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Trypanosomiasis in Mice with Naturally Occurring Immunodeficiencies

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By using mice with naturally occurring defects, we have shown that an intact macrophage system is crucial to survival with the pathogenic protozoan *Trypanosoma rhodesiense*, since a defect in these cells decreased survival by half. Deficiencies in natural killer cell function or complement levels had no effect on survival. However, the capacity to survive trypanosomiasis was not related to the levels of parasitemia achieved during infection.

The infection of mice with African trypanosomes results in an acute, fatal disease characterized by waves of parasitemia and variations in parasite antigens. Each parasitemia peak contains distinct antigenic variants which are removed by an antibody-dependent mechanism and are replaced by new variants in subsequent peaks (12). Antibody is required for the clearance of trypanosomes in a peak, and this antibody production does not require T-cells (4). However, just which cells are necessary for trypanosome clearance has not been clearly defined. We used mice with naturally occurring immunodeficiencies (Table 1) to determine which components of the immune system are most critical for resistance in vivo against the human pathogen *Trypanosoma rhodesiense*.

In all experiments, we infected mice with 10^4 *T. rhodesiense* intraperitoneally on day zero. We used a clone designated Jef Tat1, derived from *T. rhodesiense* EATRO 1886 (kindly donated by J. F. Finerty, National Institute of Allergy and Infectious Diseases, Bethesda, Md.) and produced by triply cloning the parasites in mice immunosuppressed with cyclophosphamide (25). Stabilates stored at −70°C were expanded through cyclophosphamide-treated mice for 4 days, and diluted blood or DEAE-separated trypanosomes (10) were used to infect experimental animals.

Macrophages from C3H/HeJ mice are defective in their capacity to become tumoricidal after incubation with nonspecific stimulators such as BCG or bacterial lipopolysaccharide (2). Additionally, these mice are exceedingly susceptible to salmonellosis (18). These defects have been linked to the *lps* genetic locus (18). The geometric mean survival time of C3H/HeJ mice was significantly less ($P < 0.05$) than that of the "normal" C3H/HeNCrLBR mice (Fig. 1), suggesting that macrophages are critical to survival in trypanosomiasis.

To determine whether the macrophage deficiency was reflected in the capacity of C3H/HeJ mice to clear trypanosomes from the blood, we determined the blood trypanosome concentrations at frequent intervals in both the normal and deficient mice (Fig. 2). The courses of infection, as reflected by the numbers of blood parasites, were not different between the two strains of mice. In particular, both strains had very high parasitemias (10^8 parasites per ml) by the second week of infection, but although mice of the C3H/HeJ (defective) strain died rapidly, C3H/HeN (normal) mice survived with high levels of blood parasites until the third week.

The macrophages of A/J mice, like those from C3H/HeJ mice, are nonresponsive to a number of nonspecific stimulators (2, 3), but this defect is apparently not linked to the *lps* locus (3). Furthermore, unlike C3H/HeJ mice, A/J mice are relatively resistant to *Salmonella* spp. (20). Even though A/J mice have defective macrophages, they survive trypanosomiasis as well as the normal A/WySnJ mice (Fig. 1), and the parasitemias in the two strains are similar (data not shown).

To determine whether activated macrophages were more effective against trypanosomes, we injected mice intravenously with 10^6 BCG (Trudeau Institute strain TMC 1011, lot A7) 2 weeks before infection with *T. rhodesiense*. We found that CBA/CaJ mice receiving only *T. rhodesiense* survived for 27.8 days (coefficient of variance, 5.9%), and those receiving both BCG and *T. rhodesiense* lived for 28.9 days (coefficient of variance, 11.7%). Mice receiving BCG only had enlarged spleens from which BCG could be isolated.

This observation is similar to the finding that

TABLE 1. Mouse strains

Strain	Supplier[a]	Major immunodeficiency	Reference
C3H/HeN	CR	Normal	2
C3H/HeJ	J	Macrophage: lipid A nonresponsive, salmonella sensitive	2, 7, 18
A/WySnJ	J	Normal	2
A/J	J	Macrophage: lipid A nonresponsive, salmonella resistant	2
B10.D2/nSnJ	J	Normal	16
B10.D2/oSnJ	J	Complement: lacks C5	16
C57B1/6J bg/+	J	Normal	22
C57B1/6J bg/bg	J	Natural killer cells decreased	22
CBA/CaJ	J	Normal	13
CBA/N	N	B cell: Lyb 5 cell lacking	13

[a] CR, Charles River Breeding Laboratories, Wilmington, Mass.; J, Jackson Laboratories, Bar Harbor, Maine; N, National Institutes of Health, Bethesda, Md. Mice were housed in AALAS-accredited facilities. They were rested for 2 weeks before infection and were 8 to 10 weeks old at the beginning of all experiments.

nonspecific macrophage stimulation with BCG or *Corynebacterium parvum* did not enhance the killing of amastigotes of *Leishmania donovani* (14), although such procedures readily enhanced the killing of *Toxoplasma gondii* (1) and *Trypanosoma cruzi* (17). Possibly trypanosomes, like leishmania, are most readily killed by specifically activated macrophages. However, Murray and Morrison (15) found that BCG infection enhanced (but only slightly) the survival of mice infected with *Trypanosoma brucei* or *Trypanosoma congolense*. It may be that those trypanosomes are different enough from our strain of *T. rhodesiense* to account for our somewhat disparate findings.

Mice expressing the *xid* defect (CBA/N homozygous females or hemizygous males) respond poorly to certain T-independent (13) and T-dependent (21) antigens. Although CBA/N mice are highly susceptible to some bacteria, this appears to be related to their inability to make antibody rather than to a deficiency in macrophage function (19). Both CBA/N (Fig. 1) and (CBA/N × CBA/CaJ)F1 males (data not shown) survived significantly longer than normal CBA/CaJ mice. F1 littermate females or (CBA/CaJ × CBA/N)F1 males survived as long as CBA/CaJ mice (data not shown). Once again, there was no

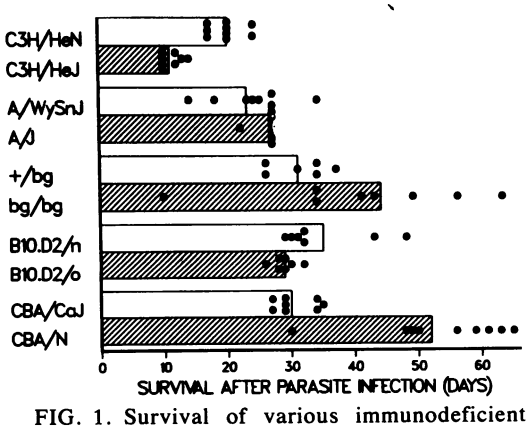

FIG. 1. Survival of various immunodeficient (hatched bars) and normal (open bars) mice after infection with 10^4 *T. rhodesiense*. Horizontal bars represent the geometric mean survival time. Each circle represents one mouse. Data are from one of three or more similar determinations.

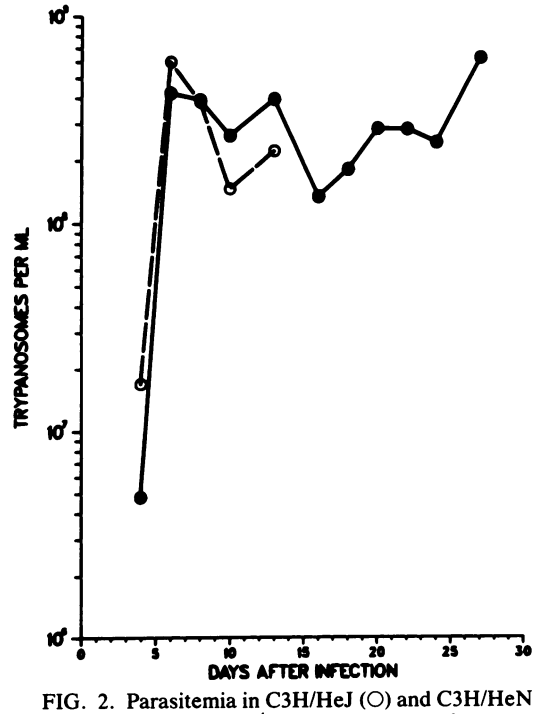

FIG. 2. Parasitemia in C3H/HeJ (○) and C3H/HeN (●) mice infected with 10^4 *T. rhodesiense* on day zero. Mean of two to four mice per time point. The geometric mean survival of C3H/HeJ mice was 13.3 days, and that of C3H/HeN mice was 23.4 days. Blood samples were diluted appropriately in a carbol-fuchsin staining reagent (15). We can detect $\geq 10^5$ parasites per ml with this reagent. Data are from one of four similar determinations.

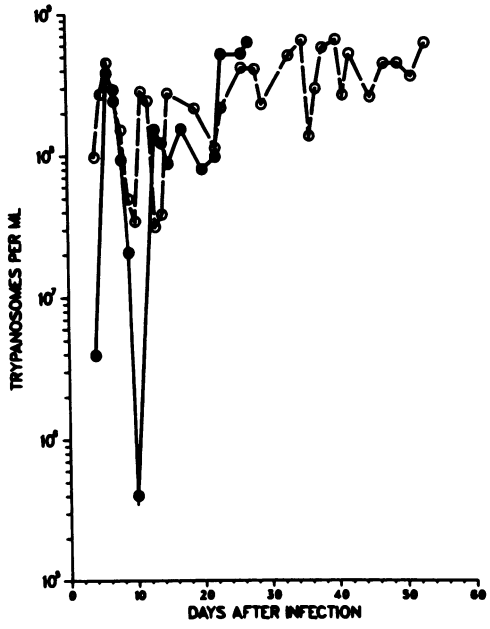

FIG. 3. Parasitemia in CBA/N (○) and CBA/CaJ (●) mice infected with 10^4 *T. rhodesiense* on day zero (see Fig. 2 legend). Mean of two to six mice per time point. The geometric mean survival of CBA/N mice was 58.1 days, and that of CBA/CaJ mice was 28.3 days. Data are from one of three similar determinations.

difference in the parasitemias between the normal and deficient mice (Fig. 3). These findings extend the observations of Gasbarre et al., who suggested that the increased survival of these mice after infection with *T. brucei* was due to their inability to make autoantibodies (8). An alternative explanation is that these mice make a more effective immune response to the parasites.

Although trypanosomes are readily lysed in vitro with antibody and complement (6; J. F. Jones, unpublished observations), a role for complement in vivo is unlikely since trypanosome infections rapidly lower the levels of serum complement (9, 24). Our findings (Fig. 1) that C5 deficient (B10.D2/o) mice survive infections as well as normal (B10.D2/n) mice support the idea that the lytic function of complement is not necessary for controlling trypanosomiasis. These findings are in accord with observations on *T. brucei* (24). Furthermore, they support the findings of Dempsey and Mansfield that complement is not required for clearance (5).

Beige mice are no more susceptible to trypanosomiasis than heterozygous bg/+ mice (Fig. 1). Since beige mice are defective in natural killer cell function (although this is a relative rather than absolute defect), antibody-dependent cell-mediated cytolysis (22), and probably cytolytic T-cell function (23), it is unlikely that these lytic processes are important in resistance to trypanosomiasis.

We are aware that there may be defects in the immunodeficient strains other than those recognized so far. Furthermore, most of these strains are not congenic, and clearly the mouse strain background has an effect on survival with trypanosomiasis (11) as well as with other infections (7). Nonetheless, our data support the idea that one major effector mechanism involved in controlling trypanosomiasis is the phagocytosis and subsequent destruction of antibody-coated trypanosomes by macrophages. This is in accord with the observations of Dempsey and Mansfield, who demonstrated that opsonized trypanosomes were rapidly cleared to the liver and suggested than an intact reticuloendothelial system was primarily involved (5). Our findings also suggest that other cellular or humoral effectors probably play a minimal role. However, the paradox remains the macrophage-defective and normal mice reach the same high levels of circulating parasites.

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