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# Major Histocompatibility Complex-Conferred Resistance to Theiler's Virus-Induced Demyelinating Disease Is Inherited as a Dominant Trait in B10 Congenic Mice

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**Intracerebral inoculation of Theiler's murine encephalomyelitis virus into susceptible strains of mice produces chronic demyelinating disease in the central nervous system characterized by persistent viral infection. Immunogenetic data suggest that genes from both major histocompatibility complex (MHC) and non-MHC loci are important in determining susceptibility or resistance to demyelination. The role of the MHC in determining resistance or susceptibility to disease can be interpretated either as the presence of antigen-presenting molecules that confer resistance to viral infection or as the ability of MHC products to contribute to pathogenesis by acting as viral receptors or by mediating immune attack against virally infected cells. These alternatives can be distinguished by determining whether the contribution of the MHC to resistance is inherited as a recessive or dominant trait. Congenic mice with different MHC haplotypes on identical B10 backgrounds were crossed and quantitatively analyzed for demyelination, infectious virus, and local virus antigen production. $F_1$ hybrid progeny derived from resistant B10 (*H-2$^b$*), B10.D2 (*H-2$^d$*), or B10.K (*H-2$^k$*) and susceptible B10.R111 (*H-2$^r$*), B10.M (*H-2$^f$*), or B10.BR (*H-2$^k$*) parental mice exhibited no or minimal demyelination, indicating that on a B10 background, resistance is inherited as a dominant trait. Although infectious virus, as measured by viral plaque assay, was cleared inefficiently from the central nervous systems of resistant $F_1$ hybrid progeny mice, we found a direct correlation between local viral antigen production and demyelination. These data are consistent with our hypothesis that the immunological basis for resistance is determined by efficient presentation of the viral antigen to the immune system, resulting in local virus clearance and absence of subsequent demyelination.**

Theiler's murine encephalomyelitis viruses (TMEV) are naturally occurring enteric pathogens belonging to the family *Picornaviridae*. Intracerebral inoculation of TMEV into susceptible strains of mice produces a characteristic biphasic disease (15, 35). The early phase is characterized by acute encephalitis during which the virus lytically infects neurons in grey matter. Strains that have a resistance phenotype are able to clear the virus and rarely develop further pathological abnormalities. In contrast, all surviving mice that have a susceptibility genotype develop chronic demyelinating disease characterized by persistent viral infection and mononuclear cell infiltration associated with myelin destruction. The pathological and immunological properties of TMEV-induced demyelination, along with the genetic predisposition for it, are similar to those seen with multiple sclerosis of humans, and thus, TMEV provides a model to study aspects of multiple sclerosis.

At least two mechanisms have been proposed to account for demyelination. That demyelination can occur from direct lysis of oligodendrocytes by virus is supported by immunoperoxidase and ultrastructural studies (2, 9, 24, 26), as well as by studies with nude mice (34). The direct correlation between viral RNA and demyelinating lesions also provides evidence that virus infection is necessary for pathogenesis (4). Evidence supporting an immunological basis for demyelination is derived from experiments in which treatment of susceptible mice with various immunosuppressive regimens (16, 29, 33) results in amelioration of demyelination.

A genetic basis for the disease is derived from immunogenetic data involving several different strain comparisons (5, 18, 28). Those studies indicated that genes from major histocompatibility complex (MHC) and non-MHC loci influence the resistance or susceptibility of mice to demyelination. Non-MHC genes which have been implicated in differential susceptibility have been mapped in or near loci encoding the β chain of the T-cell receptor (19) and, more recently, the enzyme carbonic anhydrase 2 (21). Likewise, a role for MHC-encoded genes has been demonstrated in congenic mice with resistant B10 backgrounds in which mice with *H-2$^{d,b,k}$* haplotypes are resistant to demyelination while those with *H-2$^{s,v,q,r,f}$* haplotypes are susceptible (27). The observation that congenic recombinant mice normally resistant to disease but possessing an allele derived from a susceptible haplotype at the *D* locus or, alternatively, introduction of a mutation in the *D* locus of otherwise resistant mice predisposes to demyelinating disease suggests that class I genes are critical (5, 28, 30). In addition, the development of demyelination in genetically resistant B10 mice following total-body irradiation further suggests that resistance to disease is mediated by an active process (32). On the basis of these observations, we hypothesize that in the B10 model resistance or susceptibility is determined by the ability or inability of certain class I allelic glycoproteins to present the viral antigen effectively to the immune system, resulting in either viral clearance and absence of demyelina-

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tion (resistance) or persistent viral infection and demyelination (susceptibility). On the basis of this hypothesis, we predict that in congenic mice with different MHC haplotypes on identical resistant B10 backgrounds the contribution of MHC to resistance to disease is inherited as a dominant trait and is associated with local virus clearance.

## MATERIALS AND METHODS

**Virus.** The DA strain of TMEV (6) was used for all experiments. This strain, originally obtained from J. P. Lehrich and associates of the University of Chicago, Chicago, Ill. (12), was grown to $5 \times 10^8$ PFU/ml in baby hamster kidney 21 cells (17).

**Mice.** B10.RIII, B10.M, B10.D2, and B10.K mice were obtained from the Mayo immunogenetic mouse colony. C57BL/10 (B10) mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) and used for experiments after a 1-week rest. All mice, including $F_1$ hybrid progeny mice, were raised at the Mayo Clinic facility. Mice of either sex from 4 to 8 weeks of age were injected intracerebrally with $2 \times 10^5$ PFU of virus in a 10-μl volume.

**Virus plaque assay.** Viral titers in clarified central nervous system (CNS) homogenates were determined by plaque assay as previously described (31). In brief, 0.2 ml of serial dilutions of virus in Dulbecco modified Eagle medium with 1% bovine serum albumin were plated in duplicate on confluent monolayers of L2 cells in 12-well plates. Following adsorption for 1 h at 37°C, cell cultures were overlaid with 3 ml of Dulbecco modified Eagle medium containing 2% newborn calf serum and 0.4% SeaPlaque agarose (FMC Corp., Marine Products Div., Rockland, Maine). At 2 to 3 days later, agarose was removed and cell cultures were fixed with ethanol-acetic acid-formaldehyde (6:2:1) and stained with 1% crystal violet in 20% ethanol. Plaques were enumerated, and results were expressed as $\log_{10}$ PFU per gram of CNS tissue. CNS homogenates were prepared from brains and spinal cords aseptically removed from mice killed with ether. A 10% homogenate was prepared in Dulbecco modified Eagle medium, sonicated twice for 60 s each time, and clarified by centrifugation. Virus preparations were stored at −70°C.

**Preparation of tissue for light microscopy and quantitative analysis.** Forty-five days postinfection, mice were anesthetized with 0.2 ml of pentobarbital (intraperitoneally) and perfused by intracardiac puncture with Trump's fixative (phosphate-buffered 4% formaldehyde with 1.0% glutaraldehyde, pH 7.2). Spinal cords were removed and sectioned coronally, and 12 to 15 blocks (1 to 2 mm thick) from each mouse were osmicated and embedded in 2-hydroxyethylmethacrylate (JB-4 system; Polysciences Inc., Warrington, Pa.). Two-micrometer sections were stained with a modified erichrome method with cresyl violet (25) to detect demyelination. Detailed morphological analysis was performed by examining each quadrant from 15 to 20 spinal cord coronal sections from each mouse for the presence or absence of demyelination and/or inflammation. A maximum score of 100 represented the presence of abnormalities in every quadrant of every spinal cord section examined. For immunoperoxidase studies, sections stored in 0.1 M phosphate buffer were rinsed in 0.1 M Tris buffer with 25 mM hydroxylamine (pH 7.4), treated with 10% dimethyl sulfoxide in the same buffer for 1 h, and quick-frozen in isopentane chilled in liquid nitrogen. Ten-micrometer cryostat sections were cut and transferred to gelatin-coated glass slides.

**Immunocytochemistry.** Frozen sections from Trump fixative-perfused mice were reduced with 1% sodium borohydride in 0.1 M Tris buffer with 25 mM hydroxylamine (pH 7.4) at 4°C and refixed with 95% alcohol–5% glacial acetic acid. Sections were immunostained with a polyclonal antiserum to purified TMEV strain DA virions (30) by the avidin-biotin immunoperoxidase technique (Vector Laboratories, Burlingame, Calif.). Slides were developed with a solution of 75 μg of Hanker-Yates reagent (*p*-phenylenediamine-procatechol [Polysciences]). Slides were counterstained with hematoxylin.

**Statistical analyses.** The Wilcoxon rank order sum test was used to evaluate the significance of differences in pathological-change scores between different strains of mice. Fisher's exact test was used to analyze differences in the isolation of infectious virus between strains of mice.

## RESULTS

**Inheritance of resistance to TMEV-induced demyelinating disease as a dominant trait.** To determine whether MHC-conferred resistance to TMEV-induced demyelination is inherited as a recessive or dominant trait, we infected $F_1$ hybrid progeny mice derived from crosses between congenic mice having either resistance or susceptibility haplotypes on resistant B10 backgrounds. At 45 days postinfection, spinal cords were removed and quantitative analysis was performed (Table 1). $F_1$ hybrid progeny mice derived from resistant B10 ($H$-$2^b$) and either susceptible B10.RIII ($H$-$2^r$) or B10.M ($H$-$2^k$) parental mice exhibited no or minimal demyelination; pathological-change scores were significantly lower than those of either B10.RIII or B10.M parents (Table 1). Similarly, $F_1$ hybrid progeny mice derived from a cross between resistant B10.D2 ($H$-$2^d$) and susceptible B10.M parental mice also exhibited significantly less pathological change than did the B10.M parent. The absence of pathological abnormalities in $F_1$ hybrid progeny mice was not dependent upon maternal or paternal origin of specific MHC regions nor on gender; the results were therefore pooled for presentation. Histopathological changes were characterized by meningeal and perivascular infiltration of mononuclear inflammatory cells and destruction of myelin with preservation of axons (Fig. 1A). In contrast, white matter from resistant $F_1$ progeny mice showed few pathological abnormalities; inflammatory cells were minimal, and myelin was intact (Fig. 1B). These data suggest that on a B10 background, MHC-conferred resistance is inherited as a dominant trait.

Although B10.BR ($H$-$2^k$) and B10.K ($H$-$2^k$) mice are presumed to be genetically identical, B10.BR mice developed significantly more pathological abnormalities than did B10.K mice (Table 1). This differential susceptibility to TMEV-induced demyelination was further evidenced in comparisons of pathological changes in $F_1$ progeny mice derived from crosses with resistant C57BR mice; pathological changes (11.6 ± 8.5) occurring in (C57BR × B10.BR)$F_1$ mice were statistically more severe than those in (C57BR × B10.K)$F_1$ mice (0.4 ± 0.5; $P < 0.01$). As was observed in other crosses between congenic mice, pathological-change scores for (B10.BR × B10.K)$F_1$ mice were significantly lower than those of the more susceptible B10.BR mice but not those of B10.K parental mice.

To determine whether susceptibility to TMEV-induced demyelination observed in B10.BR ($H$-$2^k$) and B10.M ($H$-$2^f$) strains of mice was due to a common genetic defect, (B10.BR × B10.M)$F_1$ hybrid mice were infected with TMEV. Although pathological-change scores for (B10.BR ×

TABLE 1. Demyelination in the spinal cords of B10 congenic and $F_1$ progeny mice

| Strain | *H-2* haplotype(s) | No. of mice | Demyelination score[a] | *P* value[b] |
|---|---|---|---|---|
| B10[c] | *b* | 13 | 0.4 ± 0.7 | <0.01 |
| B10.R111 | *r* | 10 | 9.3 ± 8.4 | NS |
| (B10 × B10.R111)$F_1$ | *b/r* | 15 | 0.9 ± 1.3 | <0.01 |
| | | | | |
| B10[c] | *b* | 13 | 0.4 ± 0.7 | <0.01 |
| B10.M[c] | *f* | 9 | 17.4 ± 7.4 | NS |
| (B10 × B10.M)$F_1$ | *b/f* | 16 | 1.0 ± 1.5 | <0.01 |
| | | | | |
| B10.D2 | *d* | 9 | 1.7 ± 2.0 | <0.01 |
| B10.M[c] | *f* | 9 | 17.4 ± 7.4 | NS |
| (B10.D2 × B10.M)$F_1$ | *d/f* | 10 | 2.7 ± 3.3 | <0.01 |
| | | | | |
| B10.K | *k* | 10 | 7.4 ± 8.2 | <0.05 |
| B10.BR[c] | *k* | 16 | 14.9 ± 7.6 | NS |
| (B10.K × B10.BR)$F_1$ | *k/k* | 17 | 3.8 ± 3.7 | <0.01 |
| | | | | |
| B10.BR[c] | *k* | 16 | 14.9 ± 7.6 | NS |
| B10.M[c] | *f* | 9 | 17.4 ± 7.4 | NS |
| (B10.BR × B10.M)$F_1$ | *k/f* | 18 | 10.8 ± 6.7 | <0.05 |

[a] Demyelination was determined by scoring each quadrant from 12 to 15 coronal spinal cord sections from each mouse for the presence or absence of inflammation and/or demyelination 45 days postinfection and expressed as a mean pathological score ± the standard deviation. A total of 2,145 spinal cord sections were examined.

[b] The Wilcoxon rank-sum test was used. NS, Not significant.

[c] For purposes of presentation, the pathological scores for these mouse strains are represented more than once.

B10.M)$F_1$ were statistically lower than those of B10.M parental mice, meaningful gene complementation resulting in resistance to disease was not convincingly demonstrated, since $F_1$ progeny mice developed significant pathological abnormalities (Table 1). This observation suggests that the gene(s) predisposing to the demyelinating disease observed in B10.BR and B10.M mice is located within the same complementation groups.

**Isolation of infectious virus from brain and spinal cord of resistant and susceptible mice.** Comparisons between levels of infectious virus isolated from CNSs of prototype resistant (B10) and susceptible (SJL/J) strains of mice (23) have demonstrated that resistance correlates with virus clearance while susceptibility correlates with persistent virus infection. To determine whether inheritance of MHC-conferred resistance to TMEV-induced demyelination is associated with virus clearance, parental mice and their $F_1$ hybrid progeny mice were infected with TMEV and infectious virus was determined in their brains and spinal cords. Although the sample size in individual comparisons was small, overall virus persistence in susceptible mice (32 of 37 mice) occurred at a significantly higher incidence (13 of 47 mice) than

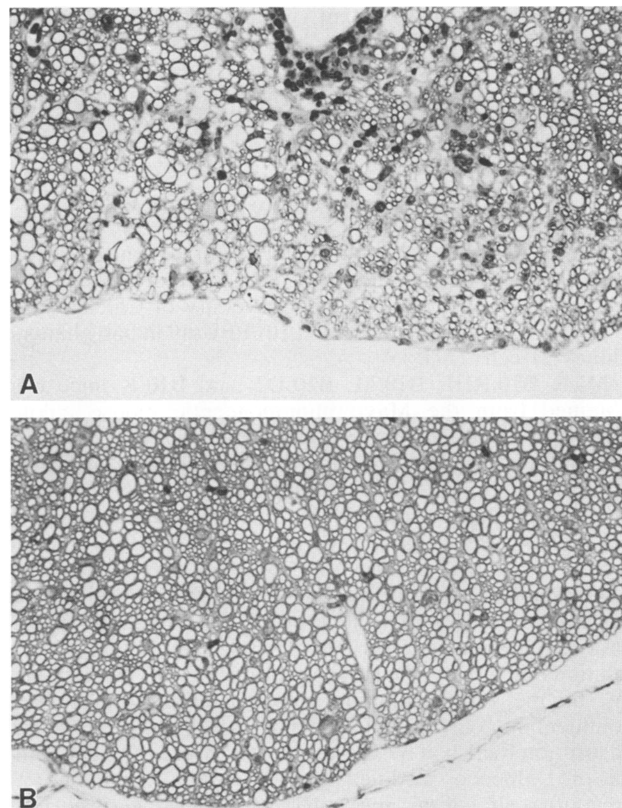


FIG. 1. (A) Inflammatory infiltrate and demyelination in the spinal cord of a B10.M mouse 45 days postinfection with TMEV. (B) Lack of pathological changes in the spinal cord of a (B10 × B10.M)$F_1$ progeny mouse. Glycol-methacrylate sections stained with erichrome-cresyl violet. Magnification, ×1,459.

in resistant mice (Fig. 2). Consistent with pathological changes, infectious virus was isolated from the CNSs of all B10.RIII and B10.M mice and was not detected in the CNSs of 10 of 10 B10 mice on 5 of 6 B10.D2 mice (Table 2; Fig. 2) examined. Clearance of virus correlated with inheritance of resistance in (B10 × B10.RIII)$F_1$ progeny mice, since fewer mice had detectable infectious virus than did susceptible B10.RIII parental mice ($P$ = 0.0192). Although (B10 × B10.M)$F_1$ and (B10.D2 × B10.M)$F_1$ progeny mice appeared to clear virus more efficiently than did susceptible B10.M parental mice (Table 2; Fig. 2), isolation of infectious virus did not appear to correlate completely with the state of resistance.

**Viral antigen production in $F_1$ hybrid progeny mice.** Immunocytochemical and in situ hybridization studies have demonstrated a strong correlation between viral antigen and RNA expression and areas of pathological change (3, 4). In one study (4), viral antigen-RNA-positive cells were detected during late disease in demyelinating lesions in SJL/J mice but no or few cells were detected in C57BL/6 mice, consistent with the lack of demyelination. In B10 mice, lack of pathological abnormalities strongly correlated with efficient virus clearance (Table 2; Fig. 2). However, although (B10 × B10.M)$F_1$ mice developed no or minimal demyelinating disease (Table 1), infectious virus was isolated from 6 of 11 mice (Table 2). To define specifically the association of local viral antigen production with demyelination in these mice, five or six spinal cord coronal sections from individual B10.M and (B10 × B10.M)$F_1$ mice known to have demyeli-

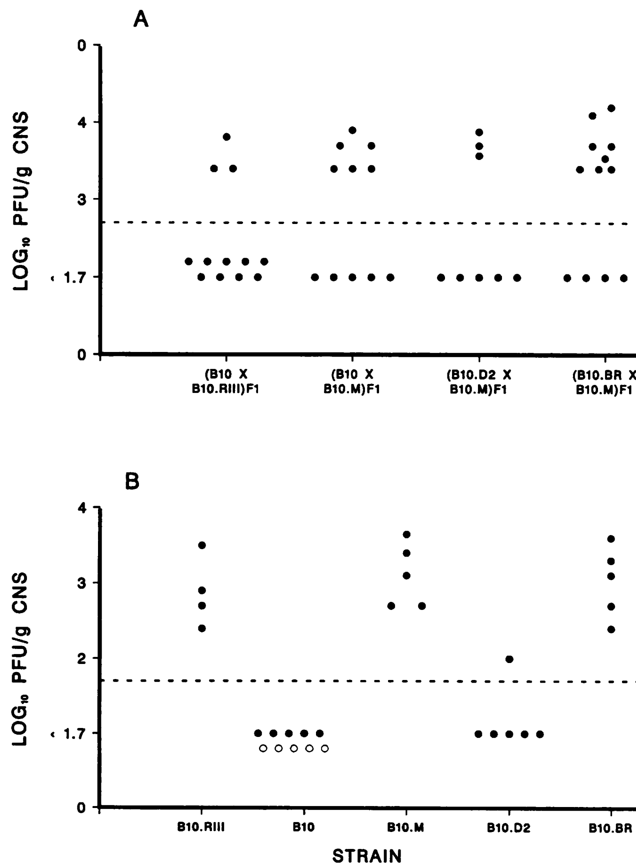


FIG. 2. Isolation of TMEV 45 (●) or 90 (○) days postinfection from the CNSs of B10 congenic mouse strains (B) and $F_1$ hybrid progeny mice (A). Infectious virus ($log_{10}$ PFU per gram of CNS tissue was determined by viral plaque assay (31). Mice from which no virus was isolated are represented below the broken lines (assay sensitivity, 1.7 $log_{10}$ PFU/g of CNS tissue).

nation, as well as (B10 × B10.M)$F_1$ mice in which pathological changes had not been detected (Table 3), were analyzed by the immunoperoxidase technique. Viral antigen-positive cells were detected in all of the B10.M mice examined and in two of three (B10 × B10.M)$F_1$ mice exhibiting pathological changes (Table 3; Fig. 3). The extent of the pathological changes observed in these mice also correlated with the number of positive cells detected; 10 to 65 cells were enumerated in more extensively affected B10.M mice, and only 2 and 5 cells, respectively, were detected in $F_1$ progeny mice which had minimal pathological changes. In contrast, no viral antigen-positive cells were detected in $F_1$ progeny mice which had no detectable pathological changes (Table 3; Fig. 3). In addition, viral antigen-positive cells were observed only within demyelinating lesions. These results are consistent with the hypothesis that MHC-conferred resistance to TMEV-induced demyelination is associated with a reduction in local viral antigen production.

## DISCUSSION

Previous results have shown that on a B10 background, resistance to TMEV-induced demyelinating disease is associated with specific *H-2* haplotypes and more specifically maps to the *D* locus, the MHC region containing genes that encode class I protein products (5, 20, 27, 28, 30). The observation that mutations within *H-2D* are sufficient to convert a resistant strain to susceptibility suggests that resistance to disease induction is mediated by an active process (30). These observations, together with the data from the present study, are consistent with our hypothesis that in the B10 model, the immunological basis for resistance is determined by the ability of class I gene products encoded by certain haplotypes, e.g., $H\text{-}2^{b,d}$, to interact with viral antigen effectively, resulting in local virus clearance and absence of demyelinating disease. Susceptibility, conse-

TABLE 2. Isolation of infectious virus from CNSs of B10 congenic and $F_1$ progeny mice 45 days postinfection

| Strain | *H-2* haplotype(s) | Virus isolation[a] | *P* value[b] | |
|---|---|---|---|---|
| B10 | *b* | 0/5 | 0.0079 | NS |
| B10.R111 | *r* | 4/4 | 0.0192 | |
| (B10 × B10.R111)$F_1$ | *b/r* | 3/12 | | |
| B10 | *b* | 0/5 | 0.0079 | 0.0934 |
| B10.M | *f* | 5/5 | 0.1186 | |
| (B10 × B10.M)$F_1$ | *b/f* | 6/11 | | |
| B10.D2 | *d* | 1/6 | 0.0152 | NS |
| B10.M | *f* | 5/5 | 0.0753 | |
| (B10.D2 × B10.M)$F_1$ | *d/f* | 3/8 | | |
| B10.BR | *k* | 5/5 | NS | NS |
| B10.M | *f* | 5/5 | NS | |
| (B10.BR × B10.M)$F_1$ | *k/f* | 8/12 | | |

[a] Number of mice with detectable virus/number of mice examined.
[b] Fisher's exact test was used. NS, Not significant.

TABLE 3. Correlation of viral antigen-positive cells with demyelination

| Mouse no. | Strain | Demyelination[a] | No. of viral antigen-positive cells[b] |
|---|---|---|---|
| 1 | B10.M | 30.2 | 65 |
| 2 | B10.M | 26.7 | 10 |
| 3 | B10.M | 20.3 | 18 |
| 4 | (B10 × B10.M)$F_1$ | 4.2 | 2 |
| 5 | (B10 × B10.M)$F_1$ | 3.1 | 0 |
| 6 | (B10 × B10.M)$F_1$ | 2.9 | 5 |
| 7 | (B10 × B10.M)$F_1$ | 0.0 | 0 |
| 8 | (B10 × B10.M)$F_1$ | 0.0 | 0 |
| 9 | (B10 × B10.M)$F_1$ | 0.0 | 0 |

[a] Demyelination was determined by scoring each quadrant from 12 to 15 coronal spinal cord sections from each mouse for the presence or absence of inflammation and/or demyelination and expressed as a mean-pathological-change score ± the standard deviation.
[b] Five or six coronal spinal cord sections from Trump fixative-perfused mice were stained by the avidin-biotin immunoperoxidase technique using polyclonal antiserum to purified TMEV strain DA virions (31).

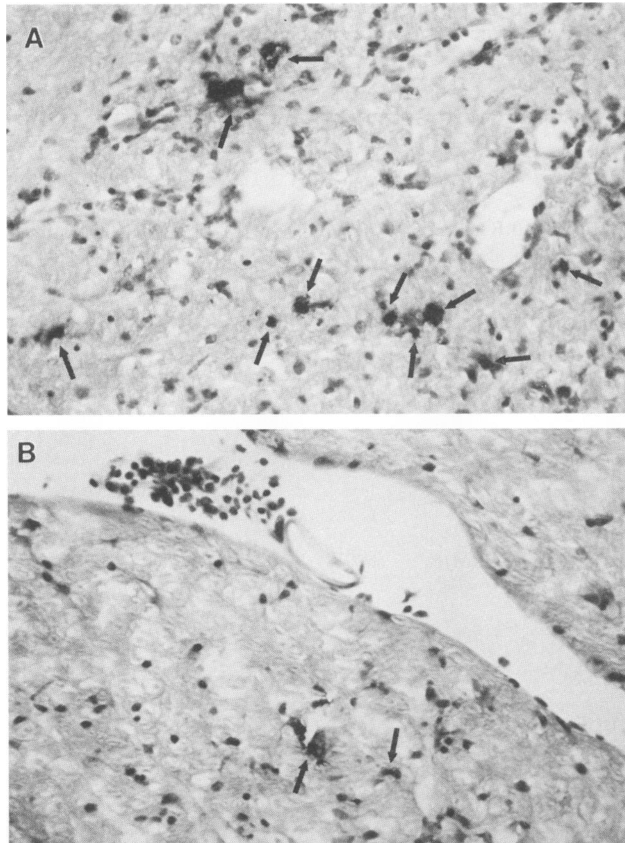


FIG. 3. Viral antigen-positive cells (arrows) within demyelinating lesions in spinal cords from a B10.M mouse (A) and a (B10 × B10.M)$F_1$ mouse (B) 45 days postinfection with TMEV. The sections were counterstained with hematoxylin. Magnification, ×1,459.

quently, is due to the inability of these products to achieve efficient virus clearance, which then predisposes to demyelinating disease. These data do not support hypotheses stating that resistance results from either lack of certain *H-2*-encoded gene products necessary for productive virus infection, e.g., viral receptors, as described for Rauscher murine leukemia virus-induced thrombocytopenia (8), or self-tolerance generated from a cross-reactive viral protein, as described by Vidovic and Matzinger (37). In these cases, susceptibility to disease would be inherited as a dominant trait.

We examined the correlation of infectious virus clearance and local viral antigen production in the CNS with the genetically determined patterns of resistance. Although virus clearance strongly correlated with resistance to demyelination in parental mice, infectious virus was cleared inefficiently in some resistant $F_1$ progeny. In contrast, we found complete absence of viral antigen-positive cells in parental and $F_1$ progeny mice that did not develop demyelinating disease. These results confirm the findings by Chamorro et al. (4), who found a direct correlation between local viral antigen or RNA production and demyelinating lesions, supporting the hypothesis that viral infection is a necessary prerequisite for disease. Although the presence or absence of infectious virus appears to be a reliable measure of susceptibility or resistance in some cases, our data suggest that local viral antigen production reflects the disease process more accurately and thus is a more relevant parameter. Discrepancies between infectious virus and viral antigen production have been noted before (38) and may be due to either cell-free virus not detected by immunocytochemistry or the presence of viral antigen in regions of the CNS not included in the analysis of pathological changes, e.g., the brain.

What regulatory mechanisms, influenced by genes encoded by *H-2*, are important in limiting viral antigen production and thus preventing subsequent demyelinating disease? Resistance may be due to an early or innate mechanism that is operative before a specific antiviral immune response develops, as has been described in B10 mice after infection with ectromelia virus (22). Our data, however, do not support this mechanism. Resistant and susceptible strains of mice are similarly affected during early grey matter disease; pathological scores, levels of infectious virus, and numbers of viral antigen-positive cells did not differ between the two groups. No difference in grey matter disease exists, despite the observation that B10 mice have twofold higher natural killer cell activity than SJL/J mice (23). However, natural killer cell depletion of resistant mice increases only the morbidity and mortality associated with grey matter disease but does not induce development of late demyelination. These observations imply that resistance to TMEV-induced demyelination depends on appropriate antiviral immune responses rather than mechanisms operating during the innate or preimmune phase of infection. An immunological basis for resistance is further supported by experiments in which immunosuppression of B10 mice by total-body irradiation predisposes to demyelinating disease (32).

Mechanisms compatible with the apparent influence of class I loci can be proposed to account for the inability of susceptible mice to limit virus production and, hence, develop demyelinating disease. Susceptible strains of mice may fail to generate an adequate cytotoxic T-lymphocyte response such that virus is never cleared and disease occurs. Relevant to this proposal are the observations that absent or low cytotoxic T-lymphocyte responsiveness to a variety of viruses, including influenza virus (1, 7), vaccinia virus (7), lymphocytic choriomeningitis virus (36), and herpes simplex virus (10), is associated with certain MHC alleles and, in many cases, correlates with inefficient virus clearance. Absent or low T-cell responsiveness may be due to either general failure in their induction or modulation of this response by immune response (*Ir*) gene products (11). Experiments suggest that genes encoded in the *I* region of *H-2* can influence the severity of TMEV-induced demyelinating disease (28). In addition, Lindsley and Rodriguez (13) have examined the numbers and times of appearance of mononuclear inflammatory cells in the CNSs of B10 and SJL/J mice. Although the number of CD8+ (class I restricted) cells peaked on day 7 in B10 mice, they were not detected in SJL/J mice until day 14 postinfection. The data also indicate that antiviral cytotoxic T lymphocytes can be detected in lymphocytes infiltrating the CNSs of B10 mice (14). The early appearance of CD8+ cells and cytotoxic activity in resistant B10 mice could play a role in virus clearance, while the delay in their appearance in susceptible mice may predispose to virus persistence and demyelination.

In summary, the B10 congenic mouse model represents a powerful tool for analyzing the contributions of genes encoded within the MHC to resistance to demyelination, as well as for extending our knowledge of interactions among the host immune system, the nervous system, and the virus. The data presented in this study confirm the observation that

resistance is an active process and suggest an important role for immune system-mediated mechanisms.

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## LITERATURE CITED

1. **Bennink, J. R., and J. W. Yewdell.** 1988. Murine cytotoxic T lymphocyte recognition of individual influenza virus proteins. High frequency of nonresponder MHC class I alleles. J. Exp. Med. **168:**1935–1939.
2. **Blakemore, W. F., C. J. R. Welsh, P. Tonks, and A. A. Nash.** 1988. Observations on demyelinating lesions induced by Theiler's virus in CBA mice. Acta Neuropathol. **76:**581–589.
3. **Brahic, M., W. G. Stroop, and J. R. Baringer.** 1981. Theiler's virus persists in glial cells during demyelinating disease. Cell **26:**123–128.
4. **Chamorro, M., C. Aubert, and M. Brahic.** 1986. Demyelinating lesions due to Theiler's virus are associated with ongoing central nervous system infection. J. Virol. **57:**992–997.
5. **Clatch, R. J., R. W. Melvold, S. D. Miller, and H. L. Lipton.** 1985. Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease in mice is influenced by the H-2D region: correlation with TMEV-specific delayed-type hypersensitivity. J. Immunol. **135:**1408–1413.
6. **Daniels, J. B., A. M. Pappenheimer, and S. Richardson.** 1952. Observations on encephalomyelitis of mice (DA strain). J. Exp. Med. **96:**517–530.
7. **Doherty, P. C., W. E. Biddison, J. R. Bennink, and B. B. Knowles.** 1978. Cytotoxic T-cell responses in mice infected with influenza and vaccinia viruses vary in magnitude with H-2 genotype. J. Exp. Med. **148:**534–543.
8. **Grau, G. E., P. Puthavathana, S. Izui, and P.-H. Lambert.** 1983. Induction of acute thrombocytopenia and infection of megakaryocytes by Rauscher murine leukemia virus reflect the genetic susceptibility to leukemogenesis. J. Exp. Med. **157:** 1028–1039.
9. **Graves, M. C., L. Bologa, L. Siegel, and H. Londe.** 1986. Theiler's virus in brain cell cultures: lysis of neurons and oligodendrocytes and persistence in astrocytes and macrophages. J. Neurosci. Res. **15:**491–501.
10. **Jennings, S. R., P. L. Rice, S. Pan, B. B. Knowles, and S. S. Tevethia.** 1984. Recognition of herpes simplex virus antigens on the surface of mouse cells of the H-$2^b$ haplotype by virus-specific cytotoxic T lymphocytes. J. Immunol. **132:**475–481.
11. **Lawson, C. M., J. E. Grundy, and G. R. Shellam.** 1987. Delayed-type hypersensitivity responses to murine cytomegalovirus in genetically resistant and susceptible strains of mice. J. Gen. Virol. **68:**2379–2388.
12. **Lehrich, J. R., B. G. W., and F. H. Hochberg.** 1976. Demyelinative myelopathy in mice induced by the DA virus. J. Neurol. Sci. **29:**149–160.
13. **Lindsley, M. D., and M. Rodriguez.** 1989. Characterization of the inflammatory response in the central nervous system of mice susceptible or resistant to demyelination by Theiler's virus. J. Immunol. **142:**2677–2682.
14. **Lindsley, M. D., and M. Rodriguez.** 1990. Cytotoxic lymphocyte activity in the CNS of Theiler's virus-infected resistant C57BL/10 mice. Neurology **40**(Suppl. 1):219.
15. **Lipton, H. L.** 1975. Theiler's virus infection in mice: an unusual biphasic disease process leading to demyelination. Infect. Immun. **11:**1147–1155.
16. **Lipton, H. L., and M. C. Dal Canto.** 1976. Theiler's virus induced demyelination: prevention by immunosuppression. Science **192:**62–64.
17. **Lipton, H. L., and M. C. Dal Canto.** 1979. The TO strains of Theiler's viruses cause "slow virus-like" infections in mice. Ann. Neurol. **6:**25–28.
18. **Lipton, H. L., and R. Melvold.** 1984. Genetic analysis of susceptibility to Theiler's virus-induced demyelinating disease in mice. J. Immunol. **132:**1821–1825.
19. **Melvold, R. W., D. M. Jokinen, R. L. Knobler, and H. L. Lipton.** 1987. Variations in genetic control of susceptibility to Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. I. Differences between susceptible SJL/J and resistant BALB/c strains map near the T cell β-chain constant gene on chromosome 6. J. Immunol. **138:**1429–1433.
20. **Melvold, R. W., D. M. Jokinen, S. D. Miller, M. C. Dal Canto, and H. L. Lipton.** 1987. H-2 genes in TMEV-induced demyelination, a model for multiple sclerosis, p. 735–745. *In* C. S. David (ed.), H-2 antigens: genes, molecules, function. Plenum Publishing Corp., New York.
21. **Melvold, R. W. D. M. Jokinen, S. D. Miller, M. C. Dal Canto, and H. L. Lipton.** 1990. Identification of a locus on mouse chromosome 3 involved in differential susceptibility to Theiler's murine encephalomyelitis virus-induced demyelinating disease. J. Virol. **64:**686–690.
22. **O'Neill, H. C., and R. V. Blanden.** 1983. Mechanisms determining innate resistance to ectromelia virus infection in C57BL mice. Infect. Immun. **41:**1391–1394.
23. **Paya, C. V., A. K. Patick, P. J. Leibson, and M. Rodriguez.** 1989. Role of natural killer cells as immune effectors in encephalitis and demyelination induced by Theiler's virus. J. Immunol. **143:**95–102.
24. **Penney, J. B., Jr., and J. S. Wolinsky.** 1979. Neuronal and oligodendroglial infection by the WW strain of Theiler's virus. Lab. Invest. **40:**324–330.
25. **Pierce, M., and M. Rodriguez.** 1989. Erichrome stain for myelin on osmicated tissue embedded in glycol methacrylate plastic. J. Histotech. **12:**35–36.
26. **Rodriguez, M.** 1985. Virus-induced demyelination in mice: "dying back" of oligodendrocytes. Mayo Clin. Proc. **60:**433–436.
27. **Rodriguez, M., and C. S. David.** 1985. Demyelination induced by Theiler's virus: influence of the H-2 haplotype. J. Immunol. **135:**2145–2148.
28. **Rodriguez, M., C. S. David, and L. R. Pease.** 1987. The contribution of the MHC gene products to demyelination by Theiler's virus, p. 747–756. *In* C. S. David (ed.), H-2 antigens: genes, molecules, function. Plenum Publishing Corp., New York.
29. **Rodriguez, M., W. P. Lafuse, J. L. Leibowitz, and C. S. David.** 1986. Partial suppression of Theiler's virus-induced demyelination in vivo by administration of monoclonal antibodies to immune response gene products (Ia antigens). Neurology **30:** 964–970.
30. **Rodriguez, M., J. Leibowitz, and C. S. David.** 1986. Susceptibility to Theiler's virus-induced demyelination. Mapping of the gene within the H-2D region. J. Exp. Med. **163:**620–631.
31. **Rodriguez, M., J. L. Leibowitz, H. C. Powell, and P. W. Lampert.** 1983. Neonatal infection with the Daniels strain of theiler's murine encephalomyelitis virus. Lab. Invest. **49:**672–679.
32. **Rodriguez, M., A. K. Patick, and L. R. Pease.** 1990. Abrogation of resistance to Theiler's virus-induced demyelination in C57BL mice by total body irradiation. J. Neuroimmunol. **26:** 189–199.
33. **Rodriguez, M., and S. Sriram.** 1988. Successful therapy of Theiler's virus-induced demyelination (DA strain) with monoclonal anti-Lyt-2 antibody. J. Immunol. **140:**2950–2955.
34. **Roos, R. P., and R. Wollmann.** 1984. DA strain of Theiler's murine encephalomyelitis virus induces demyelination in nude

mice. Ann. Neurol. **15**:494–499.

35. **Theiler, M.** 1937. Spontaneous encephalomyelitis of mice—a new disease. J. Exp. Med. **65**:705–719.
36. **Thomsen, A. R., and O. Marker.** 1989. MHC and non-MHC genes regulate elimination of lymphocytic choriomeningitis virus and antiviral cytotoxic T lymphocyte and delayed-type hypersensitivity mediating T lymphocyte activity in parallel. J. Immunol. **142**:1333–1341.
37. **Vidovic, D., and P. Matzinger.** 1988. Unresponsiveness to a foreign antigen can be caused by self-tolerance. Nature (London) **336**:222–225.
38. **Zurbriggen, A., and R. Fujinami.** 1989. A neutralization-resistant Theiler's virus variant produces an altered disease pattern in the mouse central nervous system. J. Virol. **63**:1505–1513.