A Study on Mouse Hepatitis Virus Receptor Genotype in the Wild Mouse

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1. INTRODUCTION

Whereas most of laboratory mouse strains are susceptible to mouse hepatitis virus (MHV) infection, only SJL mouse strain is resistant (Smith et al., 1984). MHV susceptible mouse strains have CEACAM1^a (MHVR1) as a receptor for MHV, while SJL mice have CEACAM1^b (MHVR2) (Ohtsuka and Taguchi, 1997). Although these molecules are functional receptors for MHV (Yokomori and Lai, 1992), there is a striking difference in receptor functionality between these two proteins. MHVR1 has 250~500 times higher receptor-binding activity relative to MHVR2 as examined by a virus overlay protein blot assay and 10~30 times higher receptor activity when expressed on cells without MHV receptor (Ohtsuka et al., 1996, Rao et al., 1997). These findings suggest that the difference in susceptibility to MHV infection between MHV-susceptible mouse strains and SJL could result from the difference in receptor functionality found between MHVR1 and MHVR2.

The Nidoviruses (Coronaviruses and Arteriviruses).
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The susceptibility of mice to MHV is determined by a single gene located on chromosome 7 and the susceptibility is dominant over the resistance (Smith et al., 1984). MHV receptor gene is also mapped on chromosome 7 (Robins et al., 1991). These facts in combination with the difference in receptor functionality detected between MHVR1 and MHVR2 suggest the possibility that the gene controlling MHV susceptibility is identical to the MHV receptor gene. To investigate this possibility, we have generated the progeny mice between BALB/c and SJL. Analysis of these progeny showed that all mice with R2/R2 genotype were resistant, while those with R1 gene were susceptible, suggesting that MHV receptor gene determines the susceptibility of mice to MHV infection (Ohtsuka and Taguchi, 1997). In order to further test above possibility using the wild mouse, we first of all examined the MHV receptor genotype of several wild mouse subspecies distributed in Asia and Europe. The results indicated that both MHVR1 and MHVR2 are common to all of the wild mouse subspecies examined.

2. MATERIALS AND METHODS

2.1 Wild mouse subspecies used in this study

Wild mice seized in Europe and Asia were used in the present study. *Mus. musculus. (M. m.) domesticus* lives in western Europe. *M. spicilegus* distributes from northern area of Black sea to the river Volga. *M. m. bactrianus* distributes from Pakistan to the west. *M. m. musculus* distributes from western Europe to northern area of China. *M. m. castaneus* distributes from Pakistan to east southern Asia. *M. m. molossinus* distributed in Japan is a hybrid between northern *M. m. musculus* and *M. m. castaneus*.

2.2 Genotyping

Genomic DNA samples were prepared from each mouse tail and N-terminal domain of MHVR was amplified from the genomic DNA by nested PCR. First PCR was performed with 5'-CCTCACTTTTAGCCTCCTGGAG -3' and 5'-ACATGAAATTGCACAGTCGC-3' primers, and second PCR was done with 5'-GCTGAAGTCACCATTGAGGC-3' and FITC labeled 5'-AGCAGGGATCCATTGCTGTA-3' primers. These products were denatured in formamide by heating at 94°C for 2 minutes. Then single strand conformation polymorphism (SSCP) of these products were analysed by electrophoresis on 6% polyacrylamide gel for 1.5 hour at 26°C.

3. RESULTS

We investigated the MHV receptor genotype of various *Mus musculus* subspecies. Since remarkable difference in DNA sequence has been reported in the N-terminal domain of MHVR1 and MHVR2 genes, we amplified this region by nested PCR. The resultant DNA products were then analyzed by SSCP. As shown in figure 1, most of wild mouse subspecies retained both MHVR1 and MHVR2 genotypes. More than half of wild mice in each subspecies had MHVR2. These results suggested that MHVR2, which is very rare in laboratory mice, is common to the wild mouse population. Although some mice contained mutations in the MHV receptor genes, none of those mutations affected the function of each MHVR1 or MHVR2 (data not shown).

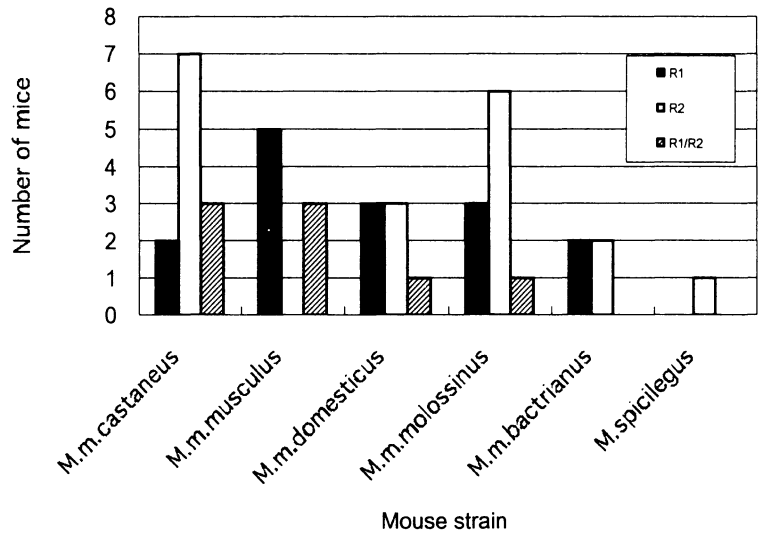

Figure 1. Typing of the MHV receptor gene in the wild mouse subspecies.

4. DISCUSSION

The present study has clearly shown that MHVR2 is common to all *Mus* subspecies examined, though that is very rare in the laboratory mouse strains. So far, only SJL mouse strain is reported to have MHVR2.

MHV strains, MHV-A59 and JHMV, multiply in cells with MHVR1 receptor 10 to 100 times better than in cells with MHVR2 (Ohtsuka et al.,

1997, Rao et al., 1997). Their preferential growth in MHVR1 cells could result from the adaptation of the virus to MHVR1 during many repeated passages in DBT or 17cl-1 cells generally used for MHV propagation. DBT and 17cl-1 cells are derived from CDF1 mouse (BALB/c and DBA hybrid) (Kumanishi, 1967) and BALB/c mouse (Sturman and Takemoto, 1972), respectively, implying that these cell lines have only MHVR1. Their adaptation to MHVR1 could account for the higher virulence of these viruses in mice expressing MHVR1 relative to those expressing MHVR2. To test this possibility, the study of MHV isolated from the wild mouse is important.

5. CONCLUSION

It was demonstrated in the present study that all of wild mouse subspecies retained the MHVR2 gene. This is very different from the distribution of MHVR2 in the laboratory mouse. Studies are in progress using the wild mouse to see whether the resistance of mice to MHV links to the MHV receptor gene.

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