Roles of the $H-2D^b$ and $H-K^b$ genes in resistance to persistent
Theiler’s murine encephalomyelitis virus infection of the
central nervous system

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Theiler’s murine encephalomyelitis virus, a member of the Picornaviridae family, persists in the spinal cord of susceptible strains of mice. Resistant strains of mice, such as the $H-2^b$ strain, clear the virus infection after an acute encephalomyelitis. The $H-2D$ locus, but not the $H-2K$ locus, has a major effect on this resistance, although both loci code for MHC class I molecules with similar general properties. For the present work, we rendered susceptible $H-2^q$ FVB/N mice transgenic for either the $H-2D^b$ gene, the $H-2K^b$ gene or a chimeric $H-2D^q/K^q$ gene in which the exons encoding the peptide-binding groove of the $H-2K^q$ gene have been replaced by those of the $H-2D^b$ gene. Mice transgenic for either the $H-2D^b$ gene or the chimeric $H-2D^q/K^q$ gene were significantly more resistant to persistent virus infection than mice transgenic for the $H-2K^q$ gene, suggesting that the difference in the effects of the $H-2D^b$ gene and the $H-2K^q$ gene are due to the nature of the peptides presented by these class I molecules.

The DA strain of Theiler’s murine encephalomyelitis virus (TMEV), a member of the Picornaviridae family, is responsible for a biphasic disease of the murine CNS. The first phase is an acute encephalomyelitis, which takes place during the first 2 weeks after intracerebral inoculation. This is followed, only in genetically susceptible animals, by a persistent infection of the white matter of the spinal cord, resulting in inflammation and chronic primary demyelination. This natural disease is one of the best animal model systems available for multiple sclerosis (Dal Canto & Lipton, 1977; Monteyne et al., 1997). Susceptibility to the second phase of disease varies greatly among inbred strains of mice (Lipton & Dal Canto, 1979; Lipton & Melvold, 1984). Several genetic loci implicated in susceptibility to virus persistence, demyelination and clinical disease have been identified (Brahic & Bureau, 1998). A locus with a major effect on demyelination was first located in the H-2D region of the MHC (Clatch et al., 1985; Lipton & Melvold, 1984; Rodriguez & David, 1985; Rodriguez et al., 1986). Resistance was dominant over susceptibility (Patick et al., 1990). Studying susceptibility to persistent TMEV infection in 17 inbred strains of mice, we later showed that the b haplotype was associated with resistance, the q haplotype was associated with full susceptibility, and the k, d, and s haplotypes were associated with intermediate susceptibility. The resistance that was brought by the b haplotype was dominant. The locus controlling virus persistence was mapped to the H-2D region of the MHC (Bureau et al., 1992). It is most likely that the $H-2$ loci that control the susceptibility to persistent TMEV infection and susceptibility to demyelination are the same (Bureau et al., 1992; Patick et al., 1990; Rodriguez et al., 1986).

Several observations suggest that the susceptibility gene present in the H-2D region is an MHC class I $H-2^b$ gene (Azoulay-Cayla et al., 1994, 2000; Fiette et al., 1993; Lin et al., 1997; Pullen et al., 1993; Rodriguez et al., 1986, 1993). The role of the $H-2D^b$ gene in resistance was formally demonstrated by showing that (H-2q) FVB/N mice, made transgenic for the $H-2D^b$ gene, are resistant to virus persistence (Azoulay-Cayla et al., 1994; Rodriguez & David, 1995) and that mutations in the $H-2D^b$ gene reduce or delay virus clearance (Lipton et al., 1995).

The highly polymorphic exons 2 and 3 of class I genes code for the peptide-binding groove of the class I molecule. The polymorphism of this groove explains the large repertoire of peptides that can be presented to CD8+ T cells. The class I genes of the mouse are located in two sub-regions of the MHC, $H-2K$ and $H-2D/L$. To date, no difference in the ability to present peptides has been demonstrated between the families of $H-2K$ and $H-2D/L$ proteins. In spite of this, resistance to both TMEV persistence and virus-induced demyelination is linked to the $H-2D$ locus, and not to the $H-2K$ locus, in strains

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**SHORT COMMUNICATION**

Monteyne et al., 1997). Susceptibility to the second phase of disease varies greatly among inbred strains of mice (Lipton & Dal Canto, 1979; Lipton & Melvold, 1984). Several genetic loci implicated in susceptibility to virus persistence, demyelination and clinical disease have been identified (Brahic & Bureau, 1998). A locus with a major effect on demyelination was first located in the H-2D region of the MHC (Clatch et al., 1985; Lipton & Melvold, 1984; Rodriguez & David, 1985; Rodriguez et al., 1986). Resistance was dominant over susceptibility (Patick et al., 1990). Studying susceptibility to persistent TMEV infection in 17 inbred strains of mice, we later showed that the b haplotype was associated with resistance, the q haplotype was associated with full susceptibility, and the k, d, and s haplotypes were associated with intermediate susceptibility. The resistance that was brought by the b haplotype was dominant. The locus controlling virus persistence was mapped to the H-2D region of the MHC (Bureau et al., 1992). It is most likely that the $H-2$ loci that control the susceptibility to persistent TMEV infection and susceptibility to demyelination are the same (Bureau et al., 1992; Patick et al., 1990; Rodriguez et al., 1986).

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The highly polymorphic exons 2 and 3 of class I genes code for the peptide-binding groove of the class I molecule. The polymorphism of this groove explains the large repertoire of peptides that can be presented to CD8+ T cells. The class I genes of the mouse are located in two sub-regions of the MHC, $H-2K$ and $H-2D/L$. To date, no difference in the ability to present peptides has been demonstrated between the families of $H-2K$ and $H-2D/L$ proteins. In spite of this, resistance to both TMEV persistence and virus-induced demyelination is linked to the $H-2D$ locus, and not to the $H-2K$ locus, in strains
with b, d and k H-2 haplotypes (Rodriguez et al., 1986). Class I genes are not expressed in cells of a normal CNS; expression is induced during infection. Interestingly, the levels of expression of the H-2D and H-2K molecules in the CNS following inoculation with TMEV are different (Altintas et al., 1993). Therefore, the exclusive role of the H-2D locus in virus clearance could be due either to particularities of gene expression in the CNS or to the nature of the viral peptides presented.

To examine if the peptide-binding groove of the H-2D<sup>b</sup> molecule bore all the determinants for virus clearance, we constructed transgenic FVB/N mice expressing a chimeric H-2K<sup>b</sup> gene in which exons 2 and 3 were replaced by the corresponding exons of the H-2D<sup>b</sup> gene.

The D1 line is an FVB/N mouse transgenic for the H-2D<sup>b</sup> gene and is resistant to persistent TMEV infection (Azoulay-Cayla et al., 1994). Two lines of FVB/N mice transgenic for the H-2K<sup>N</sup> gene, named K1 and K2, and three lines of FVB/N mice transgenic for a chimeric H-2D<sup>b</sup>/K<sup>N</sup> gene, named D/K1, D/K2 and D/K3, were constructed for the present study. To construct the chimeric gene, a BssHII–SpeI fragment containing exons 2 and 3 of the H-2D<sup>b</sup> gene was exchanged with the corresponding fragment of the H-2K<sup>N</sup> gene. All transgenes were genomic DNA segments that included the authentic class I promoter (Allen et al., 1984).

The level of expression of each transgenic class I molecule was determined and compared to that of the H-2D<sup>b</sup> and H-2K<sup>N</sup> proteins of C57BL/6 mice by FACS analysis on splenic T cells from two animals of each line. FVB/N and H-2D<sup>b</sup>−/− mice were used as controls. The monoclonal antibodies KH95 and 5F1, which are specific for H-2D<sup>b</sup> and H-2K<sup>N</sup> molecules, were used at saturating concentrations (Hasenkrug et al., 1987; Péramanu et al., 1999; Sherman & Randolph, 1981). The D1, D/K1, D/K2 and D/K3 transgenic lines expressed 31, 26.5, 49 and 34%, respectively, of the level of the H-2D<sup>b</sup> molecules measured for C57BL/6 mice. As expected, no H-2D<sup>b</sup> expression was detected in either FVB/N or H-2D<sup>b</sup>−/− mice. For the K1 line, the expression of the H-2K<sup>N</sup> molecule was 68% of that measured for C57BL/6 mice. H-2K<sup>N</sup> molecules were not detected for FVB/N mice. In summary, all transgenes were expressed at similar levels. The fact that we studied heterozygous transgenic mice might explain that the transgenes were expressed at lower levels than the H-2D<sup>b</sup> and the H-2K<sup>N</sup> genes in C57BL/6 mice.

The chimeric H-2D<sup>b</sup>/K<sup>N</sup> molecule possesses the peptide-binding groove of the H-2D<sup>b</sup> molecule within the context of an H-2K<sup>N</sup> molecule. It was important to determine if the anti-TMEV CTLs of H-2D<sup>b</sup>/K<sup>N</sup> transgenic mice recognized peptide VP<sub>212-130</sub>, an immunodominant H-2D<sup>b</sup>-restricted epitope (Dethlefs et al., 1997). D/K2 and C57BL/6 mice were inoculated intraperitoneally with 10<sup>5</sup> p.f.u. of TMEV and the cytotoxicity of splenocytes was measured using H-2<sup>b</sup> C57SV cells infected with TMEV or loaded with the VP<sub>212-130</sub> peptide as targets. As shown in Fig. 1, splenocytes from D/K2 mice lysed uninfected target cells loaded with the VP<sub>212-130</sub> peptide. Therefore, D/K2 transgenic mice raise TMEV-specific CTLs that recognize the same H-2D<sup>b</sup>-restricted VP2 epitope as the CTLs of C57BL/6 mice.

To examine if FVB/N mice transgenic for either the H-2D<sup>b</sup> gene or the H-2D<sup>b</sup>/K<sup>N</sup> gene were more resistant to persistent TMEV infection than FVB/N mice transgenic for the H-2K<sup>N</sup> gene, virus loads in the CNS of different transgenic lines were measured at various times post-inoculation (p.i.) by using a dot-blot assay, as described previously (Bureau et al., 1992) (Table 1 and Fig. 2). Pooled results for mice with the same transgene are shown in Fig. 2. At 45 days p.i. (Fig. 2C), virus loads were significantly lower for FVB/N mice transgenic for the H-2D<sup>b</sup> gene than those for FVB/N mice transgenic for the H-2K<sup>N</sup> gene (P = 0.045). For both lines, virus loads were significantly lower than those for control FVB/N mice. FVB/N mice transgenic for the H-2D<sup>b</sup>/K<sup>N</sup> chimeric gene were as resistant to persistent TMEV infection as FVB/N mice transgenic for the H-2K<sup>N</sup> gene (P = 0.9850). However, FVB/N mice transgenic for the H-2D<sup>b</sup>/K<sup>N</sup> chimeric gene were significantly more resistant than FVB/N mice transgenic for the H-2K<sup>N</sup> gene (P = 0.0145). These results indicate that the peptide-binding groove of the H-2D<sup>b</sup> molecule is an important determinant of resistance to persistent TMEV infection.

The virus load of all of the transgenic animals was lower
seven out of the nine numbers of cells positive for viral antigen (data not shown). In numerous inflammatory lesions of the white matter with large 45 days p.i. Out of 31 FVB
histopathological lesions and the presence of viral antigens. Therefore, these data were congruent with those obtained by measuring the amount of viral RNA in the spinal cord. The levels of both inflammation and viral antigen were lower in H-2D\(^b\) and H-2D\(^p\)/K\(^b\) transgenic mice than those in H-2K\(^b\) transgenic mice. The lesions in the latter were similar to those of susceptible FVB/N animals, although they were less extensive.

To shed light upon the mechanism by which the transgenes H-2D\(^b\) and H-2D\(^p\)/K\(^b\) reduce virus load, we studied virus infection in the H-2D\(^b\) and H-2D\(^p\)/K\(^b\) transgenic lines and in FVB/N controls both during acute encephalomyelitis (6 days p.i.) and at the beginning of chronic infection (21 days p.i.). At 6 days p.i., viral RNA levels in both the brain and the spinal cord were the same for the three lines of mice examined (Fig. 2A). Histopathologically, all transgenic and non-transgenic FVB/N mice showed the same pattern of infection and inflammation. Infected cells were mainly neurons of the hippocampus, the temporal cortex and the anterior horns of the spinal cord. At 21 days p.i., H-2D\(^b\) and H-2D\(^p\)/K\(^b\) transgenic mice were each infected with low levels of TMEV (Fig. 2B). Neither inflammation nor viral antigens were present in the brains of either control mice or H-2D\(^b\) and H-2D\(^p\)/K\(^b\) transgenic mice. Mild inflammation and a small number of cells positive for viral antigen were found in the spinal cord of H-2D\(^b\) and H-2D\(^p\)/K\(^b\) transgenic mice. In contrast, extensive

### Table 1. Persistent TMEV infection in the CNS at 6, 21 and 45 days p.i.

<table>
<thead>
<tr>
<th>Mouse line</th>
<th>Days p.i.</th>
<th>Organ*</th>
<th>No. of mice examined</th>
<th>Amount of viral RNA (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVB/N</td>
<td>6</td>
<td>B</td>
<td>17</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>SC</td>
<td>17</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>SC</td>
<td>20</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>SC</td>
<td>35</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>D1</td>
<td>6</td>
<td>B</td>
<td>4</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>SC</td>
<td>4</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>SC</td>
<td>9</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>SC</td>
<td>9</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>K1</td>
<td>45</td>
<td>SC</td>
<td>20</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>K2</td>
<td>45</td>
<td>SC</td>
<td>7</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>D/K1</td>
<td>6</td>
<td>B</td>
<td>7</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>SC</td>
<td>7</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>SC</td>
<td>8</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>SC</td>
<td>6</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>D/K2</td>
<td>6</td>
<td>B</td>
<td>5</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>SC</td>
<td>5</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>SC</td>
<td>11</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>SC</td>
<td>6</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>D/K3</td>
<td>6</td>
<td>B</td>
<td>5</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>SC</td>
<td>5</td>
<td>1.5 ± 0.3</td>
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<tr>
<td></td>
<td>21</td>
<td>SC</td>
<td>5</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>SC</td>
<td>6</td>
<td>0.7 ± 0.4</td>
</tr>
</tbody>
</table>

* B, Brain; SC, spinal cord.
inflammation and many cells containing viral antigen were observed in FVB/N mice. These results showed that resistance to persistent TMEV infection of FVB/N mice transgenic for either the H-2D\textsuperscript{b} gene or the H-2D\textsuperscript{b}/K\textsuperscript{b} gene corresponded to the ability to clear the infection after an early acute encephalomyelitis.

Two hypotheses can be formulated regarding the association of resistance with the H-2D and the H-2K locus. Either this is due to chance and to the small number of H-2 alleles that were studied. Accordingly, resistance will also be linked to the H-2K locus when more alleles are examined. Or it is due to an intrinsic property of the groove of the H-2D/L family of class I molecules which makes them more efficient at presenting TMEV epitopes.

In summary, our results confirm that the H-2D\textsuperscript{b} gene is essential for virus clearance, whereas the H-2K\textsuperscript{b} gene has a more modest role. Also, these data strongly suggest that the peptide-binding groove of the H-2D\textsuperscript{b} molecule contains all of the biological functions necessary to eliminate TMEV from the CNS.

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