Genetic Control of Murine Resistance to *Toxoplasma gondii*

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The genetics of murine susceptibility to *Toxoplasma gondii* was investigated in inbred mice and their F1 and F2 offspring. Among four strains of congenic mice of the B10 background, those with H-2 saturated and H-2d alleles were more susceptible than those with H-2d and H-2d heterozygous genotypes. Breeding studies utilizing three of these strains demonstrated linkage between the H-2 allele and greater susceptibility. These data suggest the existence of an H-2-linked gene affecting susceptibility to *T. gondii*.

We have previously demonstrated differences in susceptibility to *Toxoplasma gondii* among inbred strains of mice (1). Our data, along with the recently published observation by Kamei et al. (6) of a strain of mice highly susceptible to *T. gondii*, are consistent with the hypothesis that genetic factors determine the differences in susceptibility observed. In this report we present data that demonstrate that at least one of the genes determining *Toxoplasma* susceptibility is linked to the murine major (H-2) histocompatibility locus, and that another is possibly linked to the H-13 locus. Furthermore, because of the presence of phenotypic complementarity, it appears that at least two of the disease susceptibility genes may function through different mechanisms.

MATERIALS AND METHODS

**Animals.** The following inbred strains of mice were used: BALB/c, C57Bl/6J, and BALB/b from Jackson Laboratories, Bar Harbor, Me., Simonsen Laboratories, Gilroy, Calif., or local breeder colonies at Stanford University School of Medicine. At the time they were used in experiments, all mice were 2 to 4 months old; their sex is indicated in Results under the specific experiments. Congenic mice, differing only at the H-2 locus, of B10.A, B10.BR, B10, and B10.D2 strains were obtained from Jackson Laboratories and were used at 6 to 12 months of age. F1 and F2 offspring of the congenic animals were used as indicated in specific experiments at 6 to 12 months of age. In the experiments with congenic mice, use of the older age ranges of the mice was unavoidable due to the fact that it was necessary to breed most of our own animals. Space and financial constraints did not permit us to breed sufficiently to use only younger animals. These same constraints limited the numbers of mice that could be used in each experiment to 8 to 15. Mice were age-matched within each experiment.

Recombinant inbred mice of the D, G, H, I, J, and K genotypes were bred from parental strains C57Bl/6J and BALB/c were also obtained from Jackson Laboratories and were used 2 to 4 months old when used (2).

All animals were negative for *Toxoplasma* antibodies as measured in the Sabin-Feldman dye test (5) prior to their use in experiments.

**Preparation of *T. gondii*.** The C56 strain of *T. gondii* (isolated in 1961 from the ovary and oviduct of a chicken by Jacobs and Melton at the National Institutes of Health) was used in all experiments. Trophozoites were obtained from the peritoneal fluid of Swiss-Webster mice (Simonsen Laboratories) which had been inoculated 6 days earlier with brain from Swiss-Webster mice chronically infected with *T. gondii* strain C56 (8). In initial experiments the peritoneal fluid was filtered to separate the organism from peritoneal exudate cells (15). After it was shown that filtration was unnecessary for this experimental model, in subsequent experiments the peritoneal fluid was
processed by disrupting the small numbers of host peritoneal cells by repeated passage through a 27-gauge needle without subsequent filtering.

Mice were inoculated with T. gondii either intraperitoneally (i.p.) or subcutaneously, the latter method producing a less virulent infection. The inoculations were administered as a suspension of trophozoites in 0.2 ml of Hanks balanced salt solution. Mice were given water and laboratory chow ad libitum, and no medications were administered. Mortality was recorded daily for 25 to 30 days. Animals dying during this period, as well as survivors, were examined for the presence of T. gondii infection as previously described (16).

H-2 typing. H-2 typing was performed by standard hemagglutination and/or lymphocyte microcytotoxicity techniques (3, 17). Alloantisera D2, D4, D11, D21, D23, and D32, obtained from the National Institutes of Health Serum Bank, Bethesda, Md., were used to identify H-2 alleles in specific F2 experimental mice.

Statistics. Statistics were performed by the chi-square method or Mann-Whitney U test (10, 12).

RESULTS

Susceptibility and the H-2 locus. Congenic mice of the B10 background were used in initial studies to test the effect of H-2 differences. Results of a representative experiment using 5 × 10⁴ organisms and female mice are shown in Fig. 1. It is clear that B10 and B10.A mice were highly susceptible when compared to B10.D2 and B10.BR mice. Confirmatory data demonstrating these differences in susceptibility with respect to H-2 differences were also obtained in separate experiments, using a challenge inoculum of 5 × 10⁶ organisms (data not shown). Although these data support the hypothesis that H-2-linked genes affect murine susceptibility to T. gondii, formal proof of linkage requires testing of the appropriate offspring of selected matings. Therefore, (B10.A × B10.D2) F2 and (B10.A × B10.BR) F2 mice were tested for susceptibility to the parasite as a function of their inherited H-2 types. The results depicted in Fig. 2a reveal that for the former group, i.e., (H-2⁻⁺ × H-2⁻/⁻) F2 male mice, H-2⁻/⁻ genotype are more susceptible than H-2⁻/⁺ genotype animals as judged by time to death (e.g., on day 12 the difference in mortality between H-2⁻/⁻ and H-2⁻/⁺ was significant [P < 0.02]). In Fig. 2b are shown data for the male offspring of H-2⁻/⁻ × H-2⁻/⁻ breeding, and again mice of the H-2⁻/⁻ genotype were significantly more susceptible (e.g., on day 25, P < 0.04).

Susceptibility and the H-13 locus. Based on our previous studies (1), it was considered highly probable that genes at loci other than H-2 would also affect susceptibility to T. gondii. Therefore, recombinant inbred mice originating from BALB/c × C57Bl/6J were tested to seek correlations between susceptibility and several identifiable genetic markers (2). Of the two original parental strains, the C57Bl/6J mice are significantly more susceptible than the BALB/c mice; e.g., as seen in Fig. 3, cumulative mortality in males challenged with 5 × 10⁴ Toxoplasma i.p. at day 15 was 68% for C57Bl/6J and 0% for BALB/c (P < 0.01). In Fig. 4 is shown the cumulative mortality figures for males of each of the recombinant inbred lines. The BK mice were clearly most susceptible (e.g., cumulative mortality on day 14, BK versus BH significant at P < 0.01, and BK versus BD significant at P < 0.001). At higher inoculum sizes of T. gondii the difference between parental strains was

**Fig. 1.** Susceptibility of female congenic mice of the B10 background to challenge with 5 × 10⁴ T. gondii i.p. There were 10 mice in each group.

**Fig. 2.** (a) Susceptibility of male F2 offspring of B10.A (H-2⁻/⁻) by B10.D2 (H-2⁻/⁺) to challenge with 10⁴ T. gondii i.p. There were 10 mice in each group except for H-2⁻/⁺, which had 8. (b) Susceptibility of male F2 offspring of B10.A (H-2⁻/⁻) by B10.BR (H-2⁻/⁻) to challenge with 5 × 10⁴ T. gondii i.p. There were 10 to 12 mice in each group.
for BJ, 81% for BK, 25% for BH, and 25% for BD). The relationship of rank order of susceptibility to the H-13 locus will be discussed below.

Toxoplasma challenge and experiment reproducibility. Because of the technical difficulty of preparing the large numbers of organisms necessary for these experiments, the virulence of each preparation was not necessarily the same between different experiments (1). Therefore, all comparisons between groups of mice were for animals tested concurrently with the same preparation of T. gondii. As a result of this variation in virulence, the inoculum size in some experiments was not optimal for revealing differences between mouse strains, and, therefore, some differences among groups did not achieve statistical significance. This problem was most apparent in the experiments with congenic mice. For example, in some experiments comparing B10.A with B10.D2, or B10.A with B10.BR, differences in mortality between parental groups was statistically significant despite the fact that the differences between the F2 homozygotes were not present or did not achieve statistical significance. All statistically significant differences were consistent and in the direction reported.

Phenotypic complementarity. Mortality of (B10.D2/n × C57Bl/6J) F1 offspring was compared with mortality of each parental strain at different doses of Toxoplasma (Fig. 5a, b, and c). With the least virulent challenge (5 × 10⁴ organisms subcutaneously, Fig. 5a), only marginal statistically insignificant differences were observed, with all mice relatively resistant. With the inoculum of intermediate virulence (5 × 10⁴ organisms i.p., Fig. 5b), C57Bl/6J parents were slightly less susceptible than B10.D2/n parents, but F1 mice were completely resistant (P < 0.003 at day 30). At a higher i.p. challenge dose (Fig. 5c), both parents were equally and highly susceptible, whereas the F1 offspring remained distinctly less susceptible than the parents (P < 0.003 at day 14). The same phenomenon of increased resistance of hybrids was also observed (data not shown) with B10.D2 and BALB/b (congenic with BALB/c but possessing the H-2b allele instead of H-2d) and their F1 offspring.

**DISCUSSION**

Variation in susceptibility of mouse strains to a variety of bacterial and viral infections has been well known for many years (18, 19). The genetics of resistance has been explored in depth for some viral infections. For example, host immunity to intracerebral injection of lymphocytic choriomeningitis virus in mice has been shown to be controlled in part by a dominant gene
closely linked to the H-2 locus (11), a chromosomal region that contains genes governing specific immune responses, mixed leukocyte reactivity, and graft rejection (7, 9). The genetics of resistance to other nonviral pathogens which are primarily intracellular are much less clearly defined. For example, murine resistance to Salmonella typhimurium was earlier thought to be linked to the H-2 locus (14). Recent data, however, have suggested that resistance follows simple Mendelian dominance and is not linked to H-2 genes (13). Studies examining genetic control of natural resistance to Leishmania donovani in mice also failed to show a relationship to H-2 type. It is of interest that in this H-2-independent resistance, strains resistant to L. donovani organisms were also resistant to S. typhimurium (4, 13). This correspondence in relative susceptibility of mouse strains does not extend, in our hands, to resistance to T. gondii.

Our data suggest that resistance to T. gondii in mice is under multigenic control. The expression of the different genes determining this resistance is markedly influenced by the dose of organisms since at high doses all strains of mice succumbed rapidly, and at low doses almost all mice of each strain survived infection. Within the intermediate range of challenge, consistent and statistically significant differences were readily observed among different strains and among corresponding offspring. The use of a relatively avirulent strain of T. gondii similarly permitted the dissection of genetic differences that might not otherwise have been revealed (J. S. Remington and J. L. Krahenbuhl, Immunology of Toxoplasma Infections, in press). Utilizing congenic mice of a B10 background, it has been demonstrated that at least one of the murine genes determining resistance to T. gondii is linked to the H-2 locus. Utilizing recombinant inbred mice, our data also strongly suggest that another susceptibility gene is linked to the H-13 locus. For example, looking at the rank order of susceptibility at the most informative dose of infectious challenge (i.e., 5 x 10^2 organisms i.p., the dose maximizing differences among strains) the three most susceptible strains, BK, BJ, and BE, all share the H-13 allele of the susceptible C57Bl/6J parent. The four most resistant strains, BH, BI, BD, and BG, all share the H-13 allele of the resistant BALB/c parent. Neither coat color nor any of the other originally described differences (H-1, H-2, Hw13, Hw17, Hw19, Hw20, Hw35, Hw38, Hw80, Hw96) among these strains showed similar correlation with susceptibility. Furthermore, as shown by the marked resistance of F1 offspring (derived from two relatively susceptible parental strains), it appears that more than one mechanism of action

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**Fig. 5. Susceptibility of C57Bl/6J and B10.D2 females and their (B10.D2 x C57Bl/6J) F1 female offspring when challenged with (a) 5 x 10^4 T. gondii subcutaneously, 8 to 10 mice in each group; (b) 5 x 10^3 i.p., 10 to 13 mice in each group; (c) 5 x 10^4 organisms i.p., 13 mice in each group. Symbols: (C) F1, (B10.D2/n × C57Bl/6J); (□) C57Bl/6J; (△) B10.D2."
for different infection susceptibility genes must be invoked to explain the phenotypic complementarity involved.

In conclusion, our data demonstrate that murine susceptibility to *T. gondii* is under multigenic control with at least one of the genes linked to the H-2 locus, and different mechanisms of action are suggested for some of the infection susceptibility genes because of the phenomenon of genetic complementarity. The availability of congenic animals differing at only one of these disease susceptibility genes should permit development of a model in which it would be possible to isolate and identify the mechanism of action of that particular gene through a study of different aspects of the immune responses to infection of susceptible versus resistant strains.

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