

H-2-LINKED GENES INFLUENCE THE SEVERITY OF  
HERPES SIMPLEX VIRUS INFECTION OF THE  
PERIPHERAL NERVOUS SYSTEM

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In some individuals herpes simplex recrudesces periodically and between attacks the virus lies dormant (latent) in sensory neurons of the peripheral nervous system (1, 2). There are many potential reasons for individual variation in the natural history of herpes simplex. Prominent aspects are variation in the phenotype of different HSV strains and the ability of the individual host to contain the infection. With respect to the latter, both innate resistance and adaptive immunity have been extensively studied, particularly in animal models of the disease.

Genetic resistance to herpes is well established. In 1975, Lopez (3) reported that C57BL/6 mice could survive >1,000 times the dose of HSV injected intraperitoneally than a variety of other inbred mouse strains. Hybrid analysis showed that this potent innate resistance is controlled by two independently segregating autosomal genetic loci, which are not linked to the MHC (4). The location of these genes and the identity of the proteins that they encode remain unknown.

Despite the established importance of T cell-mediated mechanisms in recovery from herpes simplex (5), there have been no reports implying that genes within the MHC can influence the quality of the protective response to this infection. However, the experimental protocols used in genetic studies to date have been weighted in favor of the discovery of non-MHC-linked genes, because resistance has generally been equated with survival of the animal after intraperitoneal injection of virulent virus, which rapidly kills susceptible mice. Therefore, the activity of any gene influencing the specific immune response, which takes several days to exert a measurable effect (6), is unlikely to be uncovered, because animals that lack the powerful innate resistance genes of the C57 Black background develop an overwhelming infection of the nervous system before specific immunity has developed.

Reported here is the first clear demonstration that genes linked to the MHC can exert a substantial influence on the extent of primary herpes simplex infection of mice. To measure this effect, three major changes to previously described experimental protocols were made. First, long-term survival of animals was guaranteed by performing all experiments in H-2 congenic mice with the C57B/10 background. This also had the effect of equalizing the influence of non-MHC-linked genes. Second, instead of infecting the mice intraperitoneally, virus was inoculated into the skin,

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which results in an acute ganglionic infection by centripetal spread of virus along sensory nerves, resembling the spread of virus to the human nervous system. Third, differences between the mouse strains were judged quantitatively, according to the amount of infectious virus recovered from the sensory dorsal root ganglia.

### Materials and Methods

*Mice.* Female mice were used at 6–8 wk of age. C57B10 (H-2<sup>b</sup>), B10.D2 (H-2<sup>d</sup>), B10.Br (H-2<sup>k</sup>), and B10.A (H-2<sup>k/d</sup>) were obtained from the specific pathogen-free facility, Animal Resource Centre, Perth, Western Australia.

*Virus.* A well-characterized clone of a low passaged oral isolate of HSV type 1, strain SC16, was used (7). This virus is moderately virulent in mice (8). Stocks were grown in BHK-21 cells and stored at  $-70^{\circ}\text{C}$ .

*Infection of Mice.* The zosteriform model used in these experiments has been described in detail elsewhere (9). Briefly, after depilation with Nair (Carter-Wallace, NSW, Australia), a small patch of skin on the left flank was scarified with a 27-gauge needle through a 10- $\mu\text{l}$  drop of virus suspension containing  $5 \times 10^4$  plaque forming units (pfu).

*Titration of Virus in Tissue Samples.* The eighth to thirteenth left thoracic dorsal root ganglia were removed and stored at  $-70^{\circ}\text{C}$  until required. The pooled ganglia from each mouse were homogenized in a 1-ml glass tissue grinder (Wheaton Industries, Millville, NJ) and 10-fold dilutions of the homogenate were tested for presence of infectious virus using a standard plaque assay (10).

### Results and Discussion

Groups of H-2 congenic mice were studied 5, 7, 9, and 11 d after infection with HSV (Table I). After 5 d, a similar amount of virus was recovered from each of the strains tested. However, 7 d after infection B10.Br mice yielded 400 times more virus from their ganglia than did B10.D2 animals ( $p = <0.001$ ). B10.A mice behaved in an intermediate way. By day 9, most of the remaining B10.Br mice had died. No mice of this strain survived until day 11, whereas there were no deaths among the other strains.

In a similar experiment (Fig. 1), focusing on the seventh day after infection, concordant results were obtained.

TABLE I  
*Recovery of Virus from Ganglia of H-2 Congenic Mice  
Various Days After Infection*

Host	Days after infection			
	5	7	9	11
	<i>Log<sub>10</sub> geometric mean PFU/sample</i>			
C57B10	3.1 (2.8–3.3)*	2.9 (1.8–3.5)	<1.0	<1.0
C57B10.D2	3.1 (3.0–3.1)	1.8 (1.0–2.4)	<1.0	<1.0
C57B10.A	3.4 (3.0–4.0)	2.8 (2.5–3.4)	<1.0	<1.0
C57B10.Br	3.4 (3.0–3.5)	4.4 (3.8–4.9)	–†	–†

\* Range.

† No data owing to death of mice.

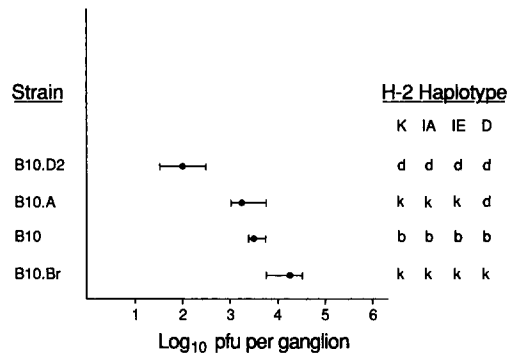


FIGURE 1. Recovery of HSV from ganglia of H-2 congenic mice 7 d after infection. Points represent the geometric mean of values from five animals. Bars indicate range.

These data show that C57BL10 H-2 congenic mice vary in their ability to control the spread of virus within the peripheral nervous system during the period 5–9 d after infection with a moderate dose of SC16. Virus clearance from the ganglia commenced between the fifth and seventh day after infection in H-2<sup>b</sup> (C57B10 parent strain) and H-2<sup>d</sup> (C57B10.D2) mice, but not in H-2<sup>k</sup> (C57B10.Br) animals. Mice of the latter haplotype were sufficiently compromised in their ability to control the disease for a fatal infection to develop, despite the innate resistance expected of the C57B10 background. C57B10.A mice, which are a natural intra-H-2 k/d recombinant, behaved in an intermediate fashion, suggesting that the introduction of k genes at the K and IA loci is sufficient to reduce the ability of the animals to control the infection at this critical period, when the specific immune response is becoming effective. In this context the finding of Jennings et al. (11), that cytotoxic T cells preferentially see HSV antigens in association with H-2K rather than H-2D, may be relevant.

Overall, the ability to control a challenge with herpes simplex has two elements. First, there is powerful innate resistance to infection, which was first demonstrated by Lopez (3), and is mediated by as yet unknown mechanisms, and second, there is an MHC-linked phase, during which certain hosts (such as C57B10.Br mice) may be significantly compromised in their ability to initiate optimum clearance of virus from the nervous system. The extent to which the nervous system is involved during primary infection may influence the subsequent behavior of the disease, because the ganglia are the main reservoir of infection between recurrences.

Attempts in humans to find HLA types that are associated with severe or frequently recurrent herpes simplex have been disappointing, although some weak associations have been reported (12, 13). However, against a variable background of powerful innate resistance genes and variations in the neurovirulence of different HSV strains (14), the chance of discovering MHC-linked effects using population studies is predictably very low. Even in studies using inbred mice, differences between H-2 haplotypes of two to three orders of magnitude have been overlooked until now.

Immunological hyporesponsiveness (in the form of a poor CTL response) to whole influenza virus proteins has recently been demonstrated in association with certain mouse H-2 haplotypes (15). The large variation in the response of H-2<sup>k</sup> and H-2<sup>d</sup> mice to challenge with HSV could therefore be the result of a defect in the specific

immune response associated with the H-2<sup>k</sup> haplotype, despite the complex nature of the antigenic challenge. This is a focus of continuing work.

Variation of two to three orders of magnitude in the ability of different inbred H-2 congenic mice to control HSV infection demonstrates the importance of genetic diversity with respect to the MHC in outbred populations. Challenge with infectious agents could of course be a significant selective force in maintaining such diversity.

### Summary

Infection of the peripheral nervous system was studied after inoculation of HSV into the flank skin of H-2 congenic mice. The amount of virus recovered from the sensory ganglia varied significantly between the mouse strains tested. Differences became apparent 7 d after infection, at which time the severity of disease in H-2<sup>k</sup> mice was two to three orders of magnitude greater than that in H-2<sup>d</sup> animals.

The association of the H-2<sup>k</sup> haplotype with impaired ability to clear HSV from the nervous system is the first clear demonstration that genes within the MHC can influence the severity of primary herpetic infection, in spite of numerous studies on genetic resistance to this disease.

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