Trypanosoma rhodesiense Infection in Mice: Sex Dependence of Resistance

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There is large variation in the survival of inbred mouse strains infected with *Trypanosoma rhodesiense* (EATRO 1886). Of those strains that survived for at least 22 days postinfection, female mice were markedly more resistant than male mice. The longer a strain survived, the greater was the difference in survival between male and female mice. Parasite counts were higher in male mice than in females, suggesting that the decreased resistance of males was due to their relative inability to control parasite growth. To determine the possible role of an X-linked resistance gene, resistant (C57BL/6) and susceptible (BALB/c) mice were mated, and their F1 progeny were infected with *T. rhodesiense*. There was no difference in the resistance between reciprocal F1 male mice (C57BL/6 × BALB/c versus BALB/c × C57BL/6), indicating that an X-linked gene does not account for the difference in resistance between susceptible and resistant mice.

African trypanosomes are protozoan blood parasites that are transmitted by the bite of an infected tsetse fly and are etiological agents of sleeping sickness; these parasites are responsible for the illness and deaths of thousands of humans and their livestock (17).

It has been demonstrated that strains of domestic animals exhibit considerable variability in their susceptibility to both natural and experimental trypanosome infections (5, 17), and it is assumed that some of the differences in resistance are regulated by mechanisms dependent on the genetic background of the host.

The results of experimental infections of various inbred mouse strains with *Trypanosoma brucei* (3, 11, 12; M. J. Clarkson, Parasitology 73:viii [part 2], 1976), *T. congolense* (15, 16), or the human pathogen *T. rhodesiense* (9, 13; Greenblatt, submitted for publication) support such a hypothesis. BALB/c mice die approximately 20 days postinfection with *T. rhodesiense* and are classified as susceptible, whereas C57BL/6 mice, which die 40 to 60 days postinfection, are classified as resistant.

In a previous study on the genetics of resistance to *T. rhodesiense* (9; Greenblatt, submitted for publication), it was observed that female F1 mice were significantly more resistant to infection than were F1 male hybrids. Since the parental female mice were also more resistant, it was not clear whether the resistance was due to an X-linked resistance gene or to a sex-dependent effect.

In this study, the basis of the sex-dependent resistance to *T. rhodesiense* in mice was analyzed. This investigation confirms that male mice are significantly less resistant to *T. rhodesiense* infection than are females, but the data do not support the existence of an X-linked gene that can account for the difference in resistance between BALB/c and C57BL/6 mice.

(Preliminary findings of this study were presented at the Fifth International Congress of Parasitology, Toronto, Canada, Aug. 7–14, 1982.)

MATERIALS AND METHODS

Mice. BALB/c, C57BL/6, CBA/J, CBA/CaJ, C57BL/KsJ, C57BL/10SnJ, C3H/He, and A.CA mice were obtained from

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the Jackson Laboratory, Bar Harbor, Maine, or from Charles River Breeding Laboratories, Inc., Wilmington, Mass. All F1 mice were bred at the Albert Einstein College of Medicine. Mice were at least 2 months of age at time of infection.

Parasite. A stabilate of *T. rhodesiense* EATRO 1886 was used for all studies (2, 6). Infection of mice with this parasite results in the eventual death of all animals.

Parasites were obtained by intraperitoneal inoculation of a sample of a stabilate into C57BL/6 mice that had been previously irradiated at 900 roentgens (Gamma-cell Ltd., Ontario, Canada). After 5 to 7 days, mice were exsanguinated; the blood was pooled and centrifuged. Trypanosomes were carefully aspirated from the surface of the pellet to limit contamination with erythrocytes and added to RPMI 1640 medium (Flow Laboratories, Rockville, Md.). Trypanosomes were counted and diluted to the appropriate concentration and kept on ice until use. Mice were injected intraperitoneally with 10^4 live parasites in a total of 0.1 ml of RPMI 1640. Nine days postinjection, lethality counts were initiated.

Parasitemia determinations. Starting on day 3, the presence of parasites was determined by examination of wet preparations of tail blood. When the number of parasites per field was low, counts were estimated by the "matching" method of Herbert and Lumsden (12). However, for higher parasitemia levels ($>10^5$), the actual parasite concentrations were determined. One microliter of blood was collected from the tail vein of an individual mouse, added to an appropriate volume of a 0.05% solution of Nile Blue sulfate (Matheson, Coleman, and Bell Manufacturing Chemist, Norwood, Ohio) (G. V. Hillyer and C. L. Diggs, J. Parasitol. 50(Suppl.):49, abstr. no. 115, 1964), and incubated for 5 min, and the trypanosomes were counted with a hemacytometer.

Statistical analyses. Differences in resistance were analyzed by use of a Student's *t* test (two-tailed).

RESULTS

Resistance of various mouse strains. Male and female mice of a number of inbred strains were infected intraperitoneally with 10^4 *T. rhodesiense* organisms, and their cumulative mortality with time was determined. There was a marked

TABLE 1. Mean survival times of male and female mice infected
with *T. rhodesiense*

Mouse strain	Mean survival time[a] (no. of mice in sample)		P
	Females	Males	
C3H/HeN	13.8 ± 0.4 (5)	15.2 ± 0.7 (5)	NS[b]
C3H/HeJ	14.6 ± 1.4 (5)	12.0 ± 0 (4)	NS
CBA/J	17.7 ± 0.84 (11)	17.3 ± 0.62 (8)	NS
BALB/c	21.5 ± 0.76 (19)	19.9 ± 0.42 (18)	NS
CBA/CaJ	26.9 ± 1.4 (16)	22.5 ± 0.43 (17)	NS
CBA/N	27.9 ± 2.2 (17)	20.3 ± 0.45 (29)	<0.05
A.CA	30.0 ± 3.3 (4)	23.7 ± 0.7 (7)	<0.05
C57BL/6J	52.2 ± 6.1 (68)	44.3 ± 1.8 (63)	<0.01
C57BL/KsJ	52.9 ± 3.3 (15)	38.7 ± 2.6 (16)	<0.01
C57BL/10SnJ	57.0 ± 2.8 (10)	46.0 ± 1.2 (14)	<0.01
(C × B6)F1	69.7 ± 2.5 (35)	54.3 ± 1.9 (38)	<0.01
(B6 × C)F1	66.8 ± 2.3 (36)	56.7 ± 2.4 (34)	<0.01

[a] Expressed in days ± standard error of the mean.
[b] NS, Not statistically significant.

variation in the resistance of the 10 inbred strains and two
sets of F1 hybrids to infection with *T. rhodesiense* (Table 1).

C3H/He, CBA/J, and BALB/c mouse strains were highly
susceptible, with mean survival times of less than 22 days;
marked differences were not found in the survival times of
male and female mice. In contrast, of those six inbred strains
that were more resistant (mean survival time, greater than 22
days), female mice were more resistant than males (Table 1).
There was a direct correlation between the increased resist-
ance of female mice and the mean survival time of the strain
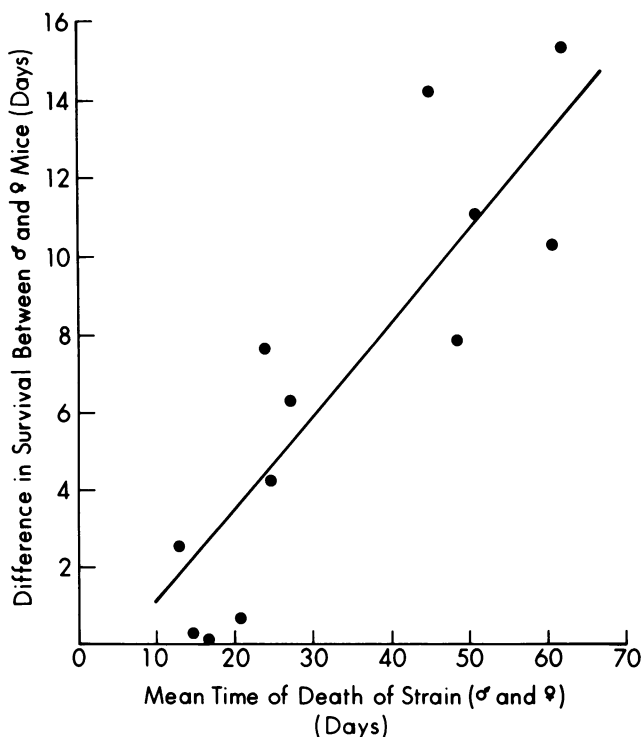(i.e., the longer the members of a strain survived, the greater

FIG. 1. Relationship between mean time of death (in days) and
increased difference in survival between male and female mice.
Each point represents the mean time of death in days of both male
and female mice of one individual mouse strain. Data were evaluat-
ed by linear regression ($r = 0.87002$; $P < 0.01$).

was the difference in survival between male and female mice
[Fig. 1]). Also, the exceptional resistance of F1 female mice
(Table 1) observed in our previous study (Greenblatt, sub-
mitted for publication) was confirmed.

The survival of male and female B6 and BALB/c mice was
examined in greater detail. The majority of BALB/c mice,
both male and female, were dead by day 20 postinfection
(Fig. 2). B6 mice did not begin to succumb until 10 days
later, but few survived past 60 days. Female B6 mice
survived significantly longer than their male counterparts (P
< 0.01). In contrast, there was no difference in survival
times of male and female BALB/c mice ($P > 0.05$).

Kinetics of parasitemia of BALB/c and C57BL/6 mice.
Blood parasitemia levels were also measured to determine
whether the differential resistance between mice was due to
failure to control parasite growth or to a specific intolerance
of certain groups of mice to high parasitic burdens.

Parasite levels of both B6 and BALB/c mice rose rapidly
after infection and peaked at approximately the same time
(day 7) and the same level (approximately 10^8 per ml) (Fig.
3). After day 7, the patterns of parasitemia in the two mouse
strains diverged. Levels in B6 mice dropped dramatically to
an undetectable range, 10^5 per ml, and remained low through
day 12. The mean parasite levels of BALB/c mice also
declined but always remained higher than those of B6 mice.
By day 12 postinfection, parasitemias began to rise again in
both strains. Parasite burdens in BALB/c mice continued to
increase until their deaths 20 to 21 days postinfection.

Mean parasite counts in male B6 mice were higher than in
females at every time point from days 14 to 33 postinfection
(Fig. 3). These differences were significant between days 17
and 24 ($P < 0.01$). Mean parasite counts of the BALB/c male
mice were also higher than the counts in females at five of six
time points between days 12 and 20. However, these differ-
ences were not statistically significant.

Resistance of male and female F1 hybrid mice. To deter-
mine whether an X-linked gene was involved in the differ-
ence in resistance between BALB/c and B6 mice, it was
necessary to test reciprocal F1 male hybrid mice. (C ×
B6)F1 and (B6 × C)F1 hybrid mice were bred and then were
infected with 10^4 *T. rhodesiense* organisms, and cumulative
mortalities were recorded (Fig. 4). As a group, the F1 mice
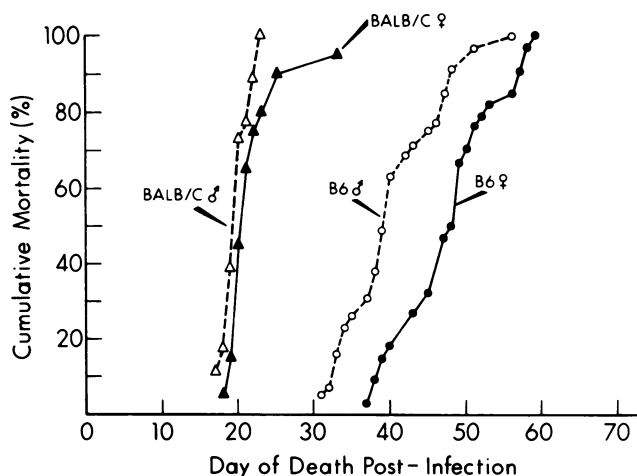were more resistant than either the BALB/c or B6 parents,

FIG. 2. Cumulative mortality of BALB/c and C57BL/6 (B6) mice
after infection with 10^4 *T. rhodesiense* parasites. Results represent a
typical experiment with 8 to 10 animals.

with few individuals succumbing before day 30. Again, F1 female mice were found to be significantly more resistant (*P* < 0.01) than F1 males. There was, however, no evidence of a difference in mortality among the reciprocal male F1 mice.

Parasite counts of F1 hybrid mice were also measured. As was seen with B6 parents, the parasite levels of the F1 male mice were higher than those in the F1 females at every time point (Fig. 5). However, there were no differences between the parasite levels of the reciprocal F1 male mice.

Thus, the mortality and parasitemia data on the reciprocal F1 males do not support the existence of an X-linked gene difference between BALB/c and B6 mice that can account for their differences in survival when infected with *T. rhodesiense*.

DISCUSSION

Sex differences of resistance to infections among humans and animals have been documented previously (4, 8). There is evidence indicating that females consistently display greater resistance to bacterial (20, 21), viral (1, 7, 14), fungal (18), and parasitic (8) infections than do males. Investigations in African trypanosomiasis have suggested a sex-dependent pattern of resistance to *T. gambiense*, *T. congolense* (F. C. Goble, E. A. Konopka, and J. L. Boyd, Abstr. Int. Conf. Protozool. 2nd, Int. Cong. Ser. **91**:54–55, 1965), and *T. brucei* (3). This study analyzed the sex dependence of murine resistance to *T. rhodesiense*.

Our earlier evidence suggested that there was an X-linked resistance gene that was responsible for the differences in survival between B6 and BALB/c mice (Greenblatt, submit-
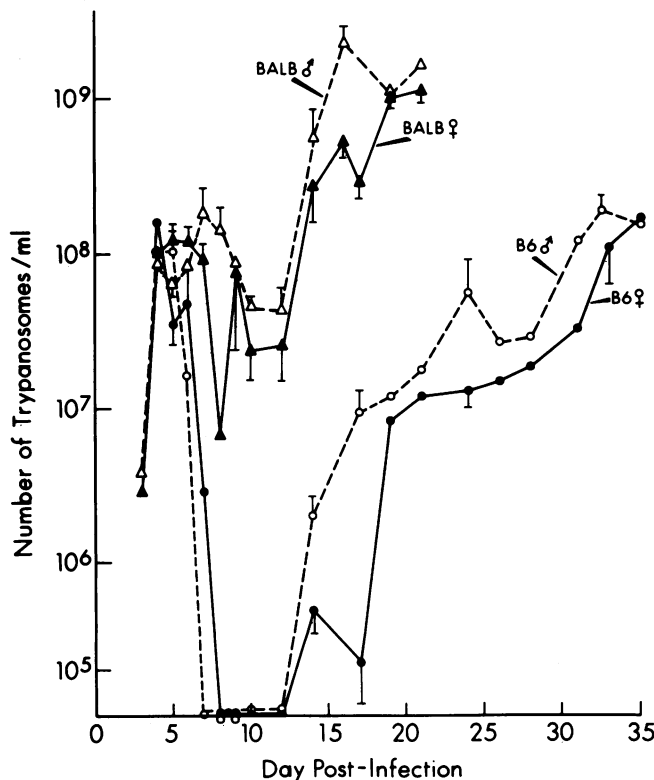

FIG. 4. Cumulative mortality of F1 hybrid mice after infection with 10^4 *T. rhodesiense* parasites. Results represent a typical experiment with 8 to 10 animals.

ted for publication). However, infection of mice with the EATRO 1886 strain of *T. rhodesiense* also resulted in significantly greater survival of female mice in comparison with males (Table 1). Increased resistance of parental female mice made it difficult to determine the existence of an X-linked resistance gene by comparing F1 male and female mice.

To overcome this problem, reciprocal [(C × B6)F1 and (B6 × C)F1] F1 male mice were compared for their resistance. These male mice are genetically identical at every locus except at the X chromosome. Any observed differences between these two groups would therefore be due to

FIG. 3. Kinetics of parasitemia in BALB/c and B6 mice after infection with 10^4 *T. rhodesiense* parasites. Results represent a typical experiment. Each point is the mean of parasite determinations of 10 individual mice.
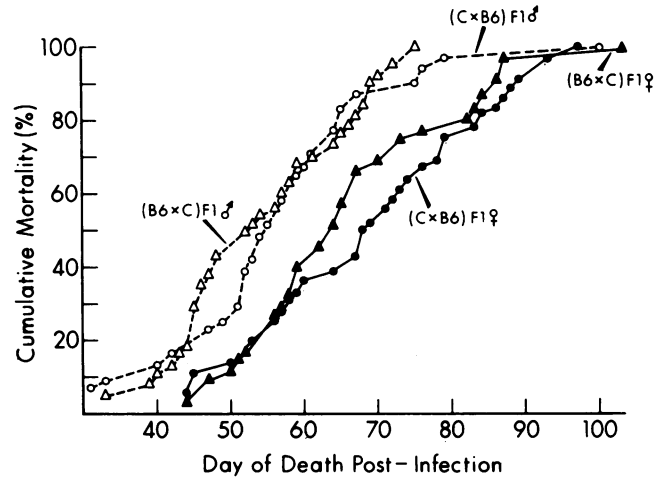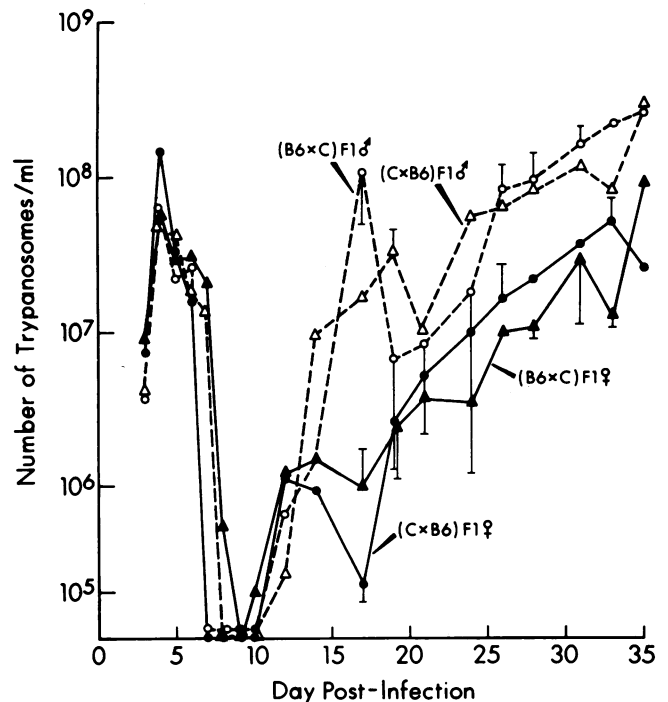

FIG. 5. Kinetics of parasitemia in F1 mice after infection with 10^4 *T. rhodesiense*. Results represent a typical experiment. Each point is the mean of parasite determinations of 10 individual mice.

an X-linked gene difference. If B6 mice carried the X-linked resistance gene, then the male progeny of a B6 × C mating would survive longer than their reciprocal (C × B6) male offspring. No significant differences were found when the resistance or parasitemias of reciprocal F1 males were compared (Fig. 4 and 5). Therefore, an X-linked gene does not account for the difference in resistance between BALB/c and B6 mice.

Analysis of parasitemias indicated that at any given time postinfection, parasite counts were higher in male mice. These data suggest that the decreased resistance of male mice may be due to an inability to control parasite growth as effectively as the females. Alternatively, male and female host defense mechanisms may be identical, but *T. rhodesiense* may grow more rapidly in males than in females for a variety of non-immunological reasons. This latter possibility is less likely given the general susceptibility of male mice to a variety of pathogens (4, 8).

The mechanisms underlying greater female resistance are not understood. Immunoglobulin levels can be regulated by X chromosome genes (10), and hormonal alteration will alter female susceptibility to autoimmune disease in mice (19). Resistance to encephalitis viruses (1, 7) has also been shown to be modulated by hormones. These findings suggest that sex reversals of mice might render males more resistant to *T. rhodesiense*, or females more susceptible. This possibility is currently under experimental investigation.

ACKNOWLEDGMENTS

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