SUSCEPTIBILITY TO THEILER'S VIRUS-INDUCED DEMYELINATION

Mapping of the Gene Within the H-2D Region

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Class I loci (K, D, and L of the murine MHC [H-2]) encode for molecules that are important in the interaction between virus-specific antigen and T-cytotoxic (Tc) effector cells. This interaction appears to be accomplished by T-cytotoxic effector cell recognition of both target H-2K/D gene products and antigen (1–3). Several virus-induced phenomena associated with immune function have been mapped to class I loci, particularly the H-2D end (3). The H-2D region harbors a group of genes that influences resistance to oncovirus-induced neoplasms such as Friend virus mouse leukemia (4, 5), murine Moloney virus leukemia (6), and radiation leukemia virus-induced tumorigenesis (7, 8). The H-2D region genes also have been implicated in susceptibility to autoimmune diseases such as experimental autoimmune thyroiditis (9). These immunogenetic studies suggest that one of the H-2D region genes or genes in close proximity may modulate the immune response of the host to a foreign virus-specific antigen or self antigen.

Theiler's murine encephalomyelitis (TME)¹ is an important model for study of viral persistence in the nervous system and immune-mediated demyelination (10, 11). This naturally occurring disease in mice has been considered an excellent model for multiple sclerosis because demyelination in the human disease may be the result of an immune-mediated response triggered by a persistent virus. The destruction of white matter is associated with inflammatory cells which play an active role in myelin breakdown. Also, the lesions are fewer and less severe in TME virus-infected (TMEV-infected) mice treated with immunosuppressive agents (12, 13) or with mAb directed at immune response gene products (14). Earlier studies demonstrated a wide range of genetic susceptibilities to TMEV-induced demyelination in mice from different strains (15, 16). We have shown, using morphologic criteria, that mice with similar genetic backgrounds, but different H-2 haplotypes, have different susceptibilities to demyelination

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¹ *Abbreviations used in this paper:* TME, Theiler's murine encephalomyelitis; TMEV, TME virus.

(17). Light and electron microscopy of spinal cord sections from mice with *s*, *f*, *p*, *r*, *v*, or *q* haplotype on a C57BL/10 background showed areas of primary demyelination in association with perivascular inflammation. In contrast, mice with identical backgrounds but *b*, *k*, or *d* haplotype showed no pathologic abnormalities in the white matter.

The present study was done to map the susceptible genes within the H-2 complex. Using mouse strains with congenic recombinant haplotypes, we demonstrate that the D region of the H-2 complex is primarily responsible for determining susceptibility or resistance to TMEV infection. In addition, mutation of the H-2D genes alters susceptibility to virus-induced demyelination. We also correlate the ability of TMEV to persist in the central nervous system and the TMEV-specific humoral response with genetic susceptibility.

Materials and Methods

Virus. The Daniels (DA) strain of TMEV was used for all experiments. The origin and passage history of the virus has been described (18–20). The brain and spinal cord from infected mice were assayed for virus by a plaque method on L2 cells (21). Virus for use as antigen was purified from infected BHK-21 cells by ultracentrifugation on cesium chloride density gradients.

ELISA To Detect TMEV-specific Antibody. Serum IgG directed against purified DA strain virus antigen was determined by ELISA. Purified DA antigen (100 ng/well) was adsorbed to 96-well microtest tissue culture plates (Falcon Labware, Oxnard, CA). The excess binding sites were saturated with 1% BSA. Plates were filled with 50% glycerol in PBS and stored frozen at -80°C .

Before the assay, plates were thawed and washed in 0.01 M PBS/0.05% Tween buffer (pH 7.4). Multiple serial dilutions of the test sera (1:800 to 1:12,800 in PBS) were made and 100 μl was added to individual wells; then the plates were incubated for 2 h at 37°C . After thorough washing of the plates, 100 μl of a 1:1,000 dilution of goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) was added; incubation was for 2 h at 37°C . After washing, 100 μl of Sigma Chemical Co. 104 phosphatase substrate was added and incubation was for 30 min. The OD at 410 nm was read in an MR 600 microplate reader (Dynatech Laboratories, Inc., Alexandria, VA). Sera from a known hyperimmune positive control as well as sera from noninfected mice were run with each plate.

Animals and Experimental Protocol. All mice were raised in the Mayo immunogenetic mouse colony. 4–10-wk-old female mice were inoculated intracerebrally with 2×10^5 PFUs of DA virus in a total volume of 10 μl . 5–20 animals from each strain were inoculated and examined daily for clinical signs of infection. All clinical observations were recorded by laboratory personnel who were unbiased as to the design of the experiment.

Preparation of Tissue for Light and Electron Microscopy. On day 45 after infection, mice were anesthetized with pentobarbital given intraperitoneally and then were sacrificed by intracardiac perfusion of phosphate-buffered 4% formaldehyde containing 1.5% glutaraldehyde (pH 7.2). Spinal cords were sectioned coronally into 15–20 blocks (1–2-mm-thick) and embedded in 2-hydroxyethyl methacrylate by using the JB4 system (Polysciences, Inc., Warrington, PA). Semithin sections were made and stained with a modified H and E method to detect inflammatory infiltrates or with our modification of the Erichrome method to stain myelin (17).

Selected spinal cord blocks were postfixated for 2 h in osmium tetroxide, dehydrated in graded alcohols, rinsed in propylene oxide, and embedded in Araldite. Selected areas were trimmed, and ultrathin sections were cut and examined by electron microscopy.

TABLE I
Mapping of Susceptible Gene to *D*^s in TMEV-induced Demyelination

Strain	K	A	E	S	D	Inflammation and demyelination*	Paralysis [‡]
B10.S	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	+	++
B10.D2	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	—	—
B10.S (9R)	<i>s</i>	<i>s</i>	<i>k</i>	<i>d</i>	<i>d</i>	—	—
B10.HTT	<i>s</i>	<i>s</i>	<i>k</i>	<i>k</i>	<i>d</i>	—	—
BSVS	<i>s</i>	<i>s</i>	<i>s</i>	<i>d</i>	<i>d</i>	—	—
B10.S (7R)	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>d</i>	—	—
B10.RSD2	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>d</i>	—	—
A.TH	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>d</i>	—	—
B10	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	<i>b</i>	—	—
B10.RSB1	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>b</i>	—	—
B10.S (26R)	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>b</i>	—	—
B10.ASR2	<i>k</i>	<i>k</i>	<i>k</i>	<i>d</i>	<i>s</i>	+	+++
A.TBR 13	<i>s</i>	<i>k</i>	<i>k</i>	<i>b</i>	<i>b</i>	—	—

* Determined by examining 15–20 spinal cord sections from each animal. +, inflammatory cells alongside demyelinated axons in all animals examined; —, no demyelination or inflammation.

[‡] +++, paralysis or death in >40% of mice inoculated with TMEV; ++, paralysis or death in 20–40% of mice; +, minimal neurologic deficits in <20% of mice; —, no clinical abnormalities and 100% survival.

Results

Influence of H-2D End. Mice (A.SW, B10.S, B10.RSS, SJL/J) with *H-2^s* haplotype were particularly susceptible to TMEV-induced demyelination. Genetic experiments using recombinant mouse strains involving a susceptible haplotype (*H-2^s*) and resistant haplotypes (*H-2^k*, *H-2^d*, *H-2^b*) indicated that genes in the *H-2D* end determine susceptibility or resistance to the demyelinating effects of TMEV (Table I). TMEV-infected B10.ASR2 mice that express the *D^s* gene product but have resistant alleles in *K*, *I*, and *S* regions showed prominent demyelination, inflammation within the spinal cord white matter, and paralysis in >40% of animals. In contrast to these findings, mice that expressed *D^d* or *D^b* genes showed no demyelination, inflammation, or paralysis, even if they also expressed *K^s*, *I^s*, or *S^s* genes (Table I).

Previous experiments have shown that mice with *q*, *v*, *p*, or *f* *H-2* haplotype are susceptible to TMEV-induced demyelination, and these animals frequently show clinical abnormalities (17). We tested to determine if the *D* region was also controlling susceptibility to demyelination in these haplotypes (Table II). B10.AKM and B10.MBR strains with a susceptible *D^q* allele but resistant *k* or *b* allele at the *K*, *I*, or *S* locus showed demyelination and inflammation in the white matter of the spinal cord in all animals tested. Similar pathologic findings were observed in B10.SM (22R) and B10.F (14R) mice with a susceptible *D^v* or *D^p* gene, respectively, but with a resistant *k* or *b* allele in other regions. In contrast, B10.F (13R) (*K^pI^pD^b*) mice, which are the reciprocal of B10.F (14R) (*K^bI^bD^p*) mice, showed no pathologic abnormalities, indicating the strong *D* region control of susceptibility to demyelination. Analogous findings were also seen in A.TFR1 (*K^sI^kS^kD^f*) mice that were susceptible.

Influence of Genes to the Right of H-2D. We examined the role of genes to the

TABLE II
D Region Control of Susceptibility to Demyelination in q, v, p, and f Haplotypes

Strain	K	A	E	S	D	Inflammation and demyelination*	Paralysis
B10.K	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	—	—
B10.Q	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	<i>q</i>	+	+
B10.AKM	<i>k</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>q</i>	+	+++
B10.MBR	<i>b</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>q</i>	+	+++
B10.SM	<i>v</i>	<i>v</i>	<i>v</i>	<i>v</i>	<i>v</i>	+	+
B10.SM (22R)	<i>k</i>	<i>k</i>	<i>k</i>	<i>v</i>	<i>v</i>	+	+
B10.F	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>	+	+
B10.F (14R)	<i>b</i>	<i>b</i>	<i>b</i>	<i>p</i>	<i>p</i>	+	+++
B10.F (13R)	<i>p</i>	<i>p</i>	<i>p</i>	<i>b</i>	<i>b</i>	—	—
B10.M	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	+	++
A.CA	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	<i>f</i>	+	+
A.TFR5	<i>f</i>	<i>f</i>	<i>f</i>	<i>k</i>	<i>d</i>	—	—
A.TFR1	<i>s</i>	<i>k</i>	<i>k</i>	<i>k</i>	<i>f</i>	+	+++

* See footnotes to Table I.

TABLE III
Absence of Role of Qa and Tla Genes in TMEV-induced Demyelination

Strain	H-2	Qa-2	Tla	Pgk	Upg	Inflammation and demyelination*	Paralysis*
A.CA	<i>f</i>	<i>b</i>	<i>d</i>	<i>b</i>	<i>f</i>	+	+
B6.AC1	<i>f</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>s</i>	+	+
B6	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>s</i>	—	—
B6.AC2	<i>b</i>	<i>a</i>	<i>d</i>	<i>b</i>	<i>f</i>	—	—
B6.K1	<i>b</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>f</i>	—	—
B6.K2	<i>b</i>	<i>a</i>	<i>a</i>	<i>b</i>	<i>f</i>	—	—
B6.T1 (+)	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>s</i>	—	—

* See footnotes to Table I.

right of the D region (*Qa*, *Tla*, *Pgk*, *UpG*) in TMEV-induced demyelination (Table III). In the strains tested, the haplotype expressed on the H-2 region was the critical determinant controlling susceptibility to demyelination. A.CA and B6.AC1 mice with susceptible *H-2^f* haplotype showed pathologic abnormalities even though these mice had alleles from resistant haplotypes to the right (*Qa*, *Tla* genes) of the D region. Similarly, B6.AC2, B6.K1, B6.K2, and B6.TL(+) mice with the resistant *H-2^b* haplotype did not show inflammation or demyelination in the spinal cord, despite expression of different genes to the right of H-2D. Therefore, genes mapping to the right of the D region did not influence susceptibility or resistance to demyelination by TMEV.

Mutation in the H-2D Gene(s) Alters Demyelination. To define the relationship between the D region loci and virus-induced demyelination, we inoculated B10.D2^{dm1} and BALB/c-dm2 mice which are known to contain deletion mutations in the D region (Fig. 1) (22). Recent data indicate that, in the *dm1* mutation, the 5' exons of the *D^d* gene are joined with 3' exons of the *L^d* gene so that there is a deletion of part of the D and L genes along with any other genes between D

Strain	K	I	S	D genes			Inflam- mation and demyelina- tion*	Paralysis*
				D	(R)	L		
B10.D2	d	d	d				-	-
B10.D2 ^{dm1}	d	d	d				+	+++
B10.D2 (R106)	b	b	b				+	++
BALB/c-H-2 ^{dm2}	d	d	d				-	-
BALB/c	d	d	d				-	-

FIGURE 1. Mutation in H-2D region genes alters susceptibility to virus-induced demyelination. In mice with the *dm1* and R106(SH) mutation, the 5' gene of *D^d* is joined with the 3' gene of *L^d* so that the 3' end of *D^d*, the 5' end of *L^d*, and the DNA between these genes are deleted. The (R) gene product has not been identified but is presumed to be a class I protein. The BALB/c-H-2^{dm1} lacks detectable H-2R and H-2L products.

* See footnotes to Table 1.

and L (23, 24). In contrast, the BALB/c-dm2 mouse has a complete deletion of the L gene along with an undefined region of adjacent DNA between the D gene and genes in the *Qa* region (25, 26). TMEV infection of the parent B10.D2 or BALB/c mice resulted in little or no inflammation or demyelination. In the spinal cords of some BALB/c mice there were occasional perivascular lymphocytes, but there was no demonstrable demyelination by 45 d. Similarly, BALB/c-dm2 mice did not show pathologic changes or clinical deficits. In contrast, all B10.D2^{dm1} mice showed large numbers of inflammatory cells in the white matter, along with primary demyelination (Fig. 2); there was paralysis in 9 of 15 infected mice. The inflammation and demyelination frequently involved 75% of the area of the spinal cord white matter. This was confirmed in a recombinant strain, B10.D2 (R106) (*K^bD^{dm1}*), that expresses the same deleted *dm1* mutation (Fig. 1). These findings suggested that the 3' end of *D^d* controls resistance or susceptibility to TMEV infection (Fig. 1).

Virology. Results of studies to isolate virus from the infected spinal cord and brain are shown in Table IV. Infectious virus was not detected at 45 d post-infection in spinal cord or brain extracts from mouse strains that showed no demyelination or inflammation (B10.D2; B10). In contrast, 3.3×10^2 – 1.7×10^4 PFUs of virus were isolated per gram of central nervous system tissue from animals with pathologic abnormalities in the spinal cord. Higher virus titers were seen in the B10.D2^{dm1} mutant mice, the strain that showed the most prominent destruction of white matter.

IgG Titers Against Purified Virus Antigen. Immunoglobulin in the serum directed against TMEV was measured at time of sacrifice of mice with mutations in the H-2D region (Fig. 3). Susceptible B10.D2^{dm1} mice ($n = 15$) had a significantly higher serum titer to virus antigen than did resistant B10.D2 ($n = 15$) mice (student's *t* test; $p < 0.05$). Resistant BALB/c-dm2 mice ($n = 5$) had lower titers. However, in general there was less than a twofold dilution difference in the titers between susceptible and resistant strains, indicating that these differences may not be biologically significant. Both the resistant and the susceptible mice had much higher serum antiviral titers compared with noninfected controls, indicating that both had replicated the virus and were able to mount an immune response to it.

FIGURE 2. (A) Large number of perivascular inflammatory cells are within the spinal cord white matter of virus-infected B10.D2^{dm1} mouse. Note inflammatory cells making contact with the endothelial cells of blood vessel. Glycol methacrylate-embedded section stained with H and E; $\times 320$. (B) Electron micrograph from a persistently infected B10.D2^{dm1} mouse, showing plasma cell in intimate association with multiple myelinated and demyelinated axons within the spinal cord. Note hyperactive astrocytic process (as) with abundant glial filaments. Ultrathin sections stained with uranyl acetate and lead citrate; $\times 18,000$.

TABLE IV
Isolation of TMEV From Brain and Spinal Cords of Mice With
Mutation in H-2D Region

Strain	Number of mice (n)	Inflammation and demyelination	Mean log ₁₀ of virus titer*
B10.D2	3	—	ND
B10.D2 ^{dm1}	5	+	3.52
B10.D2 (R106)	5	+	2.87
B10	5	—	ND

* Titer was PFU/g of central nervous system tissue. Lower limit of the assay was 50 PFU/g.

FIGURE 3. Serum Ig titers against purified TMEV antigen in mice with mutations in H-2D region molecules. Higher titers were noted in B10.D2^{dm1} mice, which were susceptible to virus-induced demyelination. However, B10.D2, BALB/c, and BALB/c-dm2 mice, which were resistant to demyelination, also had higher Ig titers than did uninfected control mice.

Discussion

The present study demonstrated that genes determining susceptibility or resistance to demyelination by TMEV are located within the D region of the H-2 complex. Involvement of the K region was excluded because A.TBR 13 mice ($K^s D^b$) did not show demyelination even though these mice express the susceptible K^s allele (but the resistant D^b gene). In these experiments there was not a strong influence of the I region in determining demyelination or infection. B10.ASR2, B10.AKM, B10.MBR, B10.F(14R), and A.TFR1 with susceptible alleles in the D gene but resistant alleles in the I region showed pathologic abnormalities. Similarly, B10.F(13R), A.TFR3, and B10.S(9R) with resistant alleles in the D gene but susceptible alleles in the I region did not demonstrate demyelination. In addition, genes to the right of the D region did not appear to be important in determining susceptibility to TMEV-induced demyelination.

The results of TMEV-induced demyelination in mouse strains with mutations in the H-2D gene were of particular interest. B10.D2^{dm1} mice showed prominent

inflammation, demyelination, and clinical deficits whereas the B10.D2 parent mice were normal. B10.D2^{dm1} was derived from B10.D2 mice treated with the mutagen diethyl sulfate (27). The mutation has been localized to the D region of H-2. Serologic and structural analyses indicate major alterations in the expression of at least three H-2D/L-linked antigens (23). The mutant antigen appears to be a structural mosaic of two parental antigens such that the 5' end of the *D^d* gene is joined with the 3' end of *L^d* so that the 3' end of *D^d*, the 5' end of *L^d*, and the DNA between *D* and *L* are deleted (24). The junction between the *H-2D^d* and *H-2L^d* encoded structures within the *H-2D/L^{dm1}* encoded antigen seems to be the α -2 domain (28). The difference in susceptibility to TMEV-induced demyelination between B10.D2 and B10.D2^{dm1} suggests that the resistant gene must map to the 3' end of *D*, the DNA between *D* and *L*, or the 5' end of *L*. To map the susceptibility to demyelination more precisely within the D region, BALB/c-dm2 mice were inoculated with TMEV. This mutant differs from the parental BALB/c strain by lacking detectable H-2R and H-2L encoded products (25). In contrast to the pathologic abnormalities noted in the spinal cords of B10.D2^{dm1} mice, the spinal cords of BALB/c-H-2^{dm2} mice showed no abnormalities.

The contrasting effects of TMEV infection in B10.D2^{dm1} and BALB/c-H-2^{dm2} indicate that the 3' end of the *D* molecule is sufficient in determining resistance or susceptibility to demyelination. However, because the deletions are being compared in mice with different backgrounds, it is possible that a non-H-2 gene in BALB negates the effect of an *R* or *L* gene in inducing the susceptibility. The deletion of the 3' end of the *D* gene in B10.D2^{dm1} also might cause a nonstructural but functional abnormality in the 5' end of the *D* gene, which then becomes the critical determinant in genetic susceptibility. In addition, other genes to the right of the *D* gene, which are deleted in *dm1* but not in *dm2*, may be important.

We attempted to correlate TMEV-specific humoral immunity to TMEV-induced demyelination. The susceptible B10.D2^{dm1} mutant mice did show higher serum Ig titers directed against purified viral antigens than did the resistant parental B10.D2 strain. The BALB/c-dm2 deletion mutant showed lower Ig titers to purified virus, but the titers were always higher than those in noninfected controls. In our studies, there was a strong correlation between the ability to isolate infectious virus from central nervous system tissue and susceptibility to demyelination. From the present virologic studies we cannot conclude whether the resistant strains are unable to infect central nervous system cells or there is rapid clearance of the virus by immune cells. The findings do indicate, however, a relationship between persistence of virus and destruction of white matter. This is in contrast to investigations by Clatch et al. (29) that did not demonstrate a correlation between central nervous system TMEV titer and control of demyelination by the H-2D region. Their studies used clinical rather than pathologic criteria to determine genetic susceptibility to disease. Because of the low incidence of clinical disease, they used crosses between SJL and several congenic recombinant mouse strains bearing different combinations of H-2 genes. Disease incidence correlated with TMEV-specific delayed-type hypersensitivity but not with TMEV-specific humoral responses or T-cell proliferation. We are currently investigating TMEV-specific cellular immunity in susceptible and resistant mice.

The mechanism by which TMEV induces demyelination is not yet known. In previous experiments (14) we found production of virus antigen within persistently infected oligodendrocytes (14), the myelin-producing cell. TMEV antigen was present within the inner and outer oligodendroglial loops that connect with myelin lamellae (20, 30). It is possible that class I-restricted (e.g., H-2D) cytotoxic T cells may attack antigen of the glial loops, thereby injuring infected oligodendrocytes and so producing demyelination. This hypothesis may be criticized because TMEV, as a member of the family Picornaviridae, is not known to bud from host cell surfaces and, therefore, may not insert virus-specific antigens into the cell membrane. However, some recent studies with coxsackievirus, another picornavirus, have shown cytotoxic T-cell responses against infected target cells (31). Another possible mechanism of immune-mediated demyelination may be autoimmune attack of normal myelin, as in experimental autoimmune encephalomyelitis (32). All attempts to transfer TMEV demyelination adoptively into naive syngeneic recipients by injecting lymphocytes from TMEV-immune lymph nodes or spleens have failed (20, 33). Also, serum or immune cells from TMEV-infected mice fail to injure myelinating cultures (33). These results suggest that, in this model, the immunopathology of demyelination is different from that of experimental autoimmune encephalomyelitis and may require virus antigen as an immunologic stimulus. Myelin breakdown also may be the result of release of nonspecific proteases from macrophages during the interaction of immune cells and infected non-myelin-producing cells (i.e., innocent bystander demyelination) (34, 35).

We can hypothesize that one of the D region genes (possibly an epitope on the 3' end of *D^d*) is involved in preventing persistent TMEV infection within the central nervous system. This may occur by two possible mechanisms. In resistant strains, oligodendrocytes and other glial cells may not have receptors for TMEV, and therefore infection never occurs. This is unlikely because resistant strains develop a normal humoral response to the virus. More likely, the restricting class I gene products may be involved in generating a cytotoxic T-lymphocyte response against TMEV, which prevents persistent viral infection in the central nervous system. In mice with susceptible haplotypes, class I H-2-encoded antigen may not be presented to the T cells in association with virus antigen, thereby allowing the virus to persist. The persistent infection may generate a new indolent immunopathologic response against viral antigens on oligodendrocytes or normal autoantigens on myelin or oligodendrocytes. Class II antigens may be involved in this secondary phase of the disease because mAbs against Ia suppress demyelination (14).

Summary

Demyelination induced by Theiler's virus was examined in mouse strains with congenic recombinant haplotypes. Light and electron microscopy of spinal cord sections from mice with *s*, *q*, *v*, *p*, and *f* H-2D alleles showed perivascular inflammation and primary demyelination. The presence of susceptible haplotypes in the K or I region did not correlate with pathologic abnormalities. The *Qa*, *Tla*, *PgK*, and *UpG* genes did not appear to be critical in determining susceptibility to disease. However, mutation in the H-2D genes altered the susceptibility to

virus-induced demyelination. B10.D2^{dm1} mice, which have deletions in the 3' end of *D^d* and the 5' end of *L^d*, showed prominent demyelination and clinical deficits. In contrast, BALB/c-dm2, which have a deletion of the entire *L* gene, showed no pathologic changes. Central nervous system virus titers correlated with susceptibility to demyelination; both resistant and susceptible strains had a strong humoral immune response to the virus. The findings in the congenic recombinant mice and in mice mutant in the H-2D region strongly suggest that at least one of the genes critical for determining virus-induced demyelination maps to the 3' end of the H-2D gene.

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