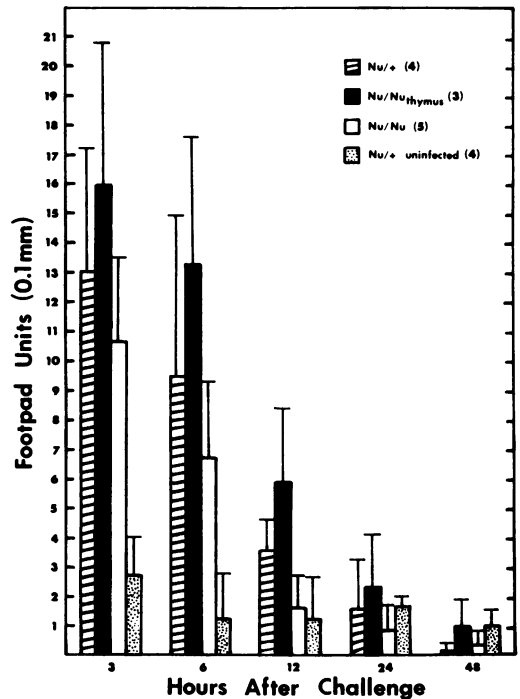


FIG. 3. Early footpad response to trypanosomal antigen in mice 30 days after infection. Values are means \pm standard deviation for the number of mice shown in parentheses.

groups. These data suggest that nu/nu mice do develop a T cell-independent antibody response to *T. musculi*, but whether this antibody has a stabilizing effect on parasitemias in nu/nu mice remains to be determined.

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