Difference in virus-binding activity of two distinct receptor proteins for mouse hepatitis virus

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The receptor proteins, MHVR1 (Bgp C or splice variant of mmCGM1 containing two ectodomains) and MHVR2 (mmCGM2) have been reported to be functional receptors for MHV, although there was a significant difference in their virus-binding activity as determined by virus overlay protein blot assay (VOPBA). To compare the receptor function of these proteins, their virus-binding capacities were tested by using soluble forms of the proteins which lacked the transmembrane and intracytoplasmic domains. To estimate the amounts of these proteins expressed, an epitope of influenza HA protein, for which specific monoclonal antibody was available, was used as a tag. Recombinant soluble MHVR1 and MHVR2, expressed in RK 13 cells using recombinant vaccinia virus were secreted into the culture fluids of infected cells expressing these proteins. The inhibitory effect on virus infectivity of MHVR1 was shown to be about 500-fold higher than that of MHVR2. A similar disparity was observed in virus binding by VOPBA. These two proteins worked as functional receptors when they were expressed on resistant BHK-21 cells. However, the efficiency of MHV infection in BHK-21 cells expressing MHVR1 was about 30-fold higher, as compared with those expressing MHVR2. These data show that the receptor function of MHVR1 was significantly higher than that of MHVR2 and suggests that the difference in susceptibility between SJL and BALB/c mice might be due to the specific receptor protein expressed in those animals.

Introduction

The cell surface receptor is the first component with which a virus interacts and is one of the major determinants of target cell specificity. Virus receptor proteins have been reported for several different viruses, including human immunodeficiency virus (HIV) (Duke et al., 1995; Maddon et al., 1986), rhinovirus (Staunton et al., 1989; Greve et al., 1989), poliovirus (Mendelsohn et al., 1989), murine leukaemia virus (Kim et al., 1991; H. Wang et al., 1991), Sindbis virus (Ubol & Griffin, 1991; K. S. Wang et al., 1991) and three different coronaviruses, porcine transmissible gastroenteritis virus (Delmas et al., 1992), human coronavirus (Yeager et al., 1992) and murine coronavirus (Dveksler et al., 1991; Williams et al., 1990). Some of these viruses are known to utilize different types of receptor proteins in different types of cells. For example, HIV is known to utilize the CD4 molecule expressed on T cells as a functional receptor (Maddon et al., 1986), but it also utilizes other receptors expressed on muscle cells and neural cells (Clapham et al., 1989; Harouse et al., 1991). It has also been shown that the HIV-binding capacity of these receptor molecules differs (Harouse et al., 1991). Sindbis virus is reported to utilize receptors of different molecular mass in different cells or tissues (Ubol et al., 1991; K. S. Wang et al., 1991). It is also documented that mouse hepatitis virus (MHV) utilizes different isoforms of the receptor protein expressed in the brain and liver (Yokomori & Lai, 1992a).

MHV belongs to the family Coronaviridae which is composed of enveloped, positive-stranded RNA viruses, associated with various diseases of economic importance in both animals and humans (Siddell, 1995; Spaan et al., 1988). MHV has a genome of about 31 kb which encodes four or five structural proteins as well as several non-structural proteins (Siddell, 1995; Spaan et al., 1988). The spike, protruding from the MHV virion, is composed of the spike (S) protein, which is a heterodimer or trimer consisting of two non-covalently bound S protein subunits (Cavanagh, 1995). The S1 protein is thought to form the globular head of the spike and the S2 its stalk portion (De Groot et al., 1987). Recently, we have shown

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that an N-terminal region comprising 330 amino acids of the S1 of MHV had the receptor-binding activity (Kubo et al., 1994), whereas the S2 subunit was not able to interact with the receptor protein by itself (Taguchi, 1995).

Several different isoforms of biliary glycoproteins have been reported to serve as a functional receptor for MHV (Dveksler et al., 1993a; McCuaig et al., 1993; Nedellec et al., 1994). All of these proteins belong to the carcinoembryonic antigen family which is classified as a member of the immunoglobulin superfamily (Dveksler et al., 1991; Williams et al., 1991). Some of these proteins have four ectodomains and others have two ectodomains (Dveksler et al., 1993a; McCuaig et al., 1993), in which the N domain located in the N-terminal region has been shown to be necessary for virus binding (Dveksler et al., 1993b). These receptor proteins can be divided into two major groups, Bgp 1a and Bgp 1b, as recently proposed by Nedellec et al. (1994). The biggest difference between these two groups is located in the C-terminal two-thirds of the N domain (McCuaig et al., 1993). The receptor proteins described in this paper, MHVR1 and MHVR2, both of which contain two ectodomains, belong to Bgp 1a and Bgp 1b, respectively (McCuaig et al., 1993; Nedellec et al., 1994).

Proteins of 110 to 120 kDa isolated from the intestine or liver of susceptible BALB/c mice were first reported to interact with MHV particles in a virus overlay protein blot assay (VOPBA). Such proteins were not detected in resistant SJL mice (Boyle et al., 1987). This protein was thereafter identified as mmCGM1, a protein with four ectodomains (classified as Bgp 1a) and also occurs as a splice variant of mmCGM1 containing two ectodomains (Bgp C; designated MHVR1 in this paper). These proteins were shown to function as receptor proteins for MHV-A59 (Dveksler et al., 1991). This finding could imply that the difference in susceptibility to SJL and BALB/c mouse strains to MHV infection may be due to the presence or absence of a functional receptor. In SJL mice however, the homologous protein to MHVR1, named mmCGM2 with two ectodomains (classified in Bgp 1a and called MHVR2 in this paper), was also found and shown to be a functional receptor (Yokomori & Lai, 1992a, b). From these results, the difference in susceptibility to MHV was not therefore explainable by the presence or absence of functional receptors. Interestingly, it was observed that MHVR1, but not MHVR2, was detected by VOPBA (Boyle et al., 1987; Williams et al., 1990). This may suggest that there is a significant, functional difference in virus-binding properties between these two receptor proteins. In the present study, we compared the virus-binding capacities of these proteins by using recombinant, soluble forms of these proteins. We demonstrated by VOPBA and virus neutralization that MHVR1 retained a very high virus-binding capacity as compared with MHVR2. Also, BHK-21 cells expressing MHVR1 (BHK-MHVR1) are more sensitive to MHV infection than BHK cells expressing MHVR2 (BHK-MHVR2). The difference in receptor function observed between these two types of cells may account for the differential susceptibility to MHV infection observed between SJL mice expressing MHVR2 and BALB/c mice expressing MHVR1.

Methods

- **Viruses and cells.** The MHV strains JHMV cl-2 (Taguchi et al., 1985) and JHMV sp-4 (Taguchi & Fleming, 1989), both of which were shown to interact with MHVR1 (Taguchi, 1995), were used in the present study. These viruses were propagated and plaque assayed on DBT cells as previously reported (Taguchi et al., 1980). The recombinant vaccinia virus (VV) vTF7.3 encoding the T7 RNA polymerase (Fuerst et al., 1986, 1987), kindly provided by B. Moss, was used to express MHV-specific receptor proteins in a VV transient expression system. vTF7.3 was propagated and plaque-assayed on RK 13 cells. These cells were grown in Dulbecco's modified Eagle's minimal essential medium (DMEM, Nissui, Tokyo) supplemented with 7% calf serum (Gibco) and 10% tryptose phosphate broth (TPB; Difco). BHK-21 cells, used to express MHV receptors with transmembrane and intracytoplasmic domains, were cultured in DMEM supplemented with 10% fetal calf serum (FCS; Gibco).

- **Construction of the vectors to express soluble receptor proteins.** Two different isoforms of MHV receptor protein were expressed using the VV transient expression system: the gene product of mL900 (designated in this paper as MHVR1), which is identical to MHVR1 (2d) (Dveksler et al., 1993a) or Bgp C (McCuaig et al., 1993), and that of SLmL900 (Yamada et al., 1993), which is designated in this paper as MHVR2 and is almost identical to mmCGM2 (Yokomori & Lai, 1992a) (Fig. 1). The genes, originally cloned into pT7 Blue vector (pT7; Novagen), were manipulated to delete their transmembrane and intracytoplasmic domains by PCR. The genes were also manipulated to incorporate at the C-terminus a linear epitope found in the haemagglutinin of influenza virus (amino acid sequence YPYDVPDYA) which can be detected using a monoclonal antibody (MAB) specific for this epitope (Fig. 1). The genes mL900 and SLmL900 were used as template for PCR with a pair of primers, oligonucleotide 1 corresponding the initiation codon of these genes (5'-AGCAGAGACATGGAGCTGGC-3') and oligonucleotide 2 corresponding to nucleotides 701-720 from the first nucleotide of the initiation codon coupled with the nucleotide sequence encoding the influenza HA epitope and termination codon (5'-TAA GCTAATCTCGGAACATCATTATGATGCTTTCTTGTGG-TCAA-3') (Fig. 1). The amplified DNA fragment (approximately 750 bp) was again cloned into the T7 vector and the correct clones for expression under the T7 promoter were selected. These vectors were designated pT7-soMHVR1-HA and pT7-soMHVR2-HA. Sequencing (Sanger et al., 1977) showed that the MHVR1 and MHVR2 genes were exactly the same as mL900 and mmCGM2 (Yokomori & Lai, 1992b), respectively.

- **Transfection and isolation of the soluble forms of receptor proteins.** RK 13 cells were used for the transient expression of soluble forms of two different receptor proteins. The subconfluent RK 13 cells grown in 10 cm dishes (Falcon) were trypsinized and suspended in DMEM containing 10 mM-dextrose and 0.1 mM-DTT at a concentration of 5 x 10^6–1 x 10^7 cells/ml. The mixture of 0.5 ml of cell suspension and 5–10 μg of recombinant plasmid, pT7-soMHVR1-HA or pT7-soMHVR2-HA, was kept on ice for 10 min and then electroporated using a Gene Pulser (Bio-Rad). The treated cells were cultured in 6 cm dishes (Falcon) with DMEM supplemented with 10% FCS for 6 to 12 h. The
cells were infected with vTF7.3 at a multiplicity of 5–10 p.f.u./cell and incubated for 1 h at 37 °C. After removing the inoculated virus material, cells were fed with PM-1000 medium (Eiken, Tokyo) for 15–24 h. Since both proteins lacked the transmembrane domain and were mostly secreted in the culture fluids, we made use of only the culture fluids for the experiments. From culture fluids of cells isolated at 15 to 24 h after vTF7.3 infection, VV was removed by centrifugation at 20 000 r.p.m. for 2 h. These materials were concentrated, when needed, by ultrafiltration with Ultra-free PF or PFL (Millipore).

**Western and dot blotting.** The size and amounts of the soluble forms of MHVR1 and MHVR2 proteins produced in the VV transient expression system were analysed by Western blotting as reported previously (Kubo et al., 1993; Taguchi, 1993). For Western blotting, aliquots of culture fluids were electrophoresed in a 10% SDS–polyacrylamide gel and the proteins were transferred onto Immobilon transfer membrane paper (Millipore). For dot blotting, 2-fold serially diluted culture fluids were prepared on membrane using vacuum apparatus (ATTO, Tokyo). The membrane paper with proteins was blocked with Block Ace (Yukijirushi, Sapporo, Japan) before incubation with antibodies. In order to detect proteins anti-HA MAb (mouse MAb clone 12CA5; Boehringer Mannheim) was used at a concentration of 400 pg/ml. After incubation with the MAb, the membrane papers were washed with PBS pH 7.2 containing 0.05% Tween 20 (PBS-Tw) and reacted with 5000– to 10 000-fold diluted anti-mouse IgG conjugated with horseradish peroxidase (Cappel Organon Teknica). The reaction was detected by enhanced chemiluminescence (ECL; Amersham).

**Virus binding to MHV receptors.** This was determined by VOPBA as described previously (Kubo et al., 1994; Taguchi, 1995). Briefly, soluble receptor proteins were prepared on the membrane paper by Western blotting or dot blotting as described above. The paper was then incubated in culture fluid of DBT cells containing 1 × 10^8–3 × 10^8 p.f.u./ml of JHMV at room temperature (22 to 24 °C) for 1 h. The binding of virus particles to the receptor proteins was monitored with MAb specific for the S proteins of JHMV (Kubo et al., 1993) as primary antibody and anti-mouse IgG conjugated with peroxidase as a secondary antibody. Peroxidase activity was detected by ECL.

**Inhibition of virus infectivity (neutralization) by receptor proteins.** The inhibitory effects of soluble receptor proteins on virus infectivity were tested as follows. The culture fluids containing MHVR1 and MHVR2 concentrated by ultrafiltration were serially 2-fold diluted with DMEM containing 10% TPB. Of each dilution, 100 µl was mixed with an equal volume of JHMV suspension containing 200–300 p.f.u. The mixture was incubated at room temperature for 50 to 60 min. The mixture was then inoculated onto DBT cells prepared in 6-well plates (Falcon) and the number of plaques produced by the remaining infectious virus was counted 1 to 2 days after virus inoculation. The degree of inhibition of each dilution was estimated as compared with the same virus titre when mixed with DMEM containing 10% TPB.

**Transient expression of MHV receptor proteins in BHK-21 cells and infection with JHMV.** The MHV receptor genes, mL900 (Kubo et al., 1994) and SlmL900, isolated from BALB/c and SJL mouse liver, respectively, were inserted in the expression vector pKS336 modified from pSV2bsr (kindly provided by K. Sakai & M. Tastumi) in the correct orientation for expression (pKS-MHVR1 and pKS-MHVR2). The recombinant plasmid was transfected into BHK-21 cells by electroporation and the cells were cultured with DMEM supplemented with
10% FCS for 1 to 2 days before virus inoculation. The expression of MfIV receptor protein in cells was confirmed by indirect immunofluorescence with rabbit anti-CEA antibodies (DAKO Japan, Kyoto) and anti-rabbit IgG labelled with FITC (Cappel). To determine the susceptibility of these cells to JHMV, cells expressing MHVR1 (BHK-MHVR1) and those expressing MHVR2 (BHK-MHVR2) were prepared in 12-well plates (Falcon) and infected with JHMV at various multiplicities of infection (10<sup>2</sup> to 10 p.f.u.). After 1 h incubation at 37 °C for virus adsorption, cells were washed with DMEM and then incubated with DMEM containing 10% FCS. To quantify the number of infected cells, cells were fixed with acetone at 9 to 10 h post-inoculation and the number of fused cells with MHV antigen was determined by immunofluorescence. Virus growth in these cells was also examined by the titration of infectious viruses in the culture fluids at 12 and 24 h post-inoculation as previously reported (Taguchi et al., 1980).

**Immunofluorescence.** RK 13 cells transfected with pT7-soMHVR1 or pT7-soMHVR2 were fixed with acetone at room temperature for 2 min and the expression of these proteins was examined with anti-influenza HA MAb as primary antibody and anti-mouse IgG conjugated with FITC as secondary antibody. BHK-21 cells transfected with pKS-MHVR1 or pKS-MHVR2 were fixed in a similar manner before or after JHMV infection. The expressed receptor proteins were detected with anti-CEA antibody and the viral antigen was detected with anti-cl-2 S MAb. They were then reacted with anti-rabbit IgG and anti-mouse IgG conjugated with FITC, respectively. The fluorescence was observed by UV microscopy.

**Results**

**Expression of soluble forms of MHVR1 and MHVR2 and their virus-binding capacity**

Recombinant soluble receptor proteins MHVR1 and MHVR2 were expressed in RK 13 cells after transfection with pT7-soMHVR1-HA and pT7-soMHVR2-HA, followed by infection with recombinant VV harbouring the T7 RNA polymerase gene, vTF7-3. In most cases, 60 to 80% of cells were shown to express the receptor proteins by immunofluorescence (data not shown). Since the proteins lacked the anchor signal and most of the proteins were expected to be secreted into the culture fluid, we examined the proteins in the culture fluids by Western blotting. The culture fluids were spun at 20000 r.p.m. for 2 h to remove VV and the supernatants were concentrated 10-fold by ultrafiltration. Ten μL of the sample diluted 2-, 4-, 8-, 16- or 32-fold was electrophoresed in an SDS-polyacrylamide gel and transferred onto membrane. The soluble receptor proteins were reacted with MAb specific for the influenza HA epitope. As shown in Fig. 2(a, b), major bands of 40 kDa and 36 kDa corresponding to MHVR1 or MHVR2, respectively, were detected in the culture fluids of RK 13 cells. Since the core proteins of soluble MHVR1 and MHVR2 encoded in the corresponding genes constructed in this study were 27756 and 27386 Da, respectively, the soluble MHV receptor proteins produced in RK 13 cells were presumably heavily glycosylated. A minor band with a slightly higher molecular mass was also found in both preparations. From the result shown in Fig. 2(a), the amount of MHVR1 was calculated to be one-sixth of the amount of MHVR2.

It has been documented that MHVR1 but not MHVR2 reacts with MHV by VOPBA when these proteins are denatured by 2-mercaptoethanol and SDS (Boyle et al., 1987; Williams et al., 1990) and we have confirmed this observation. The same amounts of MHVR1 and MHVR2 prepared on the membrane paper by Western blotting as shown in Fig. 2(b) were reacted with 100 μL of JHMV (1 x 10<sup>7</sup> p.f.u./ml) for 1 h at room temperature. The binding of virus particles was monitored by JHMV-specific MAb. As shown in Fig. 2(c), a remarkable difference in virus binding was observed; JHMV bound to MHVR1 very efficiently but not at all to MHVR2. To estimate the difference of virus-binding capacity of MHVR1 and MHVR2, we did VOPBA with a slightly modified method. We prepared soluble receptor proteins on membrane (in this case, receptor proteins were not denatured) and they were tested as to whether or not they bound the virus particles. The undiluted sample shown in lane 1 for MHVR2 contained 6-fold more receptor than that of MHVR1. As shown in Fig. 2(d), there was an apparent difference in the virus-binding capacity between these receptor proteins. The intensity of lane 1 of MHVR2 was calculated to be almost the same as lane 7 of MHVR1. This showed that MHVR1 bound to the virus particles with more than 350-fold higher efficiency than MHVR2. These results indicated that the virus-binding capacity of MHVR1 was more than 350-fold higher than that of MHVR2 by VOPBA.

**Inhibition of virus infectivity (neutralization) by soluble receptors**

The virus-binding capacities of these soluble receptor proteins were examined by neutralization of virus infectivity. The concentrated soluble MHVR1 and MHVR2, whose concentrations were estimated by Western blotting as shown in Fig. 2(a), were serially 2-fold diluted with DMEM containing 10% TPB and 100 μL of each dilution was mixed with an equal volume of JHMV containing 200–300 p.f.u. After incubation at room temperature for 60 min, the remaining infectivity was estimated by plaque assay using DBT cells. As shown in Fig. 3, there was a striking difference between these two receptors with respect to the concentration that neutralized virus infectivity. Undiluted MHVR2 neutralized more than 50% of infectivity, whereas MHVR1 completely neutralized JHMV at a dilution of more than 1:64. Even 512-fold-diluted MHVR1 neutralized JHMV infectivity to the same extent as undiluted MHVR2. This showed that MHVR1 had a 500-fold higher affinity for the virus than MHVR2. The control culture fluid showed no neutralizing activity, even when undiluted.

**Sensitivity to MHV infection of BHK-21 cells expressing MHVR1 or MHVR2**

MHVR1 and MHVR2 were reported to be functional receptors for MHV, since BHK-21 and COS-7 cells expressing these proteins were susceptible to MHV infection (Dveksler et
Fig. 2. (a) Western blot analysis of soluble MHV receptor proteins. Soluble MHVR1 and MHVR2 secreted in the culture fluids of RK 13 cells transfected with PT7-soMHVR1-HA or PT7-soMHVR2-HA were concentrated by ultrafiltration and 10 μl of MHVR1 and MHVR2 undiluted (lane 1) or diluted 2- (lane 2), 4- (lane 3), 8- (lane 4), 16- (lane 5) or 32-fold (lane 6) were electrophoresed in a 10% SDS-polyacrylamide gel. The proteins were transferred onto membrane paper and MHVR1 and MHVR2 were detected by anti-HA MAb. (b) Undiluted soluble MHVR1 (lane 1) and 6-fold diluted MHVR2 (lane 2) as well as undiluted culture fluid of RK 13 cells mock-transfected and infected with vTF7.3 were prepared by Western blotting and reacted with anti-HA MAb. (c) VOPBA with soluble MHVR1 and MHVR2. The soluble MHVR1 (lane 1), MHVR2 (lane 2) and culture fluid (lane 3) prepared as shown in Fig. 1 (b) were reacted with JHMV and the binding of the viruses to the receptor protein was monitored by anti-JHMV MAbs. (d) Quantitative VOPBA by dot blotting. Soluble MHVR1 (A), MHVR2 (B) and culture fluid from mock-transfected cells (C) were diluted in 2-fold steps and transferred to the paper by dot blotting. They were then reacted with JHMV and the binding of the virus was examined by anti-JHMV MAbs. The amounts of MHVR1 and MHVR2 in lane 1 (undiluted materials) are in the ratio 1:6 by the Western blotting analysis.

We have performed experiments to confirm this observation. MHVR1 and MHVR2 were transiently expressed in BHK-21 cells by transfecting with pKS-MHVR1 and pKS-MHVR2, respectively, by electroporation. These proteins were shown to be expressed on 60 to 80% of transfected cells and no apparent difference of intensity in expression was observed by immunofluorescence with anti-CEA antibodies (data not shown) between cells expressing MHVR1 and MHVR2. These cells were infected with various amounts of JHMV (10^6, 10^5 and 10 p.f.u. in 0.1 ml). The cells were fixed with acetone at 9 h after JHMV infection and numbers of fused cells with MHV antigen were compared between BHK-MHVR1- and BHK-MHVR2-expressing cells, respectively. Both types of BHK-21 cells were shown to be susceptible to JHMV infection as demonstrated by the presence of MHV-specific antigens in fused cells after infection with
various titres of JHMV (Fig. 4). However, it was also clearly shown in Fig. 4 that there was a striking difference in the number of fused cells with JHMV antigen between BHK-MHVR1 and BHK-MHVR2 cells infected with JHMV. JHMV produced more than 30-fold greater numbers of fusions (40-fold greater in MHV-positive cells) in BHK-MHVR1 cells as compared with BHK-MHVR2 after infection with 10⁵ p.f.u. When infected with a higher m.o.i., 10⁶ p.f.u., the difference was less remarkable; about 10-fold (Fig. 4). This showed that JHMV infection was 30-fold more efficient in BHK-MHVR1 cells than BHK-MHVR2 cells. The difference in sensitivity to MHV infection between BHK-MHVR1 and BHK-MHVR2 cells was also demonstrated by the amount of virus detected in the culture fluids of these cells, as shown in Table 1. The titres of the virus in the culture fluids of BHK-MHVR1 were 6- to 60-fold higher than those of BHK-MHVR2. Also in this case, a higher m.o.i. resulted in a less remarkable difference in virus growth (Table 1). The low titre of virus detected in the culture fluids of BHK-21 cells without MHV receptor protein may have resulted from cl-2 virus infection in BHK cells by the receptor-independent infection reported by Gallagher et al. (1992), since a minority of BHK cells without receptor protein infected with cl-2 were revealed to be antigen-positive by immunofluorescence (data not shown).

Discussion

We have compared the virus-binding capacity of two different MHV receptors, MHVR1 (mmCGM1) expressed in BALB/c mice susceptible to MHV infection and MHVR2 (mmCGM2) expressed in MHV-resistant SJL mice (Knobler et al., 1981, 1982; Smith et al., 1984; Stohlman & Frelinger, 1978). These two receptor proteins were shown in different laborato-
MHVR1 and those with MHVR2 (Yokomori & Lai, 1992a, b). They also described that the transfection of the MHVR1 gene into cells from SJL mice could not render the cells susceptible to MHV infection, which led them to speculate that another host cell factor was necessary for the resistance of SJL mice to JHMV infection (Yokomori & Lai, 1992b). Dveksler et al. (1993a) used an immunofluorescence method to examine the MHV-A59 sensitivity of BHK-21 cells expressing MHVR1 and those expressing MHVR2 and reported no substantial difference in MHV sensitivity between those cells. They also expressed MHVR1 in cells derived from SJL mice and demonstrated that such SJL cells were susceptible to MHV (Dveksler et al., 1993a), suggesting the non-involvement of SJL-specific host factors in resistance of SJL cells to MHV. We have expressed MHV receptor proteins in BHK-21 cells and found significant differences in virus infection as well as in virus growth between those cells, as shown in the present study. The differences in experimental conditions between Yokomori & Lai (1992a, b) and ourselves are the proportion of cells in culture which express receptor proteins. In our case, more than 60% of cells expressed the receptor protein. Another difference resides in the multiplicity of infection, namely they infected at an m.o.i. of 20 to 50 whereas we infected at an m.o.i. of less than 0.2. When we compared the susceptibility of BHK-MHVR1 and BHK-MHVR2 after infection at the highest multiplicity, m.o.i. 0.2, less remarkable differences were found as compared with the infection with a low multiplicity, m.o.i. 0.002, both by immunofluorescence and by titration of infectious virus (Table 1).

The present study showed a quantitative difference of MHVR1 and MHVR2 in virus-binding ability, which could account for the difference of sensitivity of cells to MHV infection. The finding that BHK-MHVR1 was 30-fold more sensitive than BHK-MHVR2 cells may favour the idea that the resistance of SJL mice to MHV could be accounted for by the low virus affinity of MHVR2 as compared with MHVR1 of other susceptible mouse strains. A 30-fold difference in virus affinity would be amplified into a huge difference in virus growth after repeated cycles of infection during the few days after initial infection, which could result in fatal disease in susceptible BALB/c mice and the survival of SJL mice. This hypothesis can be tested by interchanging these receptor genes between BALB/c and SJL mice by gene targeting (Mansour et al., 1988).

It has been documented that the resistance of SJL mice is controlled by a single recessive gene (Smith et al., 1984). MHVR2 could be the product of this recessive gene. The resistance of SJL mice to MHV infection is not absolute, since SJL mice younger than 6 weeks of age were shown to be susceptible to MHV (Stohlman & Frelinger, 1978). Further-
Table 1. Virus growths in BHK-MHVR1 and BHK-MHVR2 cells

BHK-MHVR1 and BHK-MHVR2 cells expressing MHVR1 and MHVR2 as well as BHK cells transfected with vector only (BHK-mock) were infected with various amounts of JHMV and infectivity in the culture fluids was estimated by plaque assay.

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more, adult SJL mice inoculated with a high dose of virus could not resist MHV infection (Knobler et al., 1982). These facts would suggest that some other factors may be important for the full resistance of SJL mice to MHV infections. The host immune reactions may play an important role in resistance to MHV infection, as shown in the resistance of mice to infection with other strains of MHV (Dupuy et al., 1975; Taguchi et al., 1980). Alternatively, it could be that the MHVR2 proteins are highly expressed in the young SJL mice as compared with adult mice, which may account for the higher susceptibility of young mice to JHMV and susceptibility of adult mice to infection with higher doses of virus.

MHVR1 (mmCGM1) and MHVR2 (mmCGM2) have been reported to exhibit cell adhesion activity, though the properties of this activity are different between these two proteins: the adhesion activity of mmCGM1 works in a calcium- and temperature-dependent manner, while that of mmCGM2 is calcium- and temperature-independent (McCuaig et al., 1992; Turbide et al., 1992). The adhesion activity of mmCGM1 and mmCGM2 could be mediated by the N domain located at the N terminal region of the molecules as shown in human BGP (Stanners et al., 1995; Teixeira et al., 1994). The same domain of the molecule interacts with the N-terminal region of the MHV S protein (Dveksler et al., 1993; Kubo et al., 1994). Between MHVR1 and MHVR2, one-third of the N-terminal region of the N domain is identical, while the rest of the C-terminal regions are highly distinct, where the most striking difference between MHVR1 and MHVR2 molecules exists (McCuaig et al., 1993). The biological differences of these two proteins as cell adhesion protein and receptor protein for MHV could be located in the two-thirds of the C-terminal region of the N domain. Experiments to examine this possibility are currently in progress.

It is of interest that the proteinaceous receptors for some viruses, i.e. HIV (Landau et al., 1988), poliovirus (Mendelsohn et al., 1989), rhinovirus (Staunton et al., 1989) and MHV (Dveksler et al., 1991; Williams et al., 1991) belong to the immunoglobulin superfamily and hence their structures are very similar. The virus-binding sites of these receptor proteins are known to be located in the N-terminal domain (Dveksler et al., 1993a; Koike et al., 1991; Landau et al., 1988; Peterson & Seed, 1988; Selinka et al., 1991; Staunton et al., 1990). The virus-binding site of MHV receptor should be a linear structure, since MHVR1 denatured by 2-mercaptoethanol and SDS was shown to still be functional for virus binding (Boyle et al., 1987; Williams et al., 1990). It is interesting and worthwhile for the possible prevention of virus infection to identify the precise amino acid sequence which serves as the active virus-binding site.

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References


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