MOUSE HEPATITIS VIRUS INFECTION UTILIZES MORE THAN ONE RECEPTOR AND REQUIRES AN ADDITIONAL CELLULAR FACTOR

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INTRODUCTION

Mouse hepatitis virus (MHV) causes hepatitis, encephalomyelitis, respiratory and gastrointestinal ailments. Our laboratory has been particularly interested in the mechanism of neuropathogenesis of MHV. Different MHV strains have different capacities to cause central nervous system (CNS) infection. Among the neurotropic strains, there is also a large degree of variation in their ability to cause encephalitis or demyelination. Various virus variants have been obtained from the neurotropic MHV strains which show altered neuropathogenicity; for example, many of the variants which escape neutralization by the spike (S) protein-specific monoclonal antibodies differ from the parental viruses in not causing encephalitis, but still retain the ability to cause demyelination (Fleming et al., 1986; Dalziel et al., 1986). These variants have either point mutations or deletions in the viral spike protein (Gallagher et al., 1990; Wang et al., 1992). Since the S protein presumably interacts with the viral receptor on the surface of target cells, it is reasonable to assume that the difference in the viral neurotropism is caused by the variations in their ability to interact with the receptors. Therefore, it is conceivable that the MHV receptors in different cell types, e.g. neurons, astrocytes or oligodendrocytes, in CNS might be different.

The MHV receptors have been identified as a member of murine homologue of carcinoembryonic antigen (CEA), mmCGM1 (Williams et al., 1991). It has been shown that this receptor molecule binds MHV in an in vitro virus binding assay (Boyle et al., 1987), and that SJL mouse, which is resistant to infection by A59 and JHM strains of MHV, lacks a functional receptor protein capable of binding virus in vitro (Williams et al., 1990). Thus, mmCGM1 may be the molecule controlling viral tissue tropism. However, mmCGM1 was not detected in the mouse brain; thus, the possibility arises that mmCGM1 may not be the universal receptor for MHV, and different MHV strains may utilize different receptors in different tissues.

MHV UTILIZES A DIFFERENT MEMBER OF CEA AS THE RECEPTOR IN THE BRAIN

We reasoned that if MHV utilizes an alternative receptor in the brain, this receptor would be related to mmCGM1 to a certain extent. To identify this possible receptor, we performed reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of C57BL/6 mouse brain and liver RNA by using two primers specific for the conserved sequences at the 5'- and 3'-ends of the mmCGM1 molecule. The results showed that liver RNA yielded

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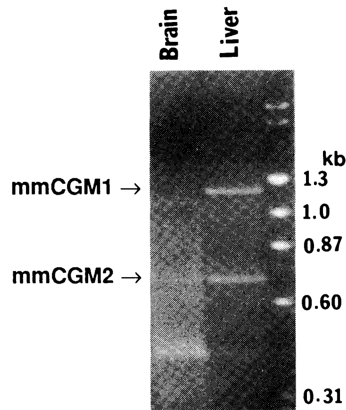

Figure 1. RT-PCR analysis of mmCGM-like molecules in C57BL/6 mouse liver and brain.

at least two PCR products, while brain RNA yielded only one of these two plus a few additional bands (Fig. 1) (Yokomori and Lai, 1992a). Cloning and sequencing of these PCR products showed that the largest PCR product of liver RNA represents the mmCGM1-derived PCR product, whereas the small one represents a similar molecule lacking 564 nucleotides in the middle. This molecule is similar in structure to another member of the CEA family, mmCGM2. Sequence analysis suggested that these two molecules probably represent the RNA species derived from alternative splicing of the same CEA gene. The identity of other molecules has not been determined.

The capacity of these CEA molecules to serve as MHV receptors was determined by cloning these PCR products into an expression vector with SV40 T antigen promoter (pECE) (Ellis et al., 1986) and transfecting them into COS cells, which were then tested for their infectability by MHV-A59 and JHM. The results indicated that both of them rendered the COS cells susceptible to MHV infection (Table 1). Thus, we concluded that both of them can be used as MHV receptors at roughly the same efficiency. Since the mouse brain expresses only mmCGM2 but not mmCGM1, the former is most likely the receptor used by MHV in CNS infection, in contrast to liver infection. This study thus shows that MHV could utilize different receptors in the brain and in the liver.

Table 1. Receptor functions of CEA molecules from B6 and SJL mice in Cos 7 cells[a].

Transfectant		Virus titer (PFU/ml) JHM	A59
mmCGM1:	B6	1.1×10^3	4.4×10^3
	SJL	2.1×10^2	2.0×10^3
mmCGM2:	B6	3.1×10^2	3.1×10^2
	SJL	3.8×10^2	2.1×10^2
Vector (pECE)		0	2.0×10^1
None		0	3.3×10^1

[a]Cos 7 cells were transfected with various DNAs and infected with either JHM or A59 at 40 h posttransfection. Viruses were harvested at 24 h after infection, and plaque assayed on DBT cells.

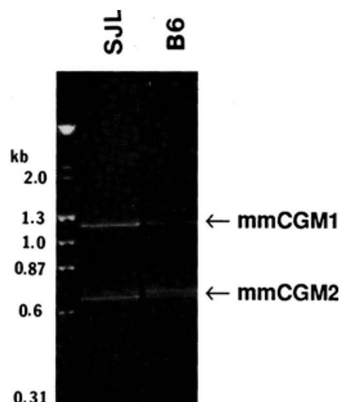

Figure 2. RT-PCR analysis of mmCGM-like molecules in SJL and C57BL/6 mouse liver and brain.

THE RESISTANCE OF SJL MICE TO MHV INFECTION IS NOT DUE TO THE DEFECTIVENESS OF THE MHV RECEPTOR

SJL mice have been known to be resistant to infection by MHV-A59 and JHM but susceptible to MHV-3 (Wilson and Dales, 1988). The viral resistance appears to be an intrinsic property of the cells, since the primary macrophages of SJL mice in culture also showed similar resistance (Stohlman et al., 1980). The mechanism of resistance has been controversial: one study indicated that the defect lies in the virus dissemination (Wilson and Dales, 1988), whereas another study revealed that the viral receptor is defective (Williams et al., 1990). The availability of cDNA probes for MHV receptors (mmCGM1 and 2) allowed us to re-examine this issue directly. We isolated the CEA-related molecules from SJL mouse liver by using RT-PCR techniques with the same set of primers as described above. Two PCR products indistinguishable from those from the susceptible mouse C57BL/6 were obtained (Fig. 2) (Yokomori and Lai, 1992b). Furthermore, these two molecules served as the receptors for MHV-A59 and JHM as efficiently as those from C57BL/6 mice, when they were transfected into COS cells (Table 1). Thus, the MHV receptor molecules from SJL mice are functional. Furthermore, Northern blot analysis of SJL mouse liver RNA indicated that these molecules are expressed in comparable amounts to that in C57BL/6 mice (data not shown). These results thus suggest that a cellular factor other than the viral receptor is defective in SJL mice, and is responsible for the viral resistance of this mouse strain.

THE EVIDENCE FOR THE REQUIREMENT OF A SECOND CELLULAR FACTOR FOR VIRUS ENTRY

To determine the mechanism of viral resistance of SJL mice, we examined several murine cell lines with regard to their virus infectability. The use of cultured cell lines simplified the parameters involved in the establishment of viral infection. We examined the established cell lines from not only the resistant SJL mice but also the susceptible C57BL and BALB/C mice. These cells were infected with either A59 or JHM viruses, and the kinetics of virus growth were then examined. Three representative cell lines are shown in Fig. 3. DBT cells, an astrocytoma cell line derived from BALB/C mice (Hirano et al., 1974), are fully susceptible to both A59 and JHM viruses. The other two cell lines are MC7, which was derived from C57BL/6, and BXS, which is derived from B10x SJL F1 mice. These two cell lines were susceptible to A59 virus infection, but, surprisingly, resistant to JHM infection. These results are different from the viral susceptibility of the parental mice. Thus, there is no strict correlation between the viral susceptibility in animal and that in cell culture, and that there is a selective resistance of most of the cell lines to

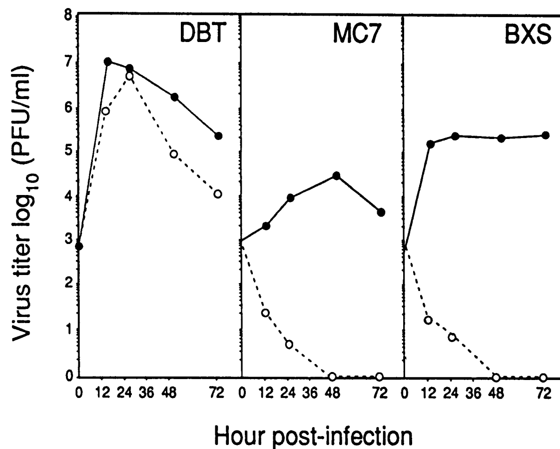

Figure 3. Viral growth kinetics on different cell lines. Virus titers released from each cell line at different time points postinfection are indicated. Solid line: A59. Dashed line: JHM.

JHM strain, but not A59. Since the viral receptors work as equally efficient receptors for both virus strains, these results suggest that there is another cellular factor which can regulate the susceptibility to viral infections.

We next examined whether these cell lines express functional MHV receptors. By RT-PCR analysis using primers specific for mmCGM2, we showed that all of these cell lines expressed at least the same or higher level of mmCGM2 molecules than that in the DBT cell line, which is highly susceptible to MHV infection (data not shown). We have also molecularly cloned the full-length mmCGM2 from one of the cultured cell lines and expressed this cDNA in COS cells. It was found that the expression of this cDNA rendered the COS cells susceptible to both A59 and JHM infection. We conclude that the MHV receptors are functional and properly expressed in these cell lines.

We then studied the step of viral replication which is blocked in these cell lines. We found that no viral RNA or protein synthesis could be detected in the JHM-infected cell lines except in DBT cells. Furthermore, the transfection of purified viral genomic RNA into these cell lines led to the production of fully infectious virus particles, indicating that viral RNA replication can proceed in these cell lines. Thus, we conclude that viral replication is blocked early in viral replication in these cell lines, most likely in the viral entry step.

PERSPECTIVES

These studies indicated that MHV can utilize more than a single CEA molecule as the viral receptor. The flexibility of MHV to utilize different molecules as the receptor in different tissues should allow the virus to infect more tissues than is otherwise possible. The CEA molecules are expressed as the alternatively spliced molecules in different tissues and possibly in different developmental periods. This spatial and temporal regulation of CEA molecules could conceivably determine the target cell specificity of MHV. Thus, further studies on the pattern of expression of CEA should shed light on the tissue tropism of MHV.

The second conclusion of this study is the demonstration that MHV entry requires an additional cellular factor other than the cellular receptor. This factor appears to be more sensitively regulated than the receptor, and is able to discriminate different virus strains. Thus, this factor exerts a very fine control on the tissue tropism of MHV strains. Conceivably, the neurotropism or hepatotropism of different MHV strains may be determined by this second factor, inasmuch as the MHV receptors expressed in the brain and liver function equally efficiently for both A59 and JHM. The nature of this factor is not yet clear. Preliminary data using recombinant viruses between A59 and JHM strains suggested that the viral gene responsible for the ability of MHV to grow in these cell lines

is the spike protein gene. Thus, the second factor may be interacting with the viral spike protein directly or indirectly, and may trigger the cellular signal to initiate the penetration or uncoating of virus particles. Future studies on this factor should shed further light on the mechanism of virus entry and viral resistance.

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