

Mouse Susceptibility to Mouse Hepatitis Virus Infection Is Linked to Viral Receptor Genotype

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We have reported that the receptor for mouse hepatitis virus (MHV) expressed in MHV-susceptible BALB/c mice (MHVR1) has 10 to 30 times the virus-binding activity of the MHV receptor expressed in MHV-resistant SJL mice (MHVR2) (N. Ohtsuka, Y. K. Yamada, and F. Taguchi, *J. Gen. Virol.* 77:1683–1992, 1996). This fact indicates the possibility that the difference in MHV susceptibility between BALB/c and SJL mice is determined by the virus-binding activity of the receptor. To test this possibility, we have examined MHV susceptibility in mice with the homozygous *MHVR1* gene (R1/R1 genotype), mice with the *MHVR1* and *MHVR2* genes (R1/R2 genotype), and mice with the homozygous *MHVR2* gene (R2/R2 genotype) produced by cross and backcross mating between BALB/c and SJL mice. All 63 F₂ and backcrossed mice with the *MHVR1* gene (R1/R1 and R1/R2) were susceptible to MHV infection, and all 57 with the homozygous *MHVR2* gene (R2/R2) were resistant. We have also examined the MHV receptor genotypes of several mouse strains that were reported to be susceptible to MHV infection. All of those mice had the *MHVR1* gene. These results suggest the possibility that the viral receptor determines the susceptibility of the whole animal to MHV infection.

Differences in susceptibility to many viral infections have been documented among mouse strains (18). These differences have been studied as models of the differences in susceptibility of individual humans to various viral infections. A number of host factors are known to be involved in such differences, from nonspecific host defense factors, such as macrophages (1, 10) and interferon (9, 12), to specific immune responses by antibody (25) and cytotoxic T cells (30). Other host factors are known to control the susceptibility of mice to viral infection in a virus-specific fashion (11, 14, 18). The host cell's viral receptor or coreceptor is also one of the factors influencing the strain difference in virus infection (24).

A virus receptor is a molecule with which the virus interacts at an initial step of infection and thus is a crucial host factor for virus infection. Virus receptor proteins have been identified for several different viruses, including mouse hepatitis virus (MHV) (13). The functional receptor for MHV is a murine biliary glycoprotein (Bgp) which is classified as a carcinoembryonic antigen of the immunoglobulin superfamily (7). Allelic genes for the MHV receptor proteins Bgp1^a (MHVR1) and Bgp1^b (MHVR2) are expressed in susceptible BALB/c (7) and resistant SJL (33) mice, respectively. The biggest difference between these two types of proteins is located in the N domain (7, 33), where the virus-binding site is located (8). In an early study by Boyle et al., MHVR1 showed virus-binding activity while MHVR2 failed to bind to the virus (2). This fact allowed them to speculate that the different viral affinities of these proteins may account for the difference in MHV susceptibility that BALB/c and SJL mice exhibit (2). However, Yokomori and Lai showed that when expressed on Cos7 cells, which are normally nonpermissible to MHV infection, both proteins function as MHV receptors without any apparent differences, suggesting that the difference in susceptibility between BALB/c

and SJL mice does not depend upon receptor function (33). Dveksler et al. (6) also found that MHVR2 functions as a receptor for MHV A-59. They suggested that small differences in the efficacy of a virus receptor which might exist between MHVR1 and MHVR2 could result in very large biological differences in the multiple cycles of infection (6). We have analyzed the receptor functionality of these proteins in detail and reached the conclusion that MHVR1 expressed on BHK-21 cells has 10 to 30 times the virus-binding activity of MHVR2 (20). Similar results were obtained by other investigators (4, 22). These MHV receptor genes have been reported to be located on chromosome 7 (23). It is of great interest that the gene controlling the susceptibility and resistance of BALB/c and SJL mice is also located on chromosome 7 (26). All of these findings prompted us to test whether or not the MHV receptor determines the susceptibility of the whole animal to MHV infection by using crossed and backcrossed BALB/c and SJL mice.

First, we examined virus growth in the brains of BALB/c and SJL mice. We used 6- to 7-week-old BALB/c (Japan CLEA Inc., Tokyo, Japan) and SJL (Gokita Breeding Inc., Tokyo, Japan) mice free of murine pathogens, including MHV and MHV-related pathogens. When inoculated intracerebrally into mice, the 50% lethal doses of the MHV JHM sp-4 strain (28) were calculated as being less than 0.7 PFU and more than 2×10^4 PFU for BALB/c and SJL mice, respectively. Based on those values, we inoculated 7×10^2 PFU of virus in 50 μ l of Eagle's minimal essential medium, which killed all of the BALB/c mice within 6 days postinoculation (p.i.) and none of the SJL mice. As shown in Fig. 1, a striking difference in virus growth was observed. In the brains of SJL mice, no virus titer higher than 10^2 PFU/g was detected within 6 days p.i. while about 10^6 PFU of virus per gram was detected in the brains of BALB/c mice as early as 2 days p.i. The 10- to 30-fold increased virus-binding activity of MHVR1 theoretically can account for the difference in virus growth between BALB/c and SJL mice shown in Fig. 1; namely, by 3 days p.i., a 10-fold difference in virus-binding activity would lead to a 10^9 -fold difference in virus titer in animals when one cycle of MHV replication is calculated to be

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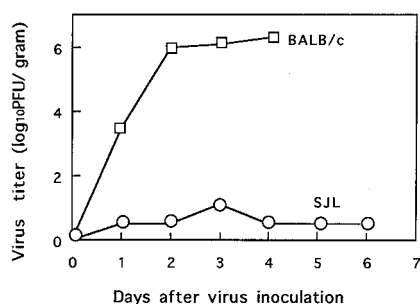


FIG. 1. Growth of JHM sp-4 virus in the brains of BALB/c (□) and SJL (○) mice. Six- to 7-week-old BALB/c and SJL mice were intracerebrally inoculated with 7×10^2 PFU of virus. Brains were collected from infected mice at intervals after inoculation, and virus titers in the brains were plaque assayed as previously described (29). Each point represents the mean virus titer from three to five samples.

8 h and other aspects of viral replication are considered equal. Thus, the difference in growth in the brains of BALB/c and SJL mice could result from the difference in receptor function.

Next, we confirmed earlier reports that BALB/c mice with an *MHVR1* genotype (R1/R1) are susceptible to MHV, that SJL with an *MHVR2* genotype (R2/R2) are resistant to MHV, and that F₁ mice (BALB/c × SJL) with an R1/R2 genotype are MHV susceptible (Table 1) (26). This dominant feature of susceptibility to MHV infection is determined by the presence of a single gene on chromosome 7 (26). If MHV susceptibility in mice is determined only by the type of receptor, then mice with one R1 allele, i.e., with the R1/R1 or R1/R2 genotype, should be susceptible to the virus while those with the homozygous R2/R2 genotype should be resistant. To see whether this is the case, we crossed BALB/c and SJL mice to obtain progeny with the receptor genotypes R1/R1, R1/R2, and R2/R2. The F₂ mice should be a mixture of R1/R1, R1/R2, and R2/R2 at a ratio of 1:2:1 and the backcross to SJL mice should generate R1/R2 and R2/R2 mice at the same ratio. We then tested MHV susceptibility and the receptor genotypes of individual mice. The susceptibility of mice to MHV infection was estimated on the basis of whether mice died within 14 days p.i. (susceptible) or survived for more than 14 days (resistant). Mice were also clinically observed for the central nervous system (CNS) symptoms that mice develop after JHM inoculation (31). A few mice that died within 24 h p.i. were excluded, since those mice died by brain destruction due to technical errors. The genotypes of individual mice, R1/R1, R1/R2, or R2/R2, were determined by a method of single-strand conformation polymorphism (SSCP) analysis by using PCR-amplified DNA fragments (Fig. 2A and B) (21). The genomic DNA isolated from the tails of BALB/c or SJL mice or their mated progenies were used as PCR templates to amplify a fragment of the MHVR N domain with the oligonucleotide 1 (5'CCTCACTT TTAGCCTCCTGGAG3') and 2 (5'ACATGAAATTGCAC AGTCGC3') primers. Plasmids harboring the *MHVR1* and *MHVR2* genes (20) were also used as templates to see the right bands amplified from *MHVR1* and *MHVR2*. A 0.5-μl sample of the PCR products was further amplified by using oligonucleotide 3 (5'GCTGAAGTCACCATTGAGGC3') and fluorescein isothiocyanate (FITC)-labeled oligonucleotide 4 (5'AGCAGG GATCCATTGCTGTA3'). One microliter of DNA amplified and labeled with FITC by the second PCR was mixed with 4 μl of formamide (DNA grade; Wako Pure Chemical Industries, Osaka, Japan) and denatured by heating at 94°C for 2 min. The sample was electrophoresed on a 6% polyacrylamide gel con-

taining 5% (wt/vol) glycerol at $26 \pm 1^\circ\text{C}$. The gel was then analyzed on a FluorImager SI (Molecular Dynamics, Sunnyvale, Calif.). As shown in Table 1, all of the 31 F₂ mice with the R1/R1 or R1/R2 genotype died with CNS symptoms like hunched posture and convulsions, as previously reported (31). All 14 of the F₂ mice with the R2/R2 genotype survived JHM virus infection. In addition, the 32 backcrossed mice with the R1/R2 genotype all died showing CNS symptoms and the 43 backcrossed mice with the R2/R2 genotype all survived infection. The surviving F₂ and backcrossed mice did not show any clinical symptoms during the observation period, nor did the parental SJL mice. These data demonstrated that the receptor type and MHV susceptibility could not be segregated in a total of 120 backcross and F₂ generation mice, suggesting that the gene responsible for susceptibility or resistance to MHV infection is the receptor gene itself or that if it is different from the MHV receptor gene, it is located fairly close to it, within less than 0.84 centimorgans of the gene. All of the BALB/c and most of the F₂ and backcrossed mice with the R1 allele died within 6 days p.i., yet a few F₂ and backcrossed mice with the R1 allele died 9 to 10 days p.i. with CNS symptoms. This difference in survival time could be affected by unknown minor factors influencing susceptibility to MHV, as reported previously (27).

It has been reported that mouse strains such as A/J, C3H, C57BL/6, and SWR (15) or NZB, DBA/2, AKR/J, and CBA/J (26) are also as susceptible to MHV infection as are BALB/c mice. We therefore examined whether these mouse strains have the *MHVR1* gene by SSCP. As shown in Fig. 3, all of these strains had the *MHVR1* gene. The *MHVR* genotype of A/J mice was not shown by SSCP, but sequence analysis demonstrated that the A/J strain had the *MHVR1* genotype (data not shown). Only the SJL mice we examined had the *MHVR2* gene, and only this strain of mice showed high resistance to MHV infection (15, 26). These facts strengthened the possibility that the susceptibility of mice to MHV is controlled by the receptor gene.

Two other proteins have been reported to function as MHV receptors in mice. MHVR-related Bgp2 was expressed in both BALB/c and SJL mice and was shown to serve as an MHV receptor, but with lower efficiency than that of MHVR (19). The other protein was a member of the pregnancy-specific

TABLE 1. Relationship between the MHV susceptibilities and MHV receptor genotypes of mice

Mouse	Genotype ^a	No. of mice ^b		
		Died	Survived	Total
BALB/c	R1/R2	33	0	33
SJL	R2/R2	0	27	27
F ₁	R1/R2	13	0	13
F ₂	R1/R1	8	0	8
	R1/R2	23	0	23
	R2/R2	0	14	14
Backcross	R1/R2	32	0	32
	R2/R2	0	43	43
Total	R1/R1, R1/R2	63	0	63
	R2/R2	0	57	57

^a The MHV receptor genotype was tested by SSCP by using DNA isolated from each mouse tail.

^b BALB/c, SJL, (BALB/c × SJL)F₁, and F₂ mice and mice backcrossed to SJL mice were inoculated intracerebrally with 7×10^2 PFU of MHV JHM sp-4, and mortality was checked for 2 weeks later.

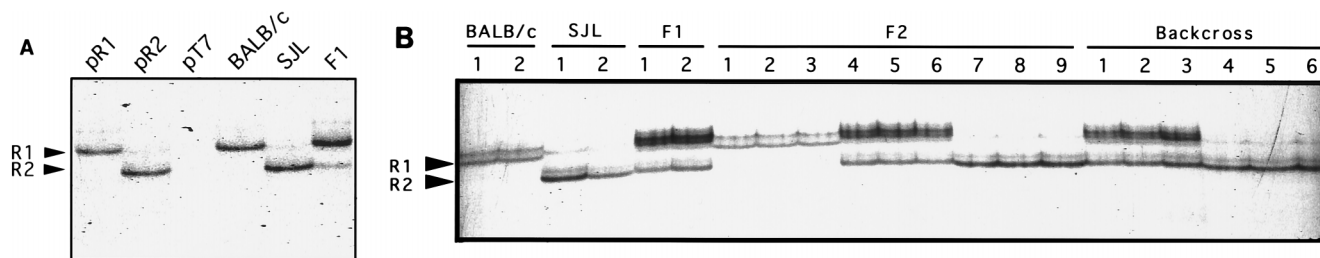


FIG. 2. Analysis of MHV receptor genotype by SSCP. Plasmids containing the *MHVRI* and *MHVR2* genes (20), as well as genomic DNA from BALB/c, SJL, or (BALB/c \times SJL) F_1 mice (A) and genomic DNA isolated from each individual mouse tail (B) were subjected to amplification of the N domain by PCR. With PCR-amplified DNA as a template, nested PCR with an FITC-labeled primer was performed and DNA fragments labeled with FITC were analyzed by SSCP (21). The labeled DNA mixed with formamide was denatured at 94°C for 2 min and electrophoresed in a 6% polyacrylamide gel containing 5% glycerol at 26 \pm 1°C. The fluorescent gel bands were analyzed on a FluorImager. pR1, pR2, and pT7 in panel A indicate the plasmids containing the *MHVRI* gene, the *MHVR2* gene, and no *MHVR* gene, respectively.

glycoprotein subgroup of the carcinoembryonic antigen gene family (3). This protein works as a receptor for MHV A-59, MHV-3, and MHV-2 but not for JHM (3). These facts suggest that the major receptor for JHM is an MHVR. Thus, the susceptibility and resistance of BALB/c and SJL mice is determined mainly by *MHVR* genes, although we cannot completely rule out the influence of Bgp2 as a determinant of MHV susceptibility.

A difference in susceptibility to MHV A-59 infection was also demonstrated between BALB/c and SJL mice (26), which suggests that the *MHVR* gene plays an important role in MHV A-59 infection as well. This also indicates that the *MHVR* gene determines susceptibility not only in the brain but also in the liver, since MHVR1 and MHVR2 are expressed in both the brain and the liver (32) and the liver is the major target organ of MHV A-59. However, in MHV-3 infection, A/J strain mice with the *MHVRI* gene showed resistance (16). The resistance of A/J mice to MHV-3 infection seems to be due to a different mechanism, since viral growth in susceptible C57BL/6 and resistant A/J mice was comparable in level (17). The resistance of A/J mice was reported to be dependent upon a host immune factor(s) (5), which explains the resistance of A/J mice to MHV-3 even if they have the *MHVRI* gene and permit high-level replication of MHV-3.

Host factors that influence viral growth are of great importance for animal resistance. The difference in virus-binding activity detected between MHVR1 and MHVR2 leads to a significant difference in virus growth in cultured cells (20, 22). The present study suggests that such a difference in receptor functionality determines the outcome of virus infection at the whole-animal level. To demonstrate this conclusively, studies are in progress to produce BALB/c mice whose *MHVRI* gene is replaced with the *MHVR2* gene, as well as SJL mice with the *MHVRI* gene in place of the *MHVR2* gene.

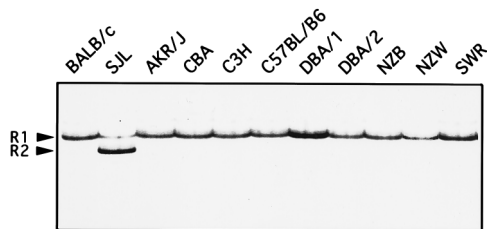


FIG. 3. Analysis of the MHV receptor genotypes of various mouse strains by SSCP. The MHV receptor genotypes of AKR/J, CBA, C3H, C57BL/6, DBA/1, DBA/2, NZB, NZW, and SWR mice were examined by SSCP by using genomic DNAs from those mouse strains as described in the legend to Fig. 2.

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