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Role of the $H-2^s$ Haplotype in Survival of Mice After Infection with *Trypanosoma cruzi*

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In studies of the resistance of inbred mice to infection with *Trypanosoma cruzi* Peru, mouse strain B10.S was the only strain which survived the infection resulting from the inoculation of 10^3 trypomastigotes. This is the only inbred mouse strain studied to survive infection. To investigate the effect of the $H-2$ haplotype on survival, C57BL/10 congenic mouse strains bearing $H-2^s$ recombinant haplotypes and mouse strains A.SWSn/J and SJL/J were tested for their ability to overcome the *T. cruzi* infection. None of the recombinant strains tested, including B10.S(7R), B10.S(8R), B10.S(9R), and B10.HTT, survived the infection, indicating that at least two or more regions of the $H-2$ locus must be $H-2^s$ to ensure survival. Strains A.SWSn/J and SJL/J with the $H-2^s$ haplotype did not survive, indicating that the genetic background outside the $H-2$ complex also influences survival. The congenic F1 hybrid (C57BL/10 × B10.S) F1 exhibited intermediate survival levels when compared with the parental strains, indicating that $H-2^s$ survival is affected by gene dosage. The F1 hybrid strain [B10.S(7R) × B10.S(8R)]F1, which possesses the complete $H-2^s$ haplotype in the *trans* configuration, did not survive *T. cruzi* infection, suggesting that $H-2^s$ -mediated survival does not operate by *trans* complementation.

Resistance to infection with various viral, bacterial, and protozoan organisms has been shown to be influenced by genetic factors in the host (1, 3, 5, 6, 13, 19, 23, 29-31). Although in most cases it appears that there are multigenic factors which influence the susceptibility or resistance to these infectious agents, in several studies specific genetic loci within the host have been mapped which govern resistance to infection (4, 9, 21).

Our previous studies of the genetics of resistance to infection with the protozoan parasite *Trypanosoma cruzi* Peru in mice have shown that inbred strains of mice can be divided into two groups, strains with high parasitemia (HP) and low parasitemia (LP), based on the level of bloodstream parasitemia attained during infection (31). This result differs from that with the less-virulent Brazil strain of *T. cruzi* in which there is a spectrum ranging from highly susceptible to resistant strains (29). Our results have also suggested that one or more genes, located outside the major mouse histocompatibility locus $H-2$, were involved in regulating the level of parasitemia reached during infection. Although in these studies we focused primarily on the inheritance of the HP-LP response, we also examined the influence of the $H-2$ region in survival of the infection. It was observed that although other inbred mice died as a result of the infection, mice of the B10.S($H-2^s$) strain survived. This implies an influence of the $H-2$ locus in survival. The results of Trischmann and Bloom (28) are in accord with our views on polygenic regulation of resistance to *T. cruzi* and the presence of an $H-2$ effect on susceptibility to the parasite.

This report represents a continuation of studies on $H-2$ influence in resistance to *T. cruzi*. We have analyzed the role of the $H-2^s$ haplotype in survival of *T. cruzi* infection by examining parasitemia levels and survival in a number of strains possessing the $H-2^s$ haplotype or recombinants be-

tween $H-2^s$ and other haplotypes. Furthermore, we examined the inheritance of genes influencing survival by constructing a number of crosses between strains possessing the $H-2^s$ haplotype and other strains not carrying $H-2^s$.

MATERIALS AND METHODS

***T. cruzi*.** The Peru strain of *T. cruzi* was used in all experiments (16). Isolation and inoculation of parasites was carried out as previously described (31). An inoculum of 10^3 trypomastigotes was used in all experiments.

Mice. Mice were 8 to 12 weeks old at the time of inoculation. The hybrid strain (C57BL/6J × A/J)F1 and mouse strains SJL/J and A.SWSn/J were purchased from the Jackson Laboratories, Bar Harbor, Maine. All other F1 hybrid strains were bred at the University of California, Irvine (12). Strains B10.S(8R) and B10.S(7R) were kindly provided by Jack Stimpfling, McLaughlin Research Institute, Great Falls, Mont. Strains B10.S(9R), B10.HTT, A.TH, and A.TL were kindly provided by M. Cohn, Salk Institute, LaJolla, Calif.

Measurement of parasitemias. The level of bloodstream parasitemia was determined as previously described by removing a blood sample from the tail and counting the number of trypomastigotes with a Neubauer hemocytometer (31).

Statistical analysis. The parasitemias observed on day 17 were used as the comparison values in all statistical tests. HP and LP strains were classified as previously described (31).

RESULTS

Influence of genetic background on survival. We wished to determine whether strains possessing the $H-2^s$ haplotype with genetic backgrounds which were unrelated to the C57BL/10 strain would survive infection with *T. cruzi*. Three mouse strains with different genetic backgrounds but all carrying the $H-2^s$ haplotype were inoculated by injecting 10^3 bloodstream trypomastigotes of *T. cruzi* Peru. Parasitemia levels at day 17 and in those mice that survived were

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TABLE 1. Responses of *H-2^s* strains to *T. cruzi* infection

Mouse strain	Mean \pm SD parasitemia levels on day 17 ($\times 10^{-6}$)		Parasitemia response phenotypes ^a	No. survivors/no. inoculated	
	Female	Male		Female	Male
B10.S	2.5 \pm 1.3	7.6 \pm 6.6	LP	12/14	8/21
SJL/J	3.6 \pm 0.5	4.7 \pm 1.8	LP	0/6	0/6
A.SWSn/J	15 \pm 4.0	25 \pm 7.0	HP	0/5	0/5

^a Classified by criteria described by Wrightsman et al. (31).

monitored. The results (Table 1) show that of the three strains examined, only the B10.S mouse strain consistently survived the infection.

Inbred mice with the A background have HP (31) and are particularly susceptible to *T. cruzi* (28, 29). We therefore studied an A congenic strain, the A.SWSn/J(*H-2^s*) strain, to determine whether the *H-2* locus alone can confer survival. This strain, which is congenic with the HP strain A/J, exhibited parasitemia levels which were identical to the background A/J strain and did not survive the infection. In addition, the SJL/J strain, which possesses the *H-2^s* haplotype, was tested for response to the *T. cruzi* infection. These mice exhibited an LP response but did not survive the infection.

Response of *H-2* recombinant strains. To map the subregion of the *H-2* complex which contributes to the survival of the B10.S strain, several C57BL/10 congenic strains carrying recombinant *H-2^s* haplotypes were tested for their response to *T. cruzi* infections (Table 2). None of the recombinant strains consistently survived as well as the B10.S strain, which carries the intact *H-2^s* haplotype.

The observation that the B10.S(7R) strain, which possesses the *H-2^s* haplotype in all regions except the D subregion, does not survive the infection indicates that the D or D-linked region(s) is necessary for survival. Results with the B10.S(8R) strain also indicate that possession of the *H-2^s* haplotype in the D region alone does not ensure survival and that the *H-2^s* haplotype must be present in some other region(s) as well as in the D region. This interpretation was further supported by the results with strains B10.S(9R) (*K^sD^d*) and B10.HTT (*K^sD^d*), neither of which survived. Interestingly, the F1 hybrid [B10.S(7R) \times B10.S(8R)]F1 did not survive, indicating that the survival effect cannot be mediated through complementation in *trans*.

Inheritance of *H-2*-mediated survival. To sort out the respective roles of the *H-2^s* haplotype and genes outside the *H-2* complex on survival, six F1 hybrids were tested for their response to *T. cruzi* infection (Table 3). Our previous work with *T. cruzi* Peru has shown that many F1 hybrid strains do not survive the infection (31). In particular, crosses between HP and LP strains frequently result in F1 hybrid progeny which exhibit LP but do not survive the infection (31) (Table 3, crosses 1 to 3). To examine whether the background genes

are dominant for survival, the (B10.S \times A.SWSn/J)F1 hybrid progeny were tested for survival. Even though parasitemia, as expected, was low, no F1 hybrid progeny survived the infection. Thus, even in strains carrying the *H-2^s* haplotype, one or more additional loci function to ensure survival.

The F1 hybrid strain (C57BL/10 \times B10.S)F1 exhibited an intermediate level of survival when compared with the parental strains. The level of survival observed in the female F1 hybrid (33%) was nearly one-half the amount observed in the B10.S females (85%). Similarly, ca. 14% of the male F1 hybrids survived the infection, or roughly one-half the amount observed in the male B10.S strain (38%).

DISCUSSION

The observation that B10.S mice survived infection with the virulent Peru strain of *T. cruzi* prompted further investigation of the *H-2^s* haplotype in conjunction with other genetic backgrounds. The results of these studies indicate that *H-2^s*-mediated survival is influenced by genes outside the *H-2* complex. For example, although the B10.S strain survived the infection, neither of two other strains (A.SWSn/J or SJL/J), both of which carry the *H-2^s* haplotype, survived infection. Furthermore, the F1 hybrid progeny of crosses between A.SWSn/J and C57BL/10 strains, as well as the (A.SWSn/J \times B10.S)F1 strain, did not survive the infection with *T. cruzi*. Thus, by placing the *H-2^s* haplotype in conjunction with C57BL/10 background, as in the case of the (C57BL/10 \times A.SWSn/J)F1, the survival of the hybrid progeny was not ensured. It is unlikely that the nonsurvival of these F1 hybrid progeny was due to high parasite burdens since survival was observed in other F1 hybrids which developed similar parasitemia levels (31). Therefore, it appears that the positive effect which the *H-2* complex can exert in response to *T. cruzi* infection results from a combination between a highly resistant (LP) strain with a "best" haplotype to just cross the survival threshold.

To determine whether the effect of the *H-2^s* haplotype on survival was inherited in a dominant fashion, the congenic F1 hybrid (C57BL/10 \times B10.S)F1 was tested for its response to *T. cruzi* infection. Hybrid (C57BL/10 \times B10.S)F1 exhibited intermediate survival levels when compared with the parental strains. The survival levels in both the female and male F1 hybrid progeny were ca. 50% of the levels observed in the B10.S strain. These results invite the suggestion that the *H-2^s*-mediated survival is affected by gene dosage. A similar effect of gene dosage has been observed in the levels of recovery from infection with Friend leukemia virus infection, in which *H-2* heterozygotes exhibited intermediate levels of recovery as compared with strains which were homozygous for *H-2* (6).

The development of strains carrying recombinant *H-2* regions has allowed the mapping of particular *H-2* regions involved in various immune response characteristics (6-8).

TABLE 2. Response of B10 congenic strains bearing recombinations within the *H-2^s* haplotype

Mouse strain	<i>H-2</i> genotype at the following regions ^a :									Mean \pm SD parasitemia levels on day 17 ($\times 10^{-6}$)	No. survivors/no. inoculated
	K	I-A	I-B	I-J	I-E	I-C	S	G	D		
B10.S(7R)	s	s	s	s	s	s	s	s	d	3.9 \pm 1.2	0/6
B10.S(8R)	k	k	+	+	+	s	s	s	s	4.5 \pm 3.0	2/14
B10.S(9R)	s	s	+	k	k	d	d	d	d	4.8 \pm 1.6	0/7
B10.HTT	s	s	s	s	k	k	k	k	d	5.8 \pm 2.5	0/4
[B10.S(7R) \times B10.S(8R)]F ₁	s/k	s/k	s/+	s/+	s/+	s/s	s/s	s/s	d/s	4.1 \pm 1.7	0/11

^a From Klein (15). +, Not determined.

TABLE 3. Responses of F1 hybrid strains to *T. cruzi* infection

Cross no.	F1 hybrid	<i>H-2</i> genotype	Mean \pm SD parasitemia level on day 17 ($\times 10^{-6}$)		No. survivors/no. inoculated	
			Female	Male	Female	Male
1	C57BL/6J \times A/J	b/a	3.0 \pm 2.8	8.9 \pm 7.2	0/6	0/6
2	B10.S \times A.SWSn/J	s/s	4.0 \pm 1.6	9.1 \pm 4.5	0/9	1/10
3	C57BL/10 \times A.SWSn/J	b/s	3.5 \pm 1.1	5.3 \pm 2.5	0/13	0/12
4	C57BL/10 \times B10.S	b/s	3.1 \pm 1.9	7.1 \pm 2.7	6/18	3/18
5	C57BL/10 \times SJL/J	b/s	1.6 \pm 1.3	5.4 \pm 4.5	5/16	4/18
6	B10.S \times SJL/J	s/s	0.7 \pm 0.4	3.3 \pm 1.6	9/9	1/10

Although our studies were limited by the availability of *H-2^s* recombinant strains and, therefore, we could not examine all possible substitutions of *H-2* regions, the results seem to indicate that at least two subregions of the *H-2^s* haplotype must be present to ensure survival. As more recombinant congenic strains become available, further dissection of the *H-2* complex will be possible. Strain B10.S(7R), which lacks the *H-2^s* genotype only in the D region, strains B10.S(9R) and B10.HTT, which possess substitutions in the I, S, G, and D regions, and strain B10.S(8R), which is *K^dD^s*, did not survive the infection; therefore, it appears that the D or D-linked region(s) and at least one other region of the *H-2^s* haplotype are important for survival. This is the first report to show that different regions within the *H-2* complex influence host resistance to *T. cruzi*. The involvement of two *H-2*-associated genes is known to occur in several immune response model systems, including recovery from Friend virus leukemia (6), autoimmune thyroiditis (27), delayed sensitivity to picryl chloride (24), and others (7, 8, 23).

The F1 hybrid strain [B10.S(7R) \times B10.S(8R)]F1 was tested for response to *T. cruzi* infection to determine whether survival would occur in a strain which possessed the complete *H-2^s* haplotype in *trans* (Table 2). These F1 hybrid progeny did not survive the infection, suggesting that *H-2^s*-mediated survival cannot operate by *trans* complementation.

From this and previous studies (28, 31), it is clear that there are multigenic factors which influence the resistance to *T. cruzi* infection in mice. Our studies, as well as those of Trischmann and Bloom (28), suggest an *H-2*-linked effect in the response to *T. cruzi* infection. *H-2*-linked effects on resistance to other parasitic, bacterial, and viral infections have also been observed (2, 6, 17, 18). In most cases, the mechanism of action mediated by the *H-2* region in the development of resistance is unclear. However, it is interesting to note that regardless of its mechanism of action, many of the *H-2* effects in resistance to infection appear to act after the initial stages of infection when the infecting agent has become established in the host.

In investigations of the immune response to *T. cruzi* infection in mice, in several studies it has been attempted to link the known differences in susceptibility of different inbred strains with possible mechanisms for the variations in resistance (10, 11, 14, 20, 22, 25, 26). However, one question remains unanswered, that is, what are the key effectors in controlling the infection with *T. cruzi*. It is hoped that information regarding the relative resistance or susceptibility of inbred strains of mice to infection will be useful in designing and interpreting experiments aimed at understanding the mechanisms operating in the development of resistance to *T. cruzi*.

In summary, it is clear that strain B10.S is unusual in that it is the only inbred mouse strain tested which survives the

infection with the Peru strain of *T. cruzi*. *H-2^s*-mediated survival may be affected by gene dosage, as seen in the intermediate survival of the (C57BL/10 \times B10.S)F1; it cannot operate by *trans* complementation, as seen in nonsurvival of the [B10.S(7R) \times B10.S(8R)]F1 hybrids.

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A portion of this work will be submitted by R. Wrightsman as partial fulfillment of the Ph.D. requirements.

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