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Genes Within the Major Histocompatibility Complex Influence Susceptibility to *Trichinella spiralis* in the Mouse

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Although genes within the major histocompatibility complex (MHC) are known to influence the immune response to many antigens and to determine the specificities for certain types of cell-cell interaction, little is known of the role played by *MHC* genes in influencing the immune response to parasites. It is recognized that various inbred strains of mice may differ in their susceptibility to infection with parasitic helminths (Liu Si-Kwang 1966, Stefanski and Kozar 1969, Ali Khan 1974, Wakelin 1975, Behnke and Wakelin 1977), but appropriate studies have not been conducted to evaluate the importance of the *H-2* gene complex in contributing to these differences. Previous attempts to show *H-2*-linked influence on susceptibility to *T. spiralis* (Tanner 1978) or other parasitic helminths (Mitchell et al. 1976 and 1977) have either not used congenic strains of mice or have tested only a few of the *H-2* haplotypes available. Tanner (1978) studying *T. spiralis* infections in inbred strains of mice concluded that *H-2*-linked genes were not important in influencing susceptibility to infection. These studies were inconclusive, however, as congenic strains of mice were not used. Deelder and co-workers (1978) noted a difference in antibody response in two congenic strains of mice infected with *Schistosoma mansoni*, but found that the number of worms in the two strains did not differ. In the following report we present data from congenic inbred and recombinant strains of mice which show that genes within the mouse MHC influence susceptibility to infection with the parasite *Trichinella spiralis*.

All congenic inbred and recombinant strains of mice used in our studies were reared in the immunogenetic mouse colony at the Mayo Clinic. Other inbred strains were either reared at the Mayo Clinic or purchased from the Jackson Laboratory, Bar Harbor, Maine. In preliminary studies, we found that when inbred strains of mice are infected with muscle larvae taken from outbred strains of guinea pigs or rats, it is often difficult to reproduce results of experiments in which the relative susceptibilities of several different strains are compared. Such problems can be resolved by using a single inbred strain of host as a source of infective larvae for all experiments (unpublished observations). This suggests that the genetic composition

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of the larval donors may influence the subsequent establishment and/or development of worms in the new host. To control for this possibility, all mice in the following experiments were infected with larvae digested from the carcasses of infected C3HeB/FeJ mice.

Our initial studies examined susceptibility of congenic strains of mice which share the same genetic background and differ only at well defined loci within the MHC. As shown in Figure 1, susceptibility to *T. spiralis* differed strikingly among the congenic strains of mice tested. The numbers of larvae recovered from the more resistant strains B10.S ($H\text{-}2^s$) and B10.Q ($H\text{-}2^q$) were significantly lower ($p < 0.001$, Mann Whitney Rank Sum Test) than the highly susceptible strains B10.BR ($H\text{-}2^k$) and B10.P ($H\text{-}2^p$). Increased numbers of larvae in mice expressing the *k* haplotype as compared to mice expressing either the *s* or *q* resistant haplotypes have been noted in each of three independent experiments. The strains B10/Sn ($H\text{-}2^b$) and B10.PL ($H\text{-}2^u$) show susceptibility intermediate between the two extremes.

To see if genes mapping outside the MHC might also influence susceptibility to infection, we infected strains of mice sharing a common *H-2* haplotype but expressing different genetic backgrounds. Larval counts from five different $H\text{-}2^k$ strains and three different $H\text{-}2^q$ strains of mice are shown in Figure 2. As predicted from results in congenic strains of mice, the $H\text{-}2^k$ strains were uniformly more susceptible to infection than the strains expressing $H\text{-}2^q$. However, differences in susceptibility attributable to non-*H-2* genes were also apparent. C3HeB/FeJ mice,

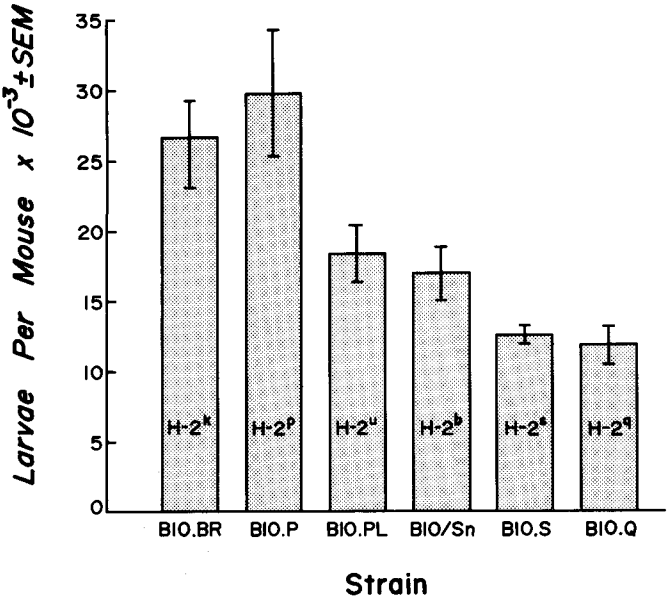

Fig. 1. Congenic strains of mice sharing the B10 background and differing only at loci within the MHC were infected with *T. spiralis* larvae obtained from infected C3HeB/FeJ mice. Mice were fed 150 larvae by esophageal intubation and total body larval counts determined 30 days later. *Vertical bars* represent the larval count means of ten mice; the standard error is given at the top of the bars. The *H-2* haplotype of each strain is indicated within the *vertical bar*.

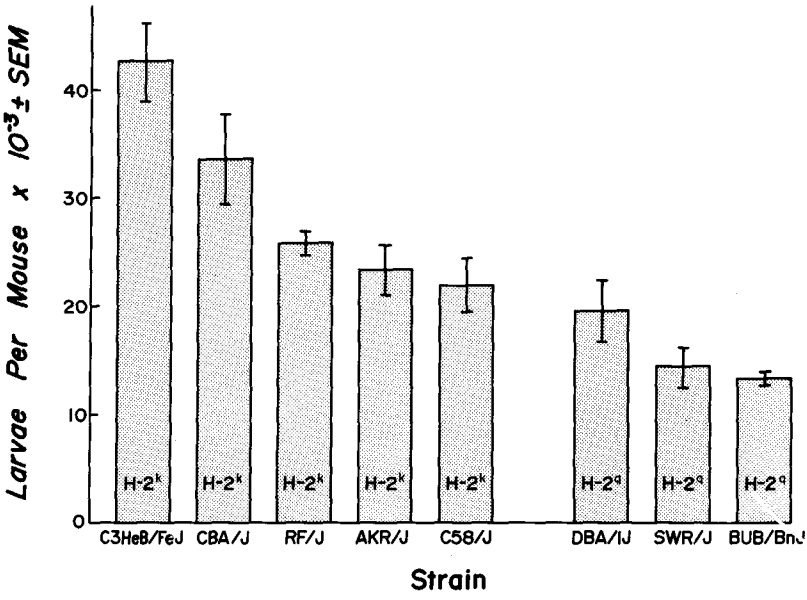

Fig. 2. Strains of mice sharing the same *H-2* haplotype but expressing different genetic backgrounds were infected with *T. spiralis* and larval counts determined 30 days later. Five strains expressing the *H-2*^{k} haplotype and three expressing the *H-2*^{q} haplotype were tested. *Vertical bars* represent the larval count means of ten mice; the standard error is given at the top of the bars. The *H-2* haplotype of each strain is indicated within the *vertical bar*.

for example, are significantly more susceptible to infection than are C58/J or AKR/J mice, and DBA/1J mice are more susceptible than are the strains SWR/J and BUB/BnJ. Furthermore, if C58/J or AKR/J (*H-2*^{k}) mice are compared to DBA/1J (*H-2*^{q}), the differences in susceptibility are not significant. These results, showing that non-*H-2* genes also influence susceptibility, clearly demonstrate the need to use congenic strains of mice in studies directed at evaluating the role of *H-2* genes in this host-parasite system. It should also be noted that the differences in susceptibility attributable to *H-2* genes (Fig. 1) were of a greater magnitude than the differences attributable to background genes alone (Fig. 2). The possible interaction of *H-2* genes with non-*H-2* genes will be explored in subsequent studies.

Having established that genes within the MHC influence susceptibility to *T. spiralis* infection, we initiated studies to map the genes involved. In these experiments, congenic *H-2* recombinant strains of mice were tested for susceptibility. The results are shown in Table 1. All strains expressing the *H-2*^{k}-derived genes to the left of the *I-E* subregion were highly susceptible to infection similar to B10.BR (*H-2*^{k}). Mice expressing the resistant *s* alleles in the *K* end were significantly more resistant even if expressing the *k* alleles in the *D* end ($p < 0.01$). This relationship is clearly shown when susceptibilities of the strains B10.S (8R) and B10.HTT are compared. Additionally, larval counts for the strain B10.S (8R) are significantly higher ($p < 0.03$) than for the strain B10.A (4R), indicating that genes mapping to the right of the *I-A* subregion may also be important. The recombinant

Table 1. Partial mapping of genes that influence susceptibility to *T. spiralis**

Strain	Haplotype									Larval Count Mean ± SEM
	K	*A*	*B*	*J*	*E*	*C*	*S*	*G*	*D*	
B10.BR	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	26 640 ± 3 236
B10.AKM	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*q*	32 760 ± 1 784
B10.AM	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*k*	*b*	26 502 ± 4 594
B10.A(1R)	*k*	*k*	*k*	*k*	*k*	*d*	*d*	?	*b*	23 334 ± 3 156
B10.M(17R)	*k*	*k*	*k*	*k*	*k*	*d*	*d*	*d*	*f*	28 436 ± 2 582
B10.S(8R)	*k*	*k*	?	?	*s*	*s*	*s*	*s*	*s*	29 120 ± 2 760
B10.A(4R)	*k*	*k*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	19 760 ± 3 023
B10.S	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	*s*	12 600 ± 646
B10.HTT	*s*	*s*	*s*	*s*	*k*	*k*	*k*	*k*	*d*	14 000 ± 1 050
B10/Sn	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	*b*	17 080 ± 1 903
B10.A(5R)	*b*	*b*	*b*	*k*	*k*	*d*	*d*	*d*	*d*	17 500 ± 2 587

* Congenic, *H-2*-recombinant strains of mice were infected with 150 *T. spiralis* larvae obtained from C3HeB/FeJ mice and total body larval counts determined 30 days later. The recombinant haplotype for each strain is shown and the susceptible *k* alleles are underscored. Larval count means represent values from at least ten mice.

strains tested in the above experiment establish that a gene mapping in the *K* end (and possibly another in the *D* end) influences susceptibility to *T. spiralis*. Further mapping studies are presently under way.

As another approach to definition of the role of *H-2* genes in *T. spiralis* infections, we tested the effect on susceptibility to infection of injecting alloantisera specific for products of *I*-region genes. Results of representative experiments are shown in Figure 3. Injection of C3HeB/FeJ mice with anti-$(ABJ)^k$ sera resulted in a significant reduction in the number of larvae when compared to NMS injected controls. Further experiments showed that injection of antiserum with specificity for $(AB)^k$ alone resulted in fewer larvae successfully encysting in the muscle. Antiserum with specificity for only $I\text{-}J^k$ gene products was incapable of inducing any significant effect. Injection of anti-$(JEC)^k$ serum also failed to alter larval counts (data not shown).

Our studies indicate that *MHC*-linked genes influence susceptibility to *Trichinella* infection in mice. We can postulate several mechanisms by which this could happen. (1) One or more *MHC*-linked immune response or immune suppressor genes may control responses to functional parasite antigens. These genes may influence responses to several different parasite antigens or susceptibility could depend on complementation or interaction between two or more genes. (2) *T. spiralis* may acquire and express host H-2 antigens on its cuticular surface, thereby inducing the stimulation of H-2-restricted effector or suppressor cell populations with specificities for parasite antigens. It is known that H-2 products are expressed on the tegument of *Schistosoma mansoni* schistosomules (Sher et al. 1978) and we are presently testing different life stages of *T. spiralis* to see if they too may bear such host determinants.

We have shown that an ongoing infection with *T. spiralis* can be influenced by injection of alloantisera with specificity for products of *I*-region genes. A probable

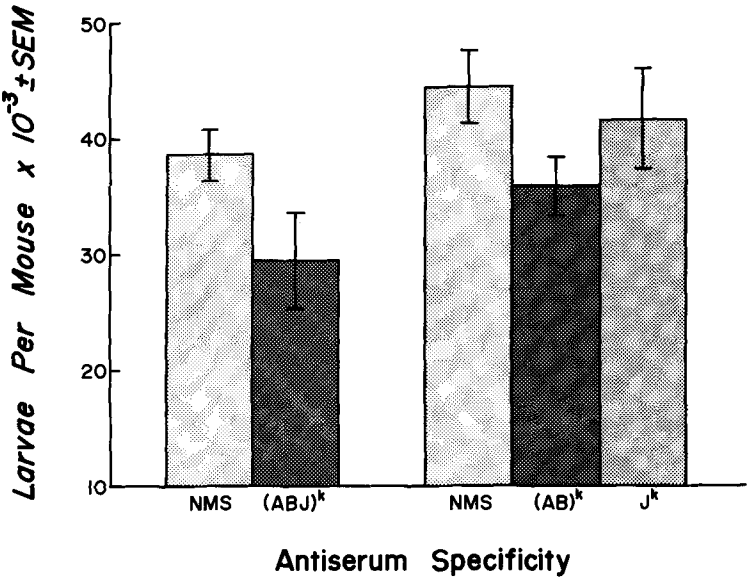

Fig. 3. Groups of ten C3HeB/FeJ ($H\text{-}2^k$) mice were infected with *T. spiralis* larvae and beginning on day 8 postinfection, and daily for 14 days thereafter, each mouse was injected i. p. with 7 µl of alloantiserum or NMS diluted in 0.25 ml sterile saline. Total body larval counts were determined 30 days postinfection. Alloantisera were made as follows: anti-$(ABJ)^k$ serum, (A.BY × B10.HTT)F_1 anti-A.TL (microcytotoxicity titer 1/800 vs donor spleen cells); anti-$(AB)^k$ serum, (B10.S(9R) × ATFR-5)F_1 anti-A.TL (microcytotoxicity titer 1/800 vs donor spleen cells); anti-$I\text{-}J^k$ serum, B10.HTT anti-B10.S(9R) (blocks MLR vs $I\text{-}J^k$ spleen cells at 1/100 dilution). Mice receiving anti-$(ABJ)^k$ serum showed significantly reduced larval burdens ($p < 0.01$, two way analysis of variance, one of three experiments shown). Injection of anti-$(AB)^k$ serum also resulted in significantly reduced larval counts ($p < 0.01$, Mann Whitney Rank Sum Test, one of two experiments shown). Injection of anti-$I\text{-}J^k$ antiserum had no effect ($p > 0.05$, one of two experiments shown).

explanation for reduced larval counts in injected mice is that the antisera in small amounts stimulate specific cell populations bearing I-A or I-B-coded antigens in the generation of effector cells or eliminate suppressor cells. Alternatively, if the parasite adsorbs and expresses host H-2 products, alloantibodies may react with the parasite directly and mediate damage via antibody-dependent cellular effectors. Further studies are required to find clues to these mechanisms.

We have shown, using congenic strains of mice, that genes within the MHC are important in controlling susceptibility to primary infection with *T. spiralis*. At least one gene mapped to the *K* end of the *H-2* complex is important in influencing this susceptibility. If infected mice are injected with specific alloantiserum directed at products of *I-A* or *I-B*-region genes, the number of larvae successfully encysting in the muscle is reduced. In addition to *H-2* genes, genes within the genetic background are also involved in controlling susceptibility to this parasite. Based on results of susceptibility trials in congenic strains of mice, it appears that *H-2* genes exert a more pronounced effect on the magnitude of differences between strains than do genes within the genetic background; however, the susceptibility of a given

individual will be determined by the cumulative effect of all alleles expressed at relevant loci both inside and outside the MHC. Susceptibility to infection, as determined by total body larval counts, may be influenced by (a) the number of worms allowed to establish in the mucosa of the small intestine, (b) the rate at which worms are expelled from the gut, (c) the fecundity of the female worms, or (d) the numbers of migrating newborn larvae that successfully encyst in the muscle. Experiments are under way to clarify the importance of each of the above factors in contributing to the *H-2*-linked differences in susceptibility observed in our studies. This work is the first demonstration that genes within the MHC are involved in influencing susceptibility to infection with a metazoan parasite. Most importantly it provides the immunogeneticist and the immunoparasitologist with a well defined genetic model which can be used to further explore immune responsiveness in host-parasite interactions.

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