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The Interaction of Two Groups of Murine Genes Determines the Persistence of Theiler's Virus in the Central Nervous System

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Theiler's murine encephalomyelitis virus is responsible for a chronic inflammatory demyelinating disease of the central nervous system of the mouse. The disease is associated with persistent viral infection of the spinal cord. Some strains of mice are susceptible to viral infection, and other strains are resistant. The effect of the genetic background of the host on viral persistence has not been thoroughly investigated. We studied the amount of viral RNA in the spinal cords of 17 inbred strains of mice and their F_1 crosses with the SJL/J strain and observed a large degree of variability among strains. The pattern of viral persistence among mouse strains could be explained by the interaction of two loci. One locus is localized in the *H-2D* region of the major histocompatibility complex, whereas the other locus is outside this complex and is not linked to the *Tcrb* locus on chromosome 6.

The DA strain of Theiler's murine encephalomyelitis virus (TMEV), a picornavirus, causes a chronic demyelinating disease in the white matter from the spinal cord of susceptible mice (11, 12). The virus persists in oligodendrocytes, astrocytes, and macrophages/microglia cells throughout the disease, which can last more than a year (1, 10, 19, 33, 42). Viral RNA replication and possibly translation are restricted in these cells (4, 5). The demyelinating lesions are infiltrated by T and B lymphocytes, macrophages, and reactive astrocytes. Meningitis is frequently observed. These activated B and T lymphocytes are directed primarily against viral components (3, 13, 15, 29, 34). However, some of the antibodies secreted by infiltrating B lymphocytes recognize a host protein (3). The pathogenesis of demyelination is still poorly understood. A direct effect of the virus (27, 39, 40, 43) as well as autoaggressive effects of the immune system (17, 18, 31, 37, 38) have been reported. On the other hand, it is clearly established that viral persistence is required for demyelination to occur (6, 16, 22, 32).

Mice of inbred strains differ in susceptibility to the demyelinating disease induced by TMEV. SJL/J and DBA/2 mice are highly susceptible, C3H and AKR mice are moderately susceptible, and BALB/c and C57BL/6 mice are resistant (8). Genetic studies demonstrated that the susceptibility to TMEV-induced demyelination is controlled by more than one locus (2, 25). One of these loci is linked to the *H-2* complex (20) and was mapped to the *H-2D* region (9, 32). *H-2* congenic strains with *b*, *d*, and *k* haplotypes are resistant, whereas strains with *f*, *p*, *q*, *r*, *v*, and *s* haplotypes are susceptible (30). The resistant haplotypes are dominant (28). Studies on recombinant inbred strains suggested the involvement of two other genes. One could be located on chromosome 6, close to the *Tcrb* locus (23), and the other could be located on chromosome 3, close to *Car-2* (24).

The *H-2* haplotype alone is not sufficient to predict whether a strain will be susceptible or resistant (25). For example, the DBA/2 strain, which is highly susceptible to the demyelinating disease, carries $H\text{-}2^d$, a resistant haplotype. Also, the study of recombinant inbred strains produced by crossing of the resistant BALB/c strain ($H\text{-}2^d$) with the susceptible SJL/J strain ($H\text{-}2^s$) did not confirm the role of an *H-2* gene (23), even though their two *H-2* haplotypes are associated with resistance and susceptibility, respectively, to demyelination (30). Furthermore, a similar study with the resistant C57BL/6 strain ($H\text{-}2^b$) and susceptible DBA/2 strain ($H\text{-}2^d$) showed that an *H-2* gene determines demyelination (24), although both strains carry resistant *H-2* haplotypes (30). These discrepancies are probably due to the multiplicity of events which determine demyelination.

To simplify the analysis, we have studied the genetic control of viral persistence, a prerequisite step for demyelination. Some fragmentary and conflicting results had been published on the subject (6, 8, 9, 20, 28); therefore, a thorough investigation seemed fully warranted. Viral persistence was studied by measuring the amount of viral RNA in the spinal cord with a semiquantitative dot blot assay. Major differences in the level of viral infection were observed among the 17 strains tested. These differences can be explained by the interaction of two loci. One locus is localized in the *H-2D* region and is highly polymorphic. The *b* haplotype confers resistance to the infection, whereas the *q* haplotype confers a high level of susceptibility. The other *H-2* haplotypes studied (*d*, *k*, and *s*) are associated with intermediate levels of susceptibility. The resistant *b* haplotype is dominant over all the others. A second locus is localized outside the major histocompatibility complex (MHC). The susceptible allele is present only in the SJL/J strain and is dominant. This locus is not linked to the *Tcrb* locus on chromosome 6.

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MATERIALS AND METHODS

Animals and virus. C57BL/6Pas, BALB/cPas, DBA/1Pas, DBA/2Pas, NZB/Pas, C3H/He/Ou//Pas, SWR/Pas, and SJL/JPas mice were bred in the Pasteur Institute animal facility. C57BL/10, B10.S, B10.D2, B10.BR, B10.Q, B10.A(2R), C3H.B10, and C3H.Q mice were obtained from the INSERM U93 animal facility (Hôpital Saint Louis). C57BR/J mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). The F_1 hybrids and backcrosses were raised in the Pasteur Institute animal facility. Mice, 3 to 4 week old, were inoculated intracranially with 10^4 PFU of TMEV strain DA in 40 μl of phosphate-buffered saline. Animals were sacrificed 45 days postinfection. Spinal cords, and brains when needed, were removed and used to prepare RNA immediately or kept frozen at −80°C.

Preparation of total RNA and dot blots. Total RNA was extracted from the spinal cords by the procedure of Chirgwin et al. (7) and quantified with a spectrophotometer. Series of fivefold dilutions of total RNA, starting from 10 μg, were dotted on Hybond C-extra filters (Amersham) according to the manufacturer's recommendations. The dot blots were hybridized overnight in 0.5 M sodium phosphate (pH 7.4)–7% sodium dodecyl sulfate (SDS) at 65°C with 10^6 cpm of a virus-specific, random-primed [^{32}P]cDNA probe (specific activity of between 10^7 and 10^8 cpm/μg). The filters were washed five times for 15 min at 65°C in 40 mM sodium phosphate (pH 7.4)–1% SDS and exposed overnight at −80°C. Dilutions of a stock solution of TMEV strain DA cDNA, diluted in genomic mouse DNA, were dotted in parallel. The results obtained with this standard were used to normalize the results of different experiments. For each mouse, the highest dilution which gave a positive signal was used as a measure of viral RNA content. Mean and standard error of the mean (SEM) were calculated for each strain of mouse.

DNA extraction and PCR. Brain DNA was extracted as described previously (21). The *Tcrb* genotype was determined by polymerase chain reaction (PCR) amplification followed by *Bam*HI and/or *Xba*I digestion of the PCR products (41). Restriction fragments were analyzed on a 4% agarose gel (NuSieve 3:1) stained with ethidium bromide.

Statistical analysis. Means were compared successively by the unpaired *t* Student test and the Mann-Whitney test. One-, two-, or three-factor analysis of variance with or without interaction (the BMDP package) was used to detect an effect of qualitative variables. The Tukey studentized range method was used to specify the action of the different MHC haplotypes.

RESULTS

Persistence of TMEV in different inbred strains. The results obtained from a representative dot blot are shown in Fig. 1. It can be seen in this example that two SJL/J mice had, respectively, 25- and 125-fold more viral RNA than did the other mice (C57BL/6, BALB/c, and DBA/2 strains). After normalization as described in Materials and Methods, the highest dilution which gave a positive hybridization signal was used as a measure of viral RNA content in the central nervous system (CNS) (Fig. 1). Means and SEMs of the RNA content were calculated for all strains and for their F_1 hybrids and are presented in Fig. 2 to 5 and Table 1. The differences observed among strains were highly reproducible and allowed an unambiguous classification. It should be noticed that the heights of the bars in the figures are directly

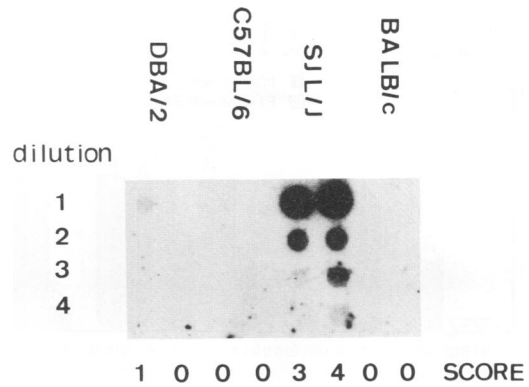

FIG. 1. Representative dot blot used to quantitate viral RNA in the CNS. Serial fivefold dilutions of 10 μg of total RNA from spinal cord were dotted and hybridized. Two infected mice were studied for each strain. For each mouse, the highest positive dilution was used as a measure of viral RNA content. The score given to individual mice is shown at the bottom.

proportional to the logarithmic transformation of the amount of viral RNA. The amount of viral RNA varied widely among the six inbred strains studied and their F_1 hybrids with the SJL/J strain (Fig. 2). Interestingly, mice of some inbred strains (DBA/2, NZB, BALB/c, and C57BL/6 strains) were infected at low levels, and their F_1 hybrids with the SJL/J strain were infected either at high levels (DBA/2, NZB, and BALB/c strains) or a low level (C57BL/6 strain), suggesting that several genes interact to determine viral persistence. In fact, we show below that our results can be explained by the interaction of only two loci.

The persistence of TMEV is determined by a gene localized in the MHC. Previous studies had shown that demyelination was determined by a gene located in the *H-2D* region of the MHC (9, 32) and that its resistant alleles were dominant (28). Since conflicting results concerning the effect of this gene on

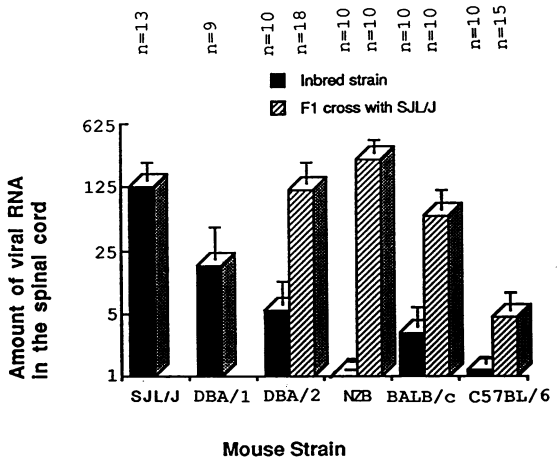

FIG. 2. Variability of TMEV persistence in different inbred mouse strains. The amount of viral RNA in the spinal cord was measured in six inbred strains (solid bars) (SJL/J [*H-2*^{s}], DBA/1 [*H-2*^{q}], DBA/2 [*H-2*^{d}], NZB [*H-2*^{d}], BALB/c [*H-2*^{d}], and C57BL/6 [*H-2*^{b}]) and their F_1 hybrids with SJL/J mice (hatched bars). The ordinate shows the titer of the highest RNA dilution which gave a positive hybridization signal. Mean and SEM are indicated for each group of mice. n, number of mice in each group.

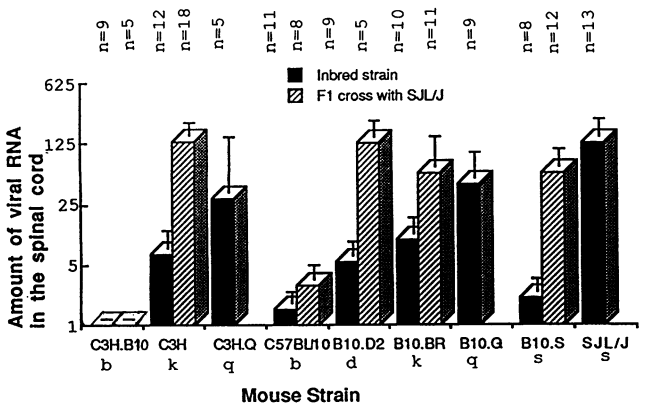

FIG. 3. Amount of viral RNA in the CNS of mice from *H-2* congenic strains. The amounts of viral RNA in the CNS of *H-2* congenic mice with C3H and C57BL/10 backgrounds (solid bars) and in their F_1 hybrids with SJL/J mice (hatched bars) are presented as in Fig. 2. The MHC haplotype is indicated below the name of each strain.

viral persistence had been obtained, we reexamined this question by using the assay described above. A set of congenic strains carrying MHC of various origins in a C57BL/10 or C3H background, as well as their F_1 hybrids with SJL/J were studied (Fig. 3). The C3H.B10 and C57BL/10 strains ($H\text{-}2^b$) and their F_1 hybrids with the SJL/J strain were infected at low levels. The levels of infection in the C3H.Q and B10.Q strains ($H\text{-}2^q$) were similar to that observed in the highly susceptible SJL/J strain. The other congenic strains ($H\text{-}2^d$, $H\text{-}2^k$, and $H\text{-}2^s$) were infected at low levels; however, their F_1 hybrids with the SJL/J strain were infected at high levels. Therefore, susceptibility to viral persistence correlated with the MHC haplotype. The same results were obtained by using mice of the C57BL/10 and C3H backgrounds. The *b* haplotype was the most resistant one, the *q* haplotype was the most susceptible, and the *d*, *k*, and *s* haplotypes corresponded to intermediate susceptibilities. The high susceptibility of the SJL/J strain ($H\text{-}2^s$) is not taken into account in this analysis, because it is due not to the *H-2D* allele but to a second, dominant locus (see below). A single-factor analysis of variance performed on congenic strains confirmed the effect of the MHC [F (4, 127) = 14.482; P = 0.001].

A gene determining susceptibility is localized in the *H-2D* region, and its resistant allele is dominant. To map the gene determining viral persistence, the recombinant B10.A(2R) strain, which carries the *k* haplotype at the *K*, *I*, and *E* loci and the *b* haplotype in the *H-2D* region, was compared with the susceptible B10.BR strain ($H\text{-}2^k$) and with the resistant C57BL/10 strain ($H\text{-}2^b$). Figure 4A shows that viral RNA levels were the same in the B10.A(2R) and C57BL/10 strains. Therefore, a gene determining viral persistence is located in the *D* region of the MHC. To examine whether the effect of this gene on resistance is dominant, we took advantage of the C3H and C3H.B10 strains. We had shown that the C3H strain ($H\text{-}2^k$) was more susceptible to TMEV persistence than was the C3H.B10 strain ($H\text{-}2^b$) (Fig. 3). Figure 4B shows that the F_1 hybrid between these two strains is resistant to infection. Therefore, for the *H-2D* locus, the resistant allele (*b*) is dominant.

A second locus, outside the MHC and not linked to the *Tcrb* locus, determines TMEV persistence. Although the B10.S and SJL/J strains carry the same $H\text{-}2^s$ MHC haplotype, the former is less susceptible to TMEV infection than is the latter (Fig. 3). The F_1 hybrids between these two strains are infected at a level similar to that of the SJL/J strain (Fig. 3). This result indicates the existence of a second locus, outside the MHC, which also determines persistence and for which the susceptible allele is dominant. The effect of this locus is

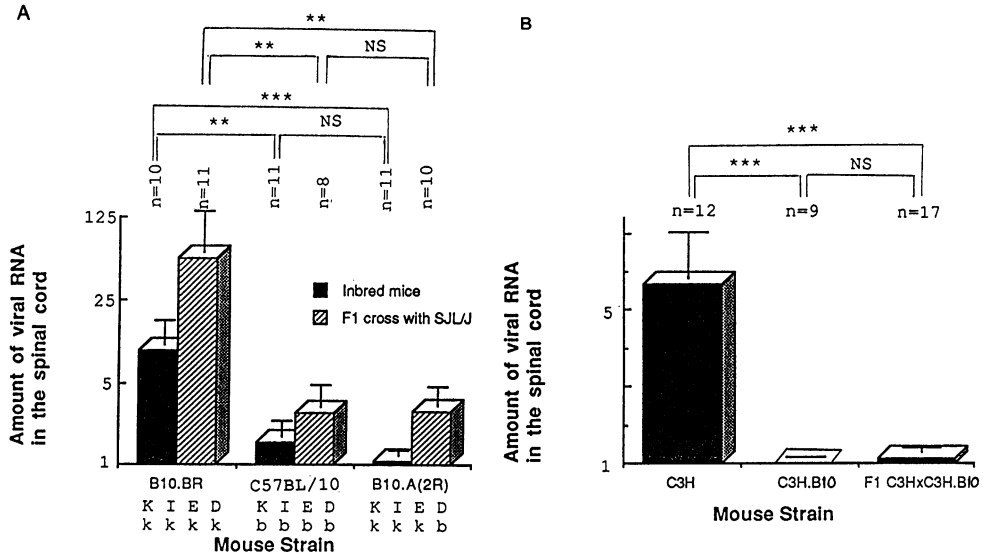

FIG. 4. Characterization of the *H-2* gene which determines viral persistence. The amounts of viral RNA in the spinal cords of mice of the different strains and their F_1 hybrids were compared successively with the unpaired Student t test and the Mann-Whitney test. ***, P = 0.001; **, P = 0.01; NS, nonsignificant. Similar results were obtained with the two tests except for the comparison of the F_1 hybrids in panel A, for which P = 0.05 with the Mann-Whitney test instead of P = 0.01 with the Student t test. n, number of mice in each group. (A) Viral persistence in the congenic B10.A(2R) strain and in the C57BL/10 and B10.BR strains. The allele in the four different regions of the MHC (*K*, *I*, *E*, and *D*) is indicated for each strain. (B) Viral persistence in the F_1 hybrids between C3H mice ($H\text{-}2^k$) and C3H.B10 mice ($H\text{-}2^b$) and their parents.

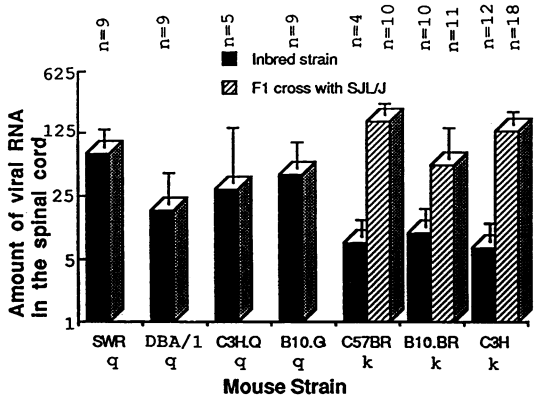

FIG. 5. Viral persistence in the SWR strain, the C57BR strain, and *H-2* matched strains. No statistically significant differences were detected between the SWR strain and the DBA/1, B10.Q, and C3H.Q strains or between the C57.BR strain (or its F_1 hybrids) and the C3H and B10.BR strain (or their F_1 hybrids). The tests used were the unpaired Student *t* test and the Mann-Whitney test.

TABLE 1. Amount of viral RNA detected in the spinal cord

Mouse strain	*H-2* haplotype	Mean amt of viral RNA in spinal cord ± SEM (no. of mice/group)[a]	
		Inbred strain	F_1 cross with SJL/J
C57BL/10	*b*	0.3 ± 0.2 (11)	0.6 ± 0.3 (8)
C57BL/6	*b*	0.1 ± 0.1 (10)	0.9 ± 0.3 (15)
C3H.B10	*b*	0.0 ± 0.0 (9)	0.0 ± 0.0 (5)
BALB/c	*d*	0.7 ± 0.3 (10)	2.5 ± 0.3 (10)
DBA/2	*d*	1.0 ± 0.3 (10)	2.9 ± 0.4 (18)
NZB	*d*	0.0 ± 0.0 (10)	3.4 ± 0.2 (10)
B10.D2	*d*	1.1 ± 0.2 (9)	3.0 ± 0.3 (5)
C3H	*k*	1.2 ± 0.3 (12)	3.0 ± 0.2 (18)
C57BR	*k*	1.2 ± 0.2 (4)	3.2 ± 0.2 (10)
B10.BR	*k*	1.4 ± 0.3 (10)	2.5 ± 0.5 (11)
DBA/1	*q*	1.8 ± 0.5 (9)	ND
SWR	*q*	2.7 ± 0.3 (9)	ND
B10.Q	*q*	2.3 ± 0.4 (9)	ND
C3H.Q	*q*	2.1 ± 0.9 (5)	ND
B10.S	*s*	0.4 ± 0.2 (8)	2.5 ± 0.3 (12)
SJL/J	*s*	3.0 ± 0.3 (13)	

[a] Data are expressed as described in Results. ND, not done.

also observed in the F_1 hybrids between the SJL/J strain (*H-2*^{s}) and strains with *H-2*^{d} or *H-2*^{k} haplotype (Fig. 3). However, in F_1 hybrids with *H-2 b/s* genotypes, the effect of the locus is undetectable (e.g., C3H.B10 and C57BL/10 mice in Fig. 3). A single-factor analysis of variance performed on all congenic strains confirmed the effect of this second locus [F (1, 130) = 28.246; P = 0.001].

It has been proposed that the deletion of about 50% of the T-cell receptor (TCR) Vβ genes of SJL/J mice, or an SJL/J-specific gene linked to the *Tcrb* locus, might be responsible for the susceptibility of this strain to demyelination induced by TMEV (23). We tested the effect of this deletion on viral persistence by using the SJL/J and B10.S strains of mice. The genotype at the *Tcrb* locus in (B10.S × SJL)F_1 × B10.S backcross mice was determined by using PCR and a restriction enzyme site polymorphism in the TCR Vβ1 gene (41). The PCR product from TCR Vβ-deleted chromosome lacks a *Bam*HI restriction site. Among 49 of these backcross mice, the mice homozygous (m_{Ho}) at the *Tcrb* locus had the same amount of virus as did the heterozygous mice (m_{He}) [m_{Ho} = 1.3 ± 0.3 (n = 23) versus m_{He} = 1.5 ± 0.3 (n = 26); $t_{47\ df}$ = 0.523; Z (23, 26) = −0.46, not significant]. This result rules out the possibility that the second locus determining persistence is localized close to the *Tcrb* locus on chromosome 6. Further, we compared C57BR (*H-2*^{k}) and SWR (*H-2*^{q}) strains, which have the same deletion as does the SJL/J strain, with strains carrying the same MHC haplotype but lacking the deletion (Fig. 5). No difference was detected. Therefore, the hypothesis that the *Tcrb* deletion is involved in the control of TMEV persistence had to be rejected.

Statistical analysis of the data is congruent with a two-loci model. Mean and SEM of the amount of viral RNA in the CNS of each strain studied are presented in Table 1. In this table, the amount of viral RNA is given not as titers, as in the figures, but as its logarithmic transformation obtained directly from the dot blots (i.e., the score shown in Fig. 1). On the basis of the data shown in Table 1, we proposed an a posteriori model which explains all of our observations on congenic and inbred strains. According to this model, alleles at two loci can explain the observed differences in viral persistence. One locus is localized in the *H-2D* region of MHC. Mice with *q* haplotype are more susceptible than are mice with *b*, *d*, *k*, and *s* haplotypes. A second locus is located outside the MHC. The allele which confers susceptibility to viral persistence is present only in the SJL/J strain. Its effect on persistence depends on the *H-2* haplotype of the mouse examined. This susceptible allele is dominant in *H-2*^{s} and in *s/d* and *s/k* hybrids and is recessive in *s/b* hybrids (Fig. 2 and 3).

A two-factor analysis of variance with interaction was performed on the data of Table 1 to test the model (Table 2). This analysis looked for an effect of the MHC, an effect of non-MHC genes of SJL/J origin in F_1 hybrids, and an interaction between these two factors. Interaction was included in the model to take into account some of our experimental results. Strains with *H-2*^{b} haplotype and their F_1 hybrids with the SJL/J strain (*H-2*^{s}) were infected at low levels, whereas strains with *H-2*^{d} and *H-2*^{k} haplotypes and the B10.S strain (*H-2*^{s}) were infected at lower levels than were their F_1 hybrids with the SJL/J strain. The results of the statistical analysis are in agreement with the model. Two loci are sufficient to explain the data of Table 1, since no background effect (other than for SJL/J) can be detected in a three-factor analysis of variance without interaction: for the *H-2* locus, F (4, 239) = 19.53, P = 0.001; for the non-*H-2* locus, F (1, 239) = 115.89, P = 0.001; effect of background, F (8, 239) = 0.73, not significant. The Tukey studentized range method confirmed that the *q* haplotype conferred the highest susceptibility to the inbred strains (Table 3) and that the highest resistance was conferred by the *b* haplotype in the F_1 hybrids with SJL/J mice (Table 4).

TABLE 2. Two-factor analysis of variance of the effects of *H-2* and non-*H-2* loci on viral persistence[a]

Source	df	Sum of squares	Mean square	F test	P value
H-2 locus	3	102.437	34.146	37.391	0.001
Non-*H-2* locus	1	129.297	129.297	141.586	0.001
Interaction	3	26.344	8.781	9.616	0.001
Error	226	206.385	0.913		

[a] Mice with *q* haplotype were not crossed with SJL/J mice, since all were infected at similar levels (Table 1). Therefore, these mice could not be included in the analysis of variance test used to look for interaction between *H-2* and non-*H-2* loci.

TABLE 3. Analysis of the effects of the different *H-2* haplotypes in inbred strains on viral persistence, using the Tukey studentized range method[a]

H-2 haplotype	Mean amt of viral RNA in spinal cord	Sample size	Result of (*P* value) statistical analysis for each haplotype				
			b	*s*	*d*	*k*	*q*
b	0.13	30		NS[b]	NS	0.01	0.01
s	0.44	8	NS		NS	NS	0.01
d	0.77	35	NS	NS		NS	0.01
k	1.27	26	0.01	NS	NS		0.01
q	2.23	32	0.01	0.01	0.01	0.01	

[a] In this population of mice, the *H-2* haplotype has a statistically significant effect [F (4, 126) = 22.07; P = 0.001].
[b] NS, not statistically significant.

DISCUSSION

Obviously, the pathogenesis of TMEV-induced disease involves a complex sequence of necessary steps, many of which are under the control of host genes. Several studies concerning genetic susceptibility to this disease have been published. In most cases, the authors monitored the appearance of clinical symptoms (8, 9, 23, 24) or histological demyelinating lesions (28, 30, 32). However, viral persistence had not yet been carefully investigated, although persistence seems to be prerequisite to the appearance of demyelination and clinical symptoms. Because studying an early event should simplify the analysis, we decided to thoroughly investigate the genetic determinism of viral persistence by using a fast, quantitative assay for viral RNA in the CNS. We showed that viral persistence could be explained by two interacting loci, one in the *H-2D* region and a second one not linked to the *Tcrb* locus.

Previous studies of the genetic determinism of viral persistence used an infectivity plaque assay to monitor CNS infection (8, 9, 20, 28, 32). Because TMEV particles are in great part associated with membranes, these assays, performed on tissue homogenates, are inaccurate. In contrast, the dot blot assay that we used gave reproducible results. Use of a plaque assay has another drawback: it measures virus production, not the persistence of viral genetic information. Host factors restrict TMEV replication at the single-cell level during persistent infection (4, 5), and the extent of restriction could vary from one mouse strain to another. Therefore, infectious titers are not necessarily proportional to the amount of viral RNA present in tissues. The dot blot test can be used to monitor any other phenotype measurable at the RNA level. For example, the expression of immunoglobulin kappa light-chain mRNA in the spinal cord can be used as marker of B-cell infiltration and, more generally, of inflammation. We found that this parameter varies in parallel with viral infection (data not shown).

TABLE 4. Analysis of the effects of the different *H-2* genotypes in SJL/J F_1 hybrids on viral persistence, using the Tukey studentized range method[a]

H-2 genotype	Mean amt of viral RNA in spinal cord	Sample size	Result (*P* value) of statistical analysis for each genotype			
			b/s	*s/s*	*d/s*	*k/s*
b/s	0.68	28		0.01	0.01	0.01
s/s	2.76	25	0.01		NS[b]	NS
d/s	2.98	43	0.01	NS		NS
k/s	2.92	39	0.01	NS	NS	

[a] In this population of mice, the *H-2* genotype has a statistically significant effect [F (3, 131) = 31.57; P = 0.001].
[b] NS, not statistically significant.

The experimental conditions that have been used to study the genetic susceptibility to TMEV-induced disease differ according to the parameter being examined. For example, to observe clinical disease in susceptible animals, it is necessary to inoculate them with 2×10^6 PFU of virus, whereas 2×10^5 is optimal to distinguish between susceptible and resistant mice by histological criteria. In our study, 10^4 PFU was optimal since it gave a full range of responses with the dot blot assay. It has been shown that the intensity of the clinical symptoms, the demyelination score, and the level of viral infectivity vary in proportion to the dose of virus inoculated (20, 30). Therefore, for the rest of this discussion, we will assume that the results of these various studies can be compared despite the differences in protocol.

Because we screened a large series of strains with a quantitative assay, we were able to clarify the effect of MHC genes on susceptibility of viral persistence and to explain some discrepancies of the literature. Using recombinant inbred strains, Melvold et al. (24) explained the difference of susceptibility to demyelination of the C57BL/6 (*H-2*^{b}) and the DBA/2 (*H-2*^{d}) strains partly by an effect of an MHC gene. However, both *H-2* haplotypes conferred resistance to the C57BL/10 (*H-2*^{b}) and B10.D2 (*H-2*^{d}) congenic strains (30). In contrast, with our quantitative assay, *H-2*^{b} confers resistance, whereas *H-2*^{d} is associated with intermediate susceptibility. Also, Melvold et al. (23) did not detect an effect of the MHC when comparing the phenotypes of BALB/c (*H-2*^{d}) and SJL/J (*H-2*^{s}) strains, although, according to the results of Rodriguez and David (30), *H-2*^{d} and *H-2*^{s} should confer resistance and susceptibility, respectively, to demyelination. In both cases, these discrepancies are explained by the existence of three classes of *H-2* haplotypes, not two, which determine resistance (*b*), intermediate susceptibility (*d*, *k*, and *s*), and full susceptibility (*q*), respectively. These three classes were uncovered because we used a quantitative assay for viral RNA. We established that the gene which affects susceptibility lies in the *H-2D* region and that the resistant allele is dominant. The fact that this gene confers a range of resistance and susceptibility degrees show that it is polymorphic, suggesting that it could code for an MHC class I molecule (2, 25, 28, 35, 36).

In our study, viral persistence does not always correlate with demyelination. For example, viral persistence is very similar in the C3H and C57BR strains, although the C3H strain is susceptible to demyelination and the C57BR strain is resistant (25). Also, one interpretation is that viral persistence is necessary but not sufficient for demyelination to occur. Viral persistence may be the first necessary event, followed by others which would trigger the onset of demyelination. In this case, other genes would determine these later steps, e.g., inflammation and demyelination. This view may also explain the low correlation observed by others between viral persistence and clinical disease (8, 20). Another interpretation is that other genes modify viral persistence at a qualitative level. For example, viral persistence could have different consequences whether it occurs in the white or the grey matter. A third interpretation is that the same MHC gene could act at different steps of the disease (e.g., persistence and inflammation and/or demyelination) and that the intensity of its pleiotropic effects could be independent of each other. Also, TMEV infects the B10.D2 strain as well as the B10.S strain, although the former is

resistant to demyelination and the latter is susceptible (9, 30).

Our results demonstrate that a second gene, outside the MHC, determines persistence. Melvold et al., using recombinant inbred strains between SJL/J and BALB/c, concluded that a gene localized close to *Tcrb* locus on chromosome 6 determines susceptibility to demyelination (23). This gene could act either through the control of viral persistence or at other steps. The first hypothesis can be ruled out since, the TCR Vβ deletion segregated independently of viral persistence in a set of (B10.S × SJL/J)F_1 × B10.S backcross animals. Moreover, the data obtained with the C57BR ($H\text{-}2^k$) and SWR ($H\text{-}2^q$) strains also exclude an influence of the deletion. It is still possible that *Tcrb* plays a role in disease at some step subsequently to the establishment of persistence. However, the localization of this second locus on mouse chromosome 6 by Melvold et al. (23) was not clearly established because the authors used an insufficient number of chromosome markers to characterize their set of recombinant inbred strains. Recently, Kappel et al. (14) reinvestigated the role of the *Tcrb* locus in order to explain the phenotype of one of the recombinant inbred strain which they had described previously. This strain (CXJ-13) was susceptible, although the *Tcrb* locus came from the resistant BALB/c strain (23). The authors explain this discrepancy by an overriding effect of the $H\text{-}2^s$ haplotype. According to their model, susceptibility to demyelination would be conferred either by the $H\text{-}2^s$ haplotype or by the *Tcrb* deletion. If this is the case, the *H-2* haplotype explains the phenotype of 4 of the 10 recombinant inbred strains examined. The correlation between phenotype and *Tcrb* genotype holds for the six other recombinant inbred strains, a number too low to establish a linkage (26).

In summary, we showed that TMEV persistence can be explained by the existence of two interacting loci. One is localized in the *H-2D* region. The *H-2* haplotype at this locus is associated with either resistance (*b*), intermediate susceptibility (*d*, *k*, and *s*), or susceptibility (*q*). The resistant allele is dominant. The second locus is localized outside the MHC and is not linked to *Tcrb*. Its susceptible allele is found only in the SJL/J strain. The interaction between these two loci is sufficient to explain the phenotypes of mice of all strains, F_1 hybrids, and backcrosses studied in this investigation.

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REFERENCES

1. **Aubert, C., M. Chamorro, and M. Brahic.** 1987. Identification of Theiler's virus infected cells in the central nervous system of the mouse during demyelinating disease. Microb. Pathog. **3**:319–326.
2. **Brahic, M., J. F. Bureau, and A. McAllister.** 1991. Mini-review: genetic determinants of the demyelinating disease caused by Theiler's virus. Microb. Pathog. **11**:77–84.
3. **Cash, E., A. Bandeira, S. Chirinian, and M. Brahic.** 1989. Characterization of B lymphocytes present in the demyelinating lesions induced by Theiler's virus. J. Immunol. **143**:984–988.
4. **Cash, E., M. Chamorro, and M. Brahic.** 1985. Theiler's virus RNA and protein synthesis in the central nervous system of demyelinating mice. Virology **144**:290–294.
5. **Cash, E., M. Chamorro, and M. Brahic.** 1986. Quantitation, with a new assay, of Theiler's virus capsid protein in the central nervous system of mice. J. Virol. **60**:558–563.
6. **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.
7. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**:5294–5299.
8. **Clatch, R. J., H. L. Lipton, and S. D. Miller.** 1987. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. II. Survey of host immune responses and central nervous system virus titers in inbred mouse strains. Microb. Pathog. **3**:327–337.
9. **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.
10. **Clatch, R. J., S. D. Miller, R. Metzner, M. C. Dal Canto, and H. L. Lipton.** 1990. Monocytes/macrophages isolated from the mouse central nervous system contain infectious Theiler's murine encephalomyelitis virus (TMEV). Virology **176**:244–254.
11. **Dal Canto, M. C., and H. L. Lipton.** 1975. Primary demyelination in Theiler's virus infection. An ultrastructural study. Lab. Invest. **33**:626–637.
12. **Dal Canto, M. C., and H. L. Lipton.** 1979. Recurrent demyelination in chronic central system infection produced by Theiler's murine encephalomyelitis virus. J. Neurol. Sci. **42**:391–405.
13. **Gerety, S. J., R. J. Clatch, H. L. Lipton, R. G. Goswami, M. K. Rundell, and S. D. Miller.** 1991. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus-induced demyelinating disease. IV. Identification of an immunodominant T cell determinant on the N-terminal end of the VP2 capsid protein in susceptible SJL/J mice. J. Immunol. **146**:2401–2408.
14. **Kappel, C. A., M. C. Dal Canto, R. W. Melvold, and B. S. Kim.** 1991. Hierarchy of effects of the MHC and T cell receptor β-chain genes in susceptibility to Theiler's murine encephalomyelitis virus-induced demyelinating disease. J. Immunol. **147**:4322–4326.
15. **Lindsley, M. D., R. Thiemann, and M. Rodriguez.** 1991. Cytotoxic T cells isolated from the central nervous system of mice infected with Theiler's virus. J. Virol. **65**:6612–6620.
16. **Lipton, H. L., M. Calenoff, P. Brandyophyay, S. D. Miller, M. Dal Canto, S. Gerety, and K. Jensen.** 1991. The 5′ noncoding sequences from a less virulent Theiler's virus dramatically attenuate GDVII neurovirulence. J. Virol. **65**:4370–4377.
17. **Lipton, H. L., and M. C. Dal Canto.** 1976. Theiler's virus induced demyelination prevention by immunosuppression. Science **192**:62–64.
18. **Lipton, H. L., and M. C. Dal Canto.** 1977. Contrasting effects of immunosuppression on Theiler's virus infection in mice. Infect. Immun. **15**:903–909.
19. **Lipton, H. L., J. Kratochvil, P. Sethi, and M. C. Dal Canto.** 1984. Theiler's virus antigen detected in mouse spinal cord 2 1/2 years after infection. Neurology **34**:1117–1119.
20. **Lipton, H. L., and R. W. Melvold.** 1984. Genetic analysis of susceptibility to Theiler's virus-induced demyelinating disease in mice. J. Immunol. **132**:1821–1825.
21. **Maniatis, R., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **McAllister, A., F. Tangy, C. Aubert, and M. Brahic.** 1990. Genetic mapping of the ability of Theiler's virus to persist and demyelinate. J. Virol. **64**:4252–4257.
23. **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.

24. **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.
25. **Miller, S., and S. Gerety.** 1990. Immunologic aspects of Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. Semin. Virol. **1:**263–272.
26. **Neumann, P. E.** 1990. Two-locus linkage analysis using recombinant inbred strains and Bayes' theorem. Genetics **126:**277–284.
27. **Ozden, S., C. Aubert, D. Gonzalez-Dunia, and M. Brahic.** 1991. In situ analysis of proteolipid protein gene transcripts during persistent Theiler's virus infection. J. Histochem. Cytochem. **39:**1305–1309.
28. **Patick, A. K., L. R. Pease, C. S. David, and M. Rodriguez.** 1990. Major histocompatibility complex-conferred resistance to Theiler's virus-induced demyelinating disease is inherited as a dominant trait in B10 congenic mice. J. Virol. **64:**5570–5576.
29. **Pena Rossi, C., A. McAllister, L. Fiette, and M. Brahic.** 1991. Theiler's virus infection induces a specific cytotoxic T lymphocyte response. Cell. Immunol. **138:**341–348.
30. **Rodriguez, M., and C. S. David.** 1985. Demyelination induced by Theiler's virus: influence of the H-2 haplotype. J. Immunol. **135:**2145–2148.
31. **Rodriguez, M., W. P. Lafuse, J. 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 **36:** 964–970.
32. **Rodriguez, M., J. L. 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.
33. **Rodriguez, M., J. L. Leibowitz, and P. W. Lampert.** 1983. Persistent infection of oligodendrocytes in Theiler's virus-induced encephalomyelitis. Ann. Neurol. **13:**426–433.
34. **Rodriguez, M., C. F. Lucchinetti, R. J. Clark, T. L. Yaksh, H. Markowitz, and V. A. Lennon.** 1988. Immunoglobulin and complement in demyelination induced in mice by Theiler's virus. J. Immunol. **140:**800–806.
35. **Rodriguez, M., C. Nickerson, A. K. Patrick, and C. S. David.** 1991. Expression of human HLA-B27 transgene alters susceptibility to murine Theiler's virus-induced demyelination. J. Immunol. **146:**2596–2602.
36. **Rodriguez, M., E. Oleszak, and J. Leibowitz.** 1987. Theiler's murine encephalomyelitis: a model of demyelination and persistence of virus. Crit. Rev. Immunol. **7:**325–365.
37. **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.
38. **Roos, R. P., S. Firestone, R. Wollmann, D. Variakojis, and B. G. W. Arnason.** 1982. The effects of short term and chronic immunosuppression on Theiler's virus demyelination. J. Neuroimmunol. **2:**223–234.
39. **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.
40. **Rosenthal, A., R. S. Fujinami, and P. W. Lampert.** 1986. Mechanism of Theiler's virus induced demyelination in nude mice. Lab. Invest. **54:**515–522.
41. **Spinella, D. G.** 1990. A rapid method for genotyping mice for T cell receptor Vβ and Vβ haplotypes by PCR analysis of whole blood. J. Immunol. Methods **135:**209–212.
42. **Theiler, M.** 1937. Spontaneous encephalomyelitis of mice, a new virus disease. J. Exp. Med. **55:**705–719.
43. **Zurbriggen, A., M. Yamada, C. Thomas, and R. Fujinami.** 1991. Restricted virus replication in the spinal cords of nude mice infected with a Theiler's virus variant. J. Virol. **65:**1023–1030.