EXPRESSION OF MHV-A59 RECEPTOR GLYCOPROTEINS IN SUSCEPTIBLE AND RESISTANT STRAINS OF MICE

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Introduction

Band and Warwick showed that inbred mouse strains differed in their susceptibility to virulent strain of mouse hepatitis virus, MHV-2, and that peritoneal macrophages cultured from these mouse strains reflected their differences in susceptibility to the virus (1). Adult SJL/J mice are highly resistant to MHV-JHM and MHV-A59 (2,12,13). Our laboratory showed that membranes from the liver and intestinal epithelial cells of BALB/c mice could bind MHV-A59 virions in a solid phase assay using undenatured membrane proteins, whereas membranes from SJL/J mice did not bind virus, suggesting that differences in binding of virus to cells from different mouse strains could account for their differences in susceptibility to MHV (3). In virus-overlay protein blot assays of intestinal brush border and liver membrane proteins, MHV-A59 virions bound strongly to a 58 kDa glycoprotein from BALB/c mice, but the virus did not recognize any proteins from SJL/J mouse tissues (3,4). Anti-receptor monoclonal antibody MAb-CC1 that blocked infection of murine cell lines with MHV-A59 recognized the 110-120 kDa and 58 kDa membrane glycoproteins of BALB/c mice, but no proteins from SJL/J membranes (6,7).

The cDNA coding for the 110 kDa glycoprotein detected by VOPBA was isolated from a BALB/c liver cDNA library and named MHVR (5). Amino acid sequence analysis and cross-reactivity with anti-human carcinoembryonic antigen (CEA) antibodies indicated that MHVR is a member of the murine CEA family of glycoproteins, in the immunoglobulin superfamily (5,6,7). MHVR is a 424-amino acid glycoprotein with four immunoglobulin-like domains, a transmembrane domain and a short intracytoplasmic tail (Figure 1). When MHVR was transfected into MHV-resistant human or hamster cells, they became susceptible to MHV-A59 as well as other MHV strains (5). Although MHV-A59-resistant adult SJL mice do not express

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a protein that binds MAb-CC1 or MHV-A59 virions, their intestinal brush border and liver membranes express two related glycoproteins that are recognized by antibody directed against the N-terminal 15 amino acids of MHVR and by antibodies to human CEA-related glycoproteins (6,7). This paper describes the cloning of cDNAs encoding for other CEA gene family members (CGMs) isolated from BALB/c, SJL/J and CD-1 mice and shows that they can also act as functional receptors for MHV-A59.

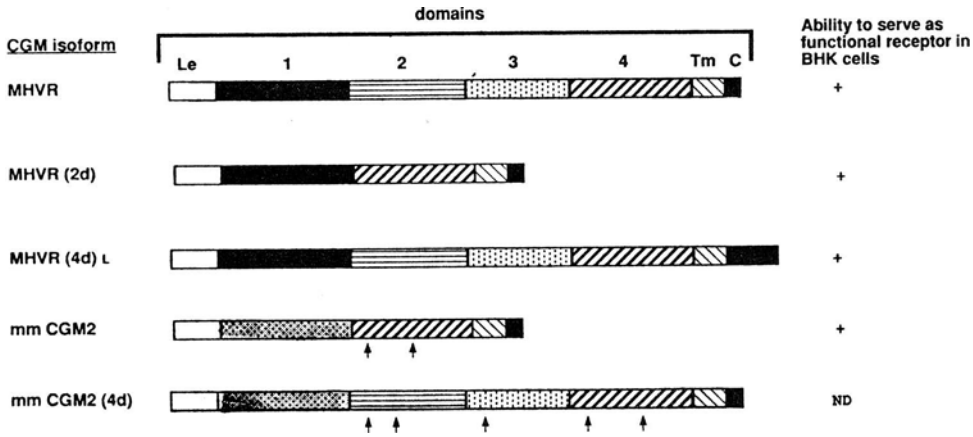

Figure 1. Schematic representation of the CEA gene family members (CGM) isolated from BALB/c and SJL/J mice. Le = leader peptide, Tm = transmembrane domain and C = cytoplasmic domain. The arrows indicate the presence of an amino acid difference between the $mmCGM_2$ isoforms and MHVR isoforms.

Results and Discussion

Characterization of splice variants of MHVR in BALB/c mice. Northern blot analysis of transcripts from adult BALB/c and SJL/J colon revealed two major MHVR-related transcripts of 3.4 and 1.7 kb and multiple additional faint bands. In these tissues, the 3.4 kb mRNA species was more abundant than the 1.7 kb species which was detected after prolonged exposure of the blots.

To isolate the cDNA encoding the 58 kDa glycoprotein detected by immunoblot analysis and VOPBA in intestine and liver membranes of MHV-A59-susceptible strains, we designed primers based on the highly conserved leader peptide and the last amino acids of MHVR, including the stop codon. A new cDNA clone, named MHVR (two domain) or MHVR(2d) was obtained by RNAPCR using BALB/c colon RNA as starting material. MHVR(2d) contains the leader, domains 1 and 4, the transmembrane sequence and the same cytoplasmic domain as MHVR. This clone is probably a splice variant of MHVR since differential splicing is a common phenomenon for members of the human CEA family (8). Besides the absence of the second and third domains in MHVR(2d), the only sequence difference between the open reading frames of the two cDNAs is the result of the splicing that created a new codon for glutamine absent in MHVR. A third member of the family that shares the same N-terminal domain of MHVR was isolated from a colon cDNA library of CD-1 mice (Figure 1). This clone, named $MHVR(4d)_L$, has the same amino

acid sequence as MHVR up to the intracytoplasmic domain. In the intracytoplasmic domain of MHVR(4d)$_L$, the last three amino acids of MHVR are substituted with D,Q, and R, and 62 additional amino acids extend the length of this domain.

Cloning and expression studies of the MHVR-related cDNAs in the SJL/J mouse strain. Immunoblots with polyclonal anti-MHVR showed that the two SJL/J glycoproteins homologous to MHVR had slightly lower molecular weights (3 to 5 kDa) in SDS-PAGE than the BALB/c proteins MHVR and MHVR(2d) (7). Although the glycoproteins have different molecular weights, Northern blot analysis of RNA isolated from colon and small intestine of the two mouse strains showed no difference in the sizes of the mRNAs that were detected upon probing with MHVR (9). On the other hand, when MHVR was used to probe Southern blots of DNA isolated from these strains, restriction fragment length polymorphisms were observed upon digestion of the DNA with BamHI, EcoRI, and SstI (unpublished). To obtain cDNA clones encoding the CGM isoforms expressed in SJL/J mice, we PCR amplified cDNAs obtained from colon and small intestine with oligonucleotide primers derived from the MHVR sequence. The open reading frames of the two SJL/J clones obtained showed numerous amino acid differences when compared to the sequence of MHVR and MHVR(2d). Like BALB/c mice, the CGMs of SJL/J mice included both a four domain type and a two domain splice variant that results from the deletion of the second and third constant domains. The sequence of the open reading frame of the SJL/J two domain protein is identical to that of mmCGM$_2$, a cDNA clone isolated from colon of CD-1 mice (10). Most of the differences between MHVR(2d) and mmCGM$_2$ mapped to the N-terminal domain (Figure 2) and two amino acid changes were found in the fourth domain. In the four domain isoforms, MHVR and mmCGM$_2$(4d), in addition to the differences in the first and fourth domains, two additional amino acids in the second domain and one amino acid in the third domain distinguished the amino acids sequences (Figure 1). This comparison between the BALB/c and SJL/J clones indicated that the lower molecular weights of the SJL/J glycoproteins probably result from the absence of the first potential N-linked glycosylation site in the N-terminal domain of MHVR (Figure 2).

CD-1 mice are outbred, and cloning showed that they express CGMs with both types of N-terminal sequences, the one present in MHVR and that in mmCGM$_2$. We investigated whether inbred mouse strains expressed only one N-terminal domain or the other, which would be expected if mmCGM$_2$ and MHVR were alleles of the same gene. For that purpose we designed oligonucleotide probes that, under special washing conditions, were specific for the MHVR or the SJL/J

N-DOMAIN

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MHVR        EVTIEAVPPQVAEDNNVLLLVHNLPLALGAFAWYKGNTTAIDKEI
                                                  ::::::
mmCGM2      EVTIEAVPPQVAEDNNVLLLVHNLPLALGAFAWYKGNPVSTNAEI

ARFVPNSNMNFTGQAYSGREIIYSNGSLLFQMITMKDMGVTTLDMTDENYRRTQATVRFHVH
::  ::: :::  : :    ::       : :: :  :  : ::     :   :   :
VHFVTGTNKTTTGPAHSGRETVYSNGSLLIQRVTVKDTGVYTIEMTDENFRRTEATVQFHVH
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Figure 2. Amino Acid sequence comparison of the N-terminal domains of MHVR and mmCGM$_2$ (5,10). Potential N-linked glycosylation sites are underlined.

sequences, and we took advantage of the different restriction enzyme site recognition sequences present in domain one of these clones. The study was extended to other inbred strains, C57/BL6 and C3H mice. The results were further confirmed by RNAse protection with RNA isolated from liver, colon and small intestine of the four mouse strains. For RNAse protection experiments, we used riboprobes specific for the N-terminal domains and a riboprobe from the fourth domain that hybridizes to all of the known isoforms. The SJL/J mice expressed only the N-terminal domain of the $mmCGM_2$ isoform, while the other strains of mice expressed only that of the MHVR isoform (Table 1) (9). Thus, the $mmCGM_2$ and MHVR isoforms and their splice variants appear to be encoded by different alleles of the murine CEA-related glycoprotein.

Table 1. Expression of CGM isoforms in different strains of mice.

N-terminal domains of MHVR and $mmCGM_2$ expressed in liver, colon and small intestine of different mouse strains

Probe specificity	BALB/c	C57BL/6	SJL/J	C3H
MHVR	+	+	-	+
$mmCGM_2$	-	-	+	-
MHVR and $mmCGM_2$	+	+	+	+

The presence (+) or absence (-) of expression of transcripts encoding the N-terminal domains of MHVR and $mmCGM_2$ was determined by RNase protection assays and RNAPCR.

Ability of splice variants of MHVR and $mmCGM_2$ to act as functional receptors for MHV-A59. Deletion mutagenesis studies showed that the N-terminal domain of MHVR is responsible for its interactions with the virus and with the MAb-CC1 (11). However, the first domain alone is not enough to act as a functional receptor when transfected into hamster cells, and the presence of either domain 2 or 4 is required (11). Since both MHVR(2d) and $MHVR(4d)_L$ share the same N-terminal domain as MHVR and have at least one extra constant domain, we expected that they would also serve as functional MHV-A59 receptors and be recognized by the MAb-CC1. The ability of the two new isoforms to act as virus receptors was studies in transient transfection assays. When MHVR(2d) and $MHVR(4d)_L$ where expressed in receptor-negative BHK-cells, they became susceptible to MHV-A59 as determined by the presence of viral antigens in the cytoplasm of the transfected cells by immunofluorescence. Our studies indicate that several MHVR isoforms from BALB/c or CD-1 mice are functional receptors for MHV-A59. The mRNAs for the 2 and 4 domain isoforms were detected by RNAPCR in all target tissues for MHV-A59 infection that were examined including brain, liver, colon, and small intestine of BALB/c mice.

We tested the two domain CGM, $mmCGM_2$ that is expressed in SJL/J mice for its ability to function as a receptor for MHV-A59. When BHK cells were

transiently or stably transfected with $mmCGM_2$, they became susceptible to MHV-A59 infection. This result was surprising since the animals are resistant to MHV infection virus and MAb-CC1 did not bind in VOPBAs to SJL/J liver and intestine membranes which express both the 55 kDa glycoprotein encoded by $mmCGM_2$ and the four domain variant $mmCGM_2(4d)$ (3,6). Anti-MHVR MAb-CC1 did not protect hamster cells transfected with $mmCGM_2$ from infection with MHV-A59, indicating that this antibody reacts with an MHVR-specific epitope and suggesting that the virus and MAb-CC1 binding domains may not be identical. Studies to determine whether the $mmCGM_2(4d)$ glycoprotein of SJL/J mice is also a functional receptor for murine coronaviruses are in progress.

A continuous cell line of SJL/J embryo fibroblasts was prepared, the mRNA encoding its MHVR-related glycoproteins was analyzed, and its ability to support MHV-A59 and MHV-JHM infection was explored. RNAPCR analysis showed that the cell line expressed both the two and four domain isoforms of $mmCGM_2$. At several passage levels, the cells were completely resistant to infection with MHV-A59, as shown by immunofluorescence with antiviral antibody at 7.5 hours after virus challenge. To determine whether resistance of the SJL/J cell line was due to an SJL/J-specific post-translational modification in processing of these glycoproteins, we transfected cDNAs encoding the MHVR, MHVR(2d), $MHVR(4d)_L$, or $mmCGM_2(2d)$ isoform into these cells and tested whether expression or overexpression of these proteins rendered the cells susceptible to infection with MHV-A59. Surprisingly, expression of each of these isoforms rendered the SJL/J cell line susceptible to infection, indicating that the SJL/J fibroblasts can process the glycoproteins in an appropriate manner to permit their use as receptors for MHV-A59. Thus, overexpression of one of the isoforms already expressed in the SJL/J tissues made the cells susceptible to infection. Quantitative studies on the binding affinities of the receptor isoforms and their relative efficiency in facilitating virus penetration in cell lines will be undertaken in order to elucidate the mechanism for resistance of the SJL/J cell line and adult animals to MHV-A59 and MHV-JHM infection.

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