INFECTION AND IMMUNITY, Apr. 1984, p. 107–111
0019-9567/84/040107-05$02.00/0

Vol. 44, No. 1

Trypanosoma rhodesiense: Analysis of the Genetic Control of Resistance Among Mice

H. C. GREENBLATT,[1]†* C. L. DIGGS,[2] AND D. L. ROSENSTREICH[1]

Department of Medicine and Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461,[1] and Department of Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20012[2]

Received 7 October 1983/Accepted 11 January 1984

Inbred mouse strains differ in their resistance to infection with the human pathogen *Trypanosoma rhodesiense*. Of the strains tested, C57BL/6 (B6) mice were the most resistant, and BALB/c (C) mice were among the most susceptible. The genetic basis underlying the different susceptibility of these two strains was analyzed. (CXB6)F1 progeny of either sex were more resistant than the BALB/c parent. Also, the backcross of F1 mice to the susceptible male or female BALB/c parent resulted in 52.0% susceptible (i.e., death on or before day 24) progeny, as compared with only 0.64% susceptible F1 progeny. The data suggested that resistance was the dominant phenotype and that the resistant allele was carried by the B6 parent. The presence of another locus regulating resistance to death was suggested by the facts that only a small percentage of F2 mice were susceptible and that a number of F1 and F2 mice were more resistant than their B6 parent. The locus responsible for these phenomena was presumably hypostatic in nature and carried by BALB/c mice, and its effects were only evident in the presence of other resistance genes. In addition, the observation that many of the susceptible individuals among F2 and backcross mice were more resistant than the BALB/c mice suggested that other minor genes also modulated the response of mice to infection. A set of CXB recombinant inbred mice was tested as well, and the individual strains within this set could also be placed into four groups: susceptible, intermediate, resistant, or hyperresistant. These findings are compatible with the multigenic model suggested by the Mendelian analyses.

Many breeds of cattle, goats, sheep, and various species of wildlife vary in their resistance to African trypanosomiasis (2, 17, 18). The probability is high that such variability is based on the genetic makeup of these animals.

To examine this possibility, we have used a mouse model since there is also variation in resistance to African trypanosomes among inbred mouse strains. Resistance varies both with the strain of the mouse infected and with the antigenic variant of the trypanosome with which the mouse is infected (1, 10, 19). Studies of the resistance of mice to *Trypanosoma brucei* (1, 4, 8, 9, 19, 20; M. J. Clarkson, Parasitology **73**:viii part 2, 1976) and the cattle parasite *T. congolense* (11, 14, 15, 16, 22) have suggested that resistance to these organisms is under genetic control. However, the exact mechanism of inheritance and the number and location of relevant gene loci and their means of action remain unknown.

Until recently (5, 6, 12, 13), few genetic analyses of African trypanosomes have utilized a human pathogen such as *T. rhodesiense* or *T. gambiense*. This report is an extension of preliminary studies (6) of the genetic control of resistance to *T. rhodesiense*. This study presents more detailed evidence that resistance in mice to infection with *T. rhodesiense* is under polygenic control.

(This paper was presented in part at the International Symposium of the Canadian Society for Immunology, Montreal, Quebec, 1980.)

MATERIALS AND METHODS

Mice. Unless noted otherwise, all mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. The recombinant inbred mice CXBD, CXBE, CXBG, CXBH, CXBI, CXBJ, and CXBK were a generous gift of B. Taylor, Jackson Laboratory. BALB/c(Dub) and C3H/He(Dub) mice were obtained from Dominion Laboratories, Dublin, Va. Some parental and all F1, F2, and backcross mice were bred at the Walter Reed Army Institute of Research, Washington, D.C. Mice were infected at 3 to 5 months of age.

Nomenclature. Strains of mice are referred to by symbols found in The Jackson Laboratory handbook (7) or by a modification of these symbols. Thus, C57BL/6J mice are designated B6, and BALB/c mice are designated C. The first letter used to designate a hybrid identifies the maternal partner of a cross. Thus, (CXB6)F1 mice are F1 mice derived from a cross between a female BALB/c mouse and a male B6 mouse. Similarly, a backcross identified as B6 × F1 would describe an individual animal derived by mating a female B6 mouse to a male F1 mouse.

Parasite. A stabilate of *T. rhodesiense* EATRO 1886 clone obtained from the Walter Reed Army Institute of Research was used throughout these studies (3). Infection of mice with this parasite resulted in the eventual death of all animals.

Parasites for all experiments were obtained by inoculating 5×10^6 parasites into individual irradiated (900 R) male C57BL/6J mice. After 5 days, mice were exsanguinated, and the blood was pooled and centrifuged. Trypanosomes were carefully aspirated off the surface of the pellet to limit contamination with erythrocytes and added to RPMI 1640 (Flow Laboratories, Inc., McLean, Va.). Trypanosomes were diluted to the appropriate concentration and kept on ice until utilized. Depending on the protocol, mice were injected intraperitoneally with 10^3, 10^4, or 10^5 live trypanosomes in a total of 0.1 ml of RPMI 1640. At 9 days postinjection, lethality counts were initiated.

Classification of mice according to survival patterns. In these studies, mice were categorized as being susceptible, intermediate, resistant, or hyperresistant to infection with *T. rhodesiense*. Mice dying on or before day 24 postinfection were considered to be susceptible since all BALB/c mice died by day 24 postinfection. Mice surviving to at least day

* Corresponding author.
† Present address: Clinical Sciences Inc., Whippany, NJ 07981.

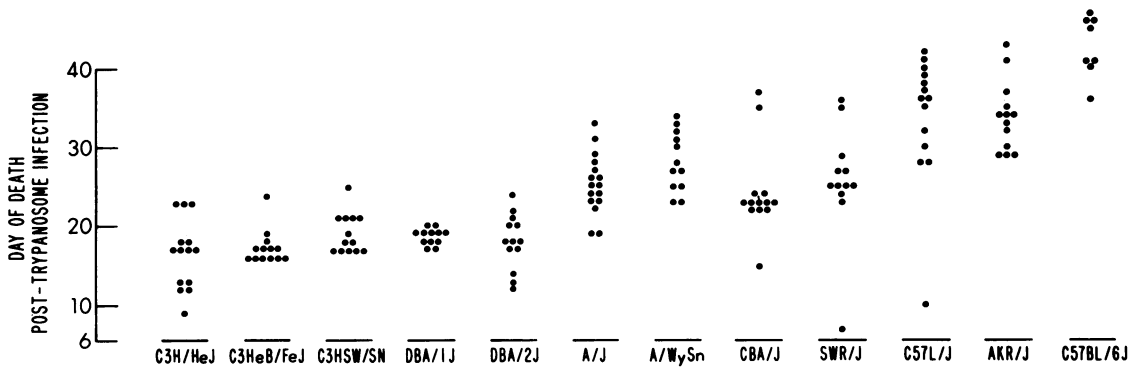

FIG. 1. Survival of inbred mouse strains after infection with 10^3 *T. rhodesiense* EATRO 1886 parasites.

41 were classified as resistant since 90% of all B6 mice survived until this date, and none died before day 35. Those mice with intervening survival times were considered intermediate. Mice surviving beyond day 65 (2 standard deviations of mean survival time [MST] of all B6 mice) were considered hyperresistant.

Statistical procedures. The MSTs of all mouse strains are presented in days plus or minus the standard error of the mean.

Cumulative frequency distributions for survival of infection with *T. rhodesiense* were analyzed with the Kolmogorov-Smirnov test (21). Differences between two sample populations were considered significant at $P < 0.05$.

RESULTS

Comparison of survival among inbred mouse strains infected with *T. rhodesiense*. Twelve inbred mouse strains were surveyed for their resistance to *T. rhodesiense* by injecting male mice of each strain intraperitoneally with 10^3 or 10^5 organisms. The data showed a spectrum of resistance to infection among these strains after inoculation of 10^3 parasites (Fig. 1). A similar pattern was obtained at a dose of 10^5 (data not shown). C3H and DBA mice were among the least resistant, with MSTs of <20 days postinfection. By day 25 postinfection, 65 of 66 (98.5%) of these mice died. There was no significant difference in the survival of any C3H or DBA strain. C57BL/6J mice were the most resistant, with an MST of 46.1 ± 2 days, with no mice dying before day 35 and 47.3% living beyond day 50 postinfection. The other inbred strains exhibited resistance between these extremes.

In addition to the testing described above, we tested male BALB/c(Dub) and B6 mice at challenges of 10^2, 10^3, and 10^4 parasites. At all doses BALB/c mice were markedly less resistant than B6 mice (Fig. 2). At an infectious dose of 10^4 trypanosomes, BALB/c mice had an MST of 17.8 ± 4.6 days, and B6 mice had an MST of 47.9 ± 1.2 days. The eventual mortality of these two strains was not dependent on the level of initial infection with *T. rhodesiense*.

Resistance of F1 progeny. To determine the genetic basis of resistance, Mendelian analyses were performed. B6 and BALB/c mice were chosen as the respective resistant and susceptible parental strains. Over a 1-year period, a series of experiments was carried out. Parental controls were included in each individual experiment. Since there was little difference in the patterns of survival for individual groups, results were pooled (Fig. 3). The MSTs of BALB/c and B6 mice were 19.6 ± 1.2 and 44.2 ± 1.4 days, respectively. Both the male and female (C × B6)F1 offspring were more resistant (males, 42.4 ± 1.5 days; females, 60.2 ± 2 days) than the BALB/c parents, indicating that resistance was the dominant phenotype. However, there was a wide range in survival times in the F1 male group, and 31.7% of these mice fell into the intermediate category. As a group (Fig. 3), the F1 female mice were significantly more resistant ($P < .001$) and had a broader range of survival times than either the B6 male or female mice, with some female F1 individuals surviving as long as 100 days.

Resistance of backcross progeny. If the resistant phenotype was determined by a single gene, the resistant allele carried by the B6 parent, Mendelian analysis would predict that a backcross of the F1 offspring to the strain carrying the recessive allele, BALB/c, would result in a 1:1 ratio of susceptible and resistant mice. A backcross to the strain carrying the dominant resistance allele would result in exclusively resistant progeny.

(C × B6)F1 hybrids were backcrossed to both the susceptible and the resistant parental strains. The resulting progeny were injected intraperitoneally with 10^4 trypanosomes, and mouse survival time was determined. Backcrosses of F1 mice with the susceptible BALB/c parent produced progeny with patterns of survival ranging from susceptible to markedly resistant (Fig. 4). Of these backcross mice, 52% fell into the susceptible range (i.e., death on or before day 24). Backcross to the resistant B6 parent also resulted in off-

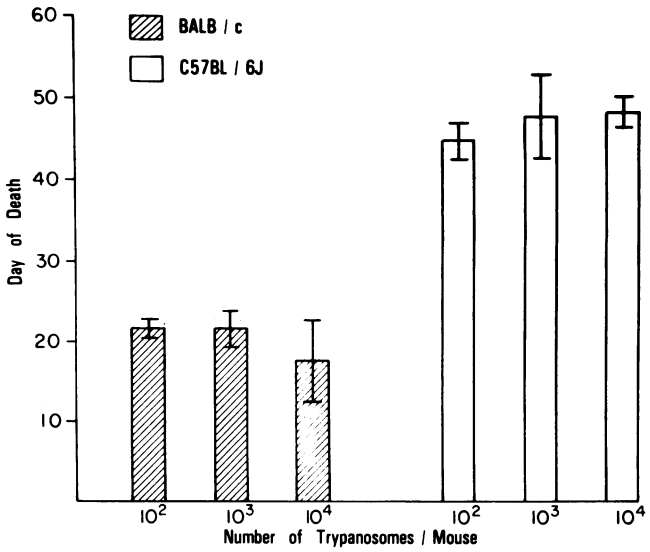

FIG. 2. Survival of BALB/c and B6 mice after infection with 10^2, 10^3, or 10^4 *T. rhodesiense* parasites.

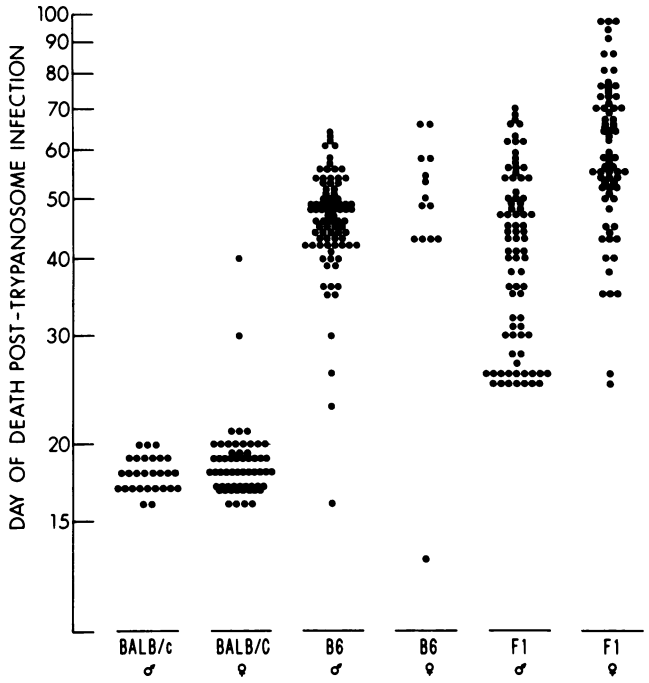

FIG. 3. Survival of parental mice and F1 progeny after infection with 10^4 *T. rhodesiense* parasites.

spring with a wide range of survival (Fig. 5), but 86.7% fell into the nonsusceptible category.

The data suggested that susceptibility to early death was regulated by at least one major autosomal recessive gene. However, as a group, the susceptible subpopulation among the backcross progeny was significantly more resistant than the BALB/c mice ($P < 0.001$), which suggested that resistance to infection was regulated by other genes.

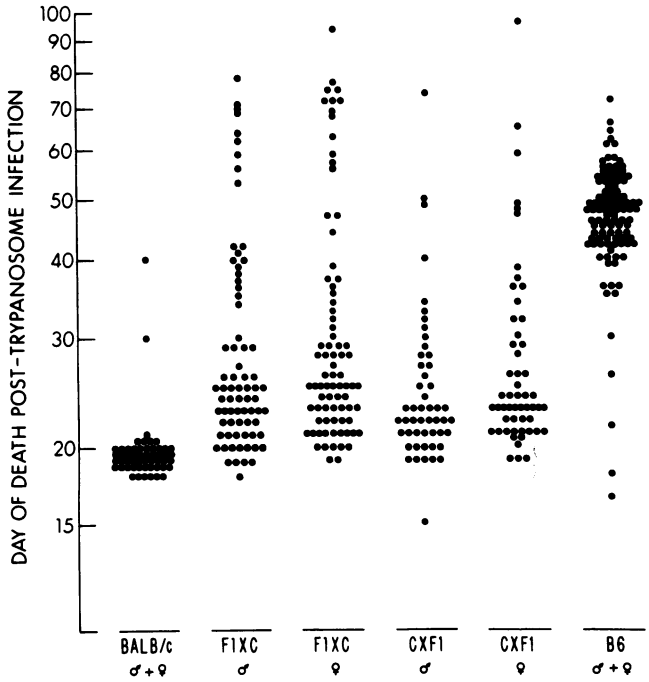

FIG. 4. Survival of F1 × C and C × F1 backcross mice after infection with 10^4 *T. rhodesiense* parasites.

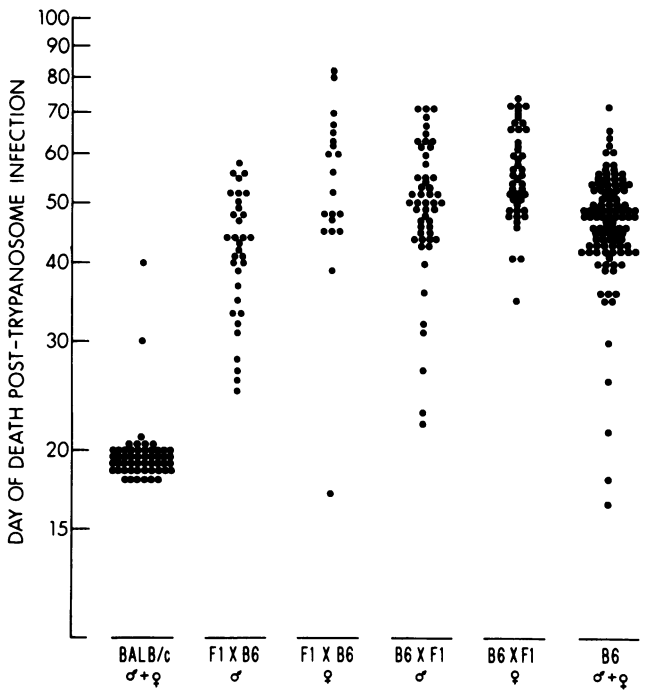

FIG. 5. Survival of F1 × B6 and B6 × F1 backcross mice after infection with 10^4 *T. rhodesiense* parasites.

Resistance of F2 progeny. F1 mice were mated to one another, and their resulting F2 progeny (160 animals) were infected with 10^4 *T. rhodesiense* and tested for resistance. Only 13.8% of F2 mice fell into the susceptible category, instead of 25% that would be expected with a single gene (Fig. 6). These data support the existence of at least two genes that regulate susceptibility to early death. In addition, 9.0% of male F2 mice and 15.85% of female F2 mice were hyperresistant (i.e., survived for >64 days). The resistant

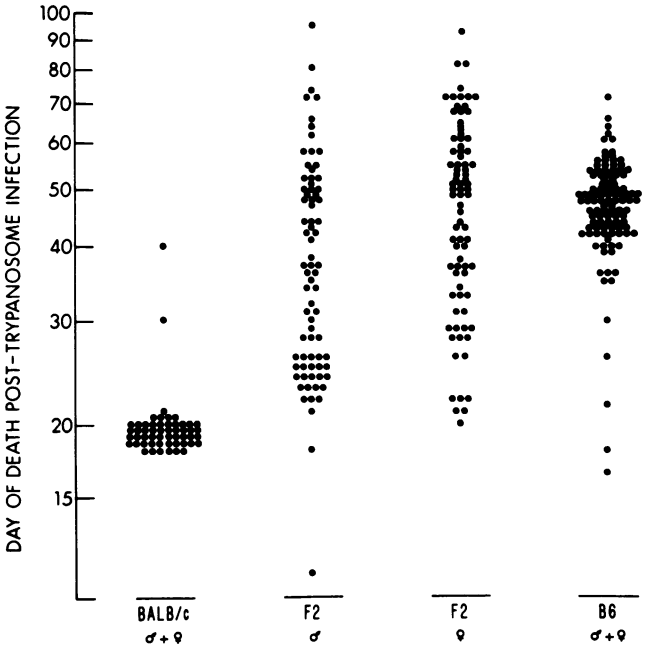

FIG. 6. Survival of F2 progeny after infection with 10^4 *T. rhodesiense* parasites.

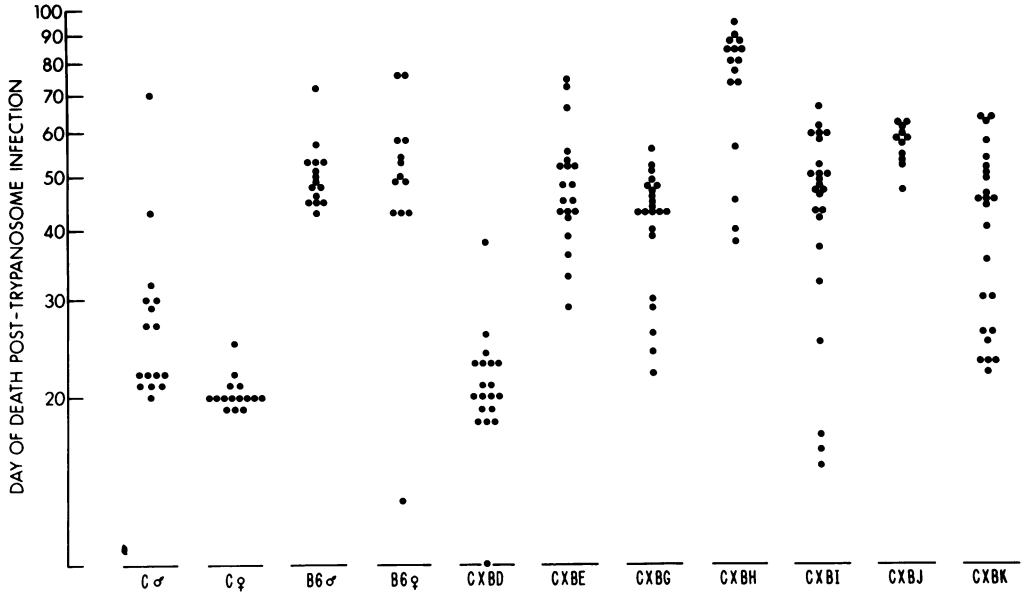

FIG. 7. Survival of female recombinant inbred mouse strains after infection with 10^4 *T. rhodesiense* parasites.

subgroup of F2 mice was significantly more resistant than the B6 parents. These latter data are consistent with the presence of a hypostatic resistance gene locus carried by the susceptible BALB/c parent.

Resistance of CXB recombinant inbred mice. Female CXB mice were injected intraperitoneally with 10^4 trypanosomes in two separate experiments, and the results were pooled (Fig. 7). In this experiment, the female C57BL/6ByJ mice were more resistant (MST, 49.1 ± 3.5 days) than the female BALB/cByJ mice (MST, 20.4 ± 0.4 days).

There were marked differences in mortality patterns among various CXB strains. Two strains proved highly resistant. CXBH mice had an MST of 81.8 ± 3.7 days, with 84.6% of these mice surviving for more than 70 days. CXBJ mice were also highly resistant, with an MST of 61.3 ± 2.8 days; both strains were significantly ($P < 0.01$) more resistant than B6/ByJ mice. Only one strain, CXBD, demonstrated a low resistance (MST, 21.2 ± 1.2 days) comparable to that of BALB/c mice. The CXBE strain exhibited an MST of 48.5 ± 2.6 days, which was similar ($P > 0.05$) to that of the B6 progenitor strain. Finally, three strains, CXBG, CXBI, and CXBK, exhibited a wide range of survival; i.e., some mice of these three strains died soon after infection, whereas others survived well into the resistant range.

DISCUSSION

This investigation reports in detail marked interstrain variation among mice in resistance to the EATRO 1886 strain of *T. rhodesiense*. After infection with this agent, there was a continuous distribution between highly susceptible and markedly resistant mice. BALB/cByJ, C3HeB/FeJ, and CBA/J mice were among the most susceptible mice, and C57BL/6 mice were among the most resistant. The findings of Levine and Mansfield (13) support our work.

There are a number of complicating factors in studying the genetics of resistance to African trypanosomiases. One major problem is the frequent fluctuation in daily total parasite loads. Levels of *T. rhodesiense* can reach 10^8 parasites per ml in the peripheral blood and then subside to below 10^5 for many days (5). At each peak, some mice will die, but others will survive until the next peak, which may not recur for several days. Because of this phenomenon, even genetically homogeneous inbred mouse strains may exhibit an extended span of time between the earliest and latest deaths in a group. Therefore, it is extremely difficult to perform a traditional Mendelian analysis in which the exact phenotype of every individual in a given generation must be delineated.

Despite these complexities, it is possible to infer from the data in this study the presence of several distinct resistance genes.

F1, F2, and backcross mice were more resistant than the susceptible BALB/c parent. This suggested the presence of an autosomal dominant resistance gene carried by the B6 parent that controlled the resistance of mice to death early after infection with *T. rhodesiense*. Further analysis of the data also supported the presence of other resistance genes contributed by the B6 parent which modulated early death. The existence of such loci would explain why susceptible groups among the backcross and F2 populations survived longer than the susceptible BALB/c parent.

The data reported in this study also suggested that there was another, interrelated locus that regulated resistance to death late after infection with *T. rhodesiense*. The resistant allele for this locus appeared to be carried by the otherwise susceptible BALB/c mice. The existence of such a gene would explain why many F1, F2, and backcross mice survived significantly longer than the B6 parent. Presumably, this locus was hypostatic in nature, and its effects were only evident when other resistance genes (from B6) were present. In *T. congolense* infection of mice, there is evidence of a similar phenomenon (14).

Finally, a sex dependence of resistance was again noted (5). Female mice were more resistant than males in all progeny groups. Part of this was due to an enhanced resistance of female B6 parental mice (8; Fig. 3). Clayton (1) noted a sex dependence in resistance to *T. brucei* infection as well.

Although the increased resistance of F1 female mice as compared with F1 male mice was consistent with the exis-

tence of an X-linked gene, this does not appear to be the case (5). In a study examining the resistance between BALB/c and B6 mice, it was found that there was no difference in the resistance between reciprocal F1 male mice (B6 × BALB/c versus BALB/c × B6). These data suggested that an X-linked gene did not account for the differences in resistance between susceptible and resistant strains of mice.

The CXB set of recombinant inbred mouse strains contains a reservoir of genes derived from the C57BL/6ByJ and BALB/cBJ progenitor inbred strains; however, unlike F1 mice, the mice making up the CXB series are homozygous at every gene locus. The CXB mice are useful both for determining the linkage of unknown resistance genes to known gene loci and for comparing gene expression between homozygous and heterozygous allelic states.

The survival patterns of the CXB recombinant inbred strains to infection with *T. rhodesiense* were consistent with the probable existence of three different gene loci, as suggested above. The CXBD strain was as susceptible as the BALB/c parent and was therefore probably homozygous recessive at the major autosomal dominant gene that controlled early death. The CXBE strain that exhibited survival levels similar to those of the B6 parent probably possesses resistance alleles at two gene loci. CXBH and CXBJ, the highly resistant recombinant inbred strains, probably carried resistant alleles at three interacting gene loci. B6 mice contributed the two resistant alleles that modulated early death, and BALB/c contributed the third hyperresistance gene. The finding of multiple patterns of susceptibility among these related, but distinct, recombinant inbred strains is also consistent with the existence of multiple gene loci that control resistance to *T. rhodesiense* EATRO 1886.

ACKNOWLEDGMENTS

We are grateful to Penny Bouchier for her excellent work in helping to set up and maintain our breeding colony and her expert technical assistance in this investigation. We thank T. V. Rajan for his thoughtful review of the manuscript.

This work was performed in part while the senior author was a National Research Council Resident Research Associate at the Walter Reed Army Institute of Research, Washington, D.C. This work was supported in part by Public Health Service grant AI17934-02 from the National Institute of Allergy and Infectious Diseases and the Trypanosomiasis Component of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

LITERATURE CITED

1. **Clayton, C. E.** 1978. *Trypanosoma brucei*: influence of host strain and parasite antigenic type on infections in mice. Exp. Parasitol. **44**:202–208.
2. **Dargie, J. D., P. K. Murray, M. Murray, and W. I. M. McIntyre.** 1979. The blood volumes and erythrokinetics of Ndama and Zebu cattle experimentally infected with *Trypanosoma brucei*. Res. Vet. Sci. **26**:245–247.
3. **Duxbury, R. E., E. H. Sadun, and J. S. Anderson.** 1972. Experimental infections with African trypanosomes. II. Immunization of mice and monkeys with a gamma-irradiated, recently isolated human strain of *Trypanosoma rhodesiense*. Am. J. Trop. Med. Hyg. **21**:885–888.
4. **Gasbarre, L. C., J. F. Finerty, and J. A. Louis.** 1981. Nonspecific immune responses in CBA/N mice infected with *Trypanosoma brucei*. Parasite Immunol. **4**:273–282.
5. **Greenblatt, H. C., and D. L. Rosenstreich.** 1984. *Trypanosoma rhodesiense* infection in mice: sex dependence of resistance. Infect. Immun. **43**:337–340.
6. **Greenblatt, H. C., D. L. Rosenstreich, and C. L. Diggs.** 1980. Genetic control of natural resistance to *T. rhodesiense* in mice, E. Skamene, P. A. L. Kongshaun, and M. Landy (ed.), p. 89–95. *In* Genetic control of natural resistance to infection and malignancy. Academic Press, Inc., New York.
7. **Heiniger, H.-J., and J. J. Dorey (ed.).** 1980. Handbook on genetically standardized Jax mice. The Jackson Laboratory, Bar Harbor, Maine.
8. **Herbert, W. J., and W. H. R. Lumsden.** 1967. Differences in the response of mice of two strains to infection with trypanosomes. Trans. R. Soc. Trop. Med. Hyg. **61**:140–141.
9. **Herbert, W. J., and W. H. R. Lumsden.** 1968. Infectivity and virulence of *Trypanosoma* (trypanozoon) *brucei* for mice. I. Comparison of two mouse strains. J. Comp. Pathol. **78**:341–344.
10. **Inverso, J. A., and J. M. Mansfield.** 1983. Genetics of resistance to the African trypanosomes. II. Differences in virulence associated with VSSA expression among clones of *Trypanosoma rhodesiense*. J. Immunol. **130**:412–417.
11. **Jennings, F. W., D. D. Whitelaw, P. H. Holmes, and G. M. Urquhart.** 1978. The susceptibility of strains of mice to infection with *Trypanosoma congolense*. Res. Vet. Sci. **25**:399–400.
12. **Jones, J. F., and G. E. Hancock.** 1983. Trypanosomiasis in mice with naturally occurring immunodeficiencies. Infect. Immun. **42**:848–851.
13. **Levine, R. F., and J. M. Mansfield.** 1981. Genetics of resistance to African trypanosomes: role of the *H-2* locus in determining resistance to infection with *Trypanosoma rhodesiense*. Infect. Immun. **34**:513–518.
14. **Morrison, W. I., and M. Murray.** 1979. *Trypanosoma congolense*: inheritance of susceptibility to infection in inbred strains of mice. Exp. Parasitol. **48**:364–374.
15. **Morrison, W. I., G. E. Roelants, K. S. Mayor-Withey, and M. Murray.** 1978. Susceptibility of inbred strains of mice to *Trypanosoma congolense*: correlation with changes in spleen lymphocyte populations. Clin. Exp. Immunol. **32**:25–40.
16. **Morrison, W. I., G. E. Roelants, T. W. Pearson, and M. Murray.** 1979. Genetic control of susceptibility to *T. congolense* infection in inbred strains of mice. Adv. Exp. Med. Biol. **114**:709–714.
17. **Murray, M., W. I. Morrison, P. K. Murray, D. J. Clifford, and J. C. M. Trail.** 1979. Trypanotolerance—a review. World Anim. Rev. **31**:2–6.
18. **Ormerod, W. E.** 1979. Human and animal trypanosomiases as world public health problems. Pharmacol. Ther. **6**:1–40.
19. **Sacks, D. L., M. E. Selkirk, B. M. Ogilvie, and B. A. Askonas.** 1980. Intrinsic immuno-suppressive activity of different trypanosome strains varies with parasite virulence. Nature (London) **283**:476–478.
20. **Selkirk, M. E., and D. L. Sacks.** 1980. Trypanotolerance in inbred mice: an immunological basis for variation in susceptibility to infection with *Trypanosoma brucei*. Tropenmed. Parasitol. **31**:436–438.
21. **Siegel, S.** 1956. Nonparametric statistics for the behavioral sciences, p. 148–152. McGraw-Hill Book Co., New York.
22. **Whitelaw, D. D., J. A. Macaskill, P. H. Holmes, F. W. Jennings, and G. M. Urquhart.** 1980. Genetic resistance to *Trypanosoma congolense* infections in mice. Infect. Immun. **27**:707–713.