A Role for Naturally Occurring Variation of the Murine Coronavirus Spike Protein in Stabilizing Association with the Cellular Receptor

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Murine hepatitis virus (MHV), a coronavirus, initiates infection by binding to its cellular receptor (MHVR) via spike (S) proteins projecting from the virion membrane. The structures of these S proteins vary considerably among MHV strains, and this variation is generally considered to be important in determining the strain-specific pathologies of MHV infection, perhaps by affecting the interaction between MHV and the MHVR. To address the relationships between S variation and receptor binding, assays capable of measuring interactions between MHV and MHVR were developed. The assays made use of a novel soluble form of the MHVR, sMHVR-Ig, which comprised the virus-binding immunoglobulin-like domain of MHVR fused to the Fc portion of human immunoglobulin G1. sMHVR-Ig was stably expressed as a disulfide-linked dimer in human 293 EBNA cells and was immobilized to Sepharose-protein G via the Fc domain. The resulting Sepharose beads were used to adsorb radiolabelled MHV particles. At 4°C, the beads specifically adsorbed two prototype MHV strains, MHV JHM (strain 4) and a tissue culture-adapted mutant of MHV JHM, the JHMX strain. A shift to 37°C resulted in elution of JHIM but not JHMX. This in vitro observation of JHM (but not JHMX) elution from its receptor at 37°C was paralleled by a corresponding 37°C elution of receptor-associated JHIM (but not JHMX) from tissue culture cells. The basis for this difference in maintenance of receptor association was correlated with a large deletion mutation present within the JHMX S protein, as sMHVR-Ig exhibited relatively thermostable binding to vaccinia virus-expressed S proteins containing the deletion. These results indicate that naturally occurring mutations in the coronavirus S protein affect the stability of the initial interaction with the host cell and thus contribute to the likelihood of successful infection by incoming virions. These changes in virus entry features may result in coronaviruses with novel pathogenic properties.

As the first step in the infection of the host cell, the interaction of virions with plasma membrane receptors plays a key role in determining the outcome of infection. To deliver the genome, viral and cellular ligands must bind and then virions must undergo a series of uncoating events in the appropriate cellular location. The ligands involved in the initiation of infection by animal coronaviruses are known (34), thereby establishing these viruses as models capable of contributing to our understanding of these early infection events.

Murine hepatitis virus (MHV) is a coronavirus; virions contain a large (27- to 32-kb) positive-strand RNA genome enclosed within a membrane envelope. These viruses are assembled in the host cell when progeny RNA genomes associate closely with nucleocapsid proteins and then bud into the lumenal cavities of intracellular membrane compartments (38, 61). Envelopment of the ribonucleoprotein complex involves the participation of the following three or four virus-encoded membrane proteins: spike (S), membrane, small membrane, and (in some MHV strains) hemagglutinin (53, 54). Assembled virions are then released from the host cell after transport through the exocytic pathway. The subsequent delivery of RNA genomes from assembled virions to neighboring host cells is dependent primarily on the functions of the S protein. S proteins bind to the MHV receptor (MHVR) (13, 27) and induce the fusion of virion and cell membranes (62), thereby permitting exposure of the infectious ribonucleoprotein complex to the host cytosol.

Biochemical investigations of MHV S proteins have shown that they are large (180-kDa) type I integral membrane glycoproteins that exist as homo-oligomeric, 20-nm projections on the virion surface (15, 16). For some MHV strains, the S protein monomers that make up these projections are composed of two polypeptide chains. This is because S monomers are subjected to cleavage by a host cell protease during intracellular transport (25, 56), thereby generating a peripheral N-terminal S1 fragment and membrane-embedded C-terminal S2 fragment. The S1 fragment binds directly to the MHVR (58, 60), and the noncovalent interaction between S1 and S2 (8, 57) ensures that virions are kept near the cell surface after inoculation.

RNA sequences encoding the S1 fragment are subject to mutation, the most striking of which results in the deletion of about 450 nucleotides near the center of the S1 gene (3, 9, 48, 64). These deletion mutations are remarkably large for an RNA virus, eliminating about 20% of S1 amino acid residues. Once formed, these S1 deletion mutants appear to be selectively amplified over that of the parent virus in tissue culture (28, 49). Emerging S1 deletion mutants are also relevant to coronavirus infections in animals, as evidenced by studies of pigs infected with the highly pathogenic transmissible gastroenteritis virus. Natural deletion mutations within the S1 gene of transmissible gastroenteritis virus is tightly correlated with the generation of an attenuated pneumotropic variant that causes respiratory disease (40).

The mechanisms by which S1 deletions provide selective growth advantages to coronaviruses remain unclear. Relative
to the parent viruses, these mutants lack both antibody epitopes (14, 28, 40, 59) and T-cell epitopes (7), thus suggesting that S1 deletion can provide a means for escape from immunologic surveillance. However, the in vivo growth kinetics of deletion mutants differs from that of the parent virus even when measured within 1 day of infection (23), before the onset of adaptive immune responses. Additionally, these mutants exhibit significant growth advantages in tissue culture (28, 49), thereby pointing to the involvement of nonimmune factors during the selective process. In this regard, one might hypothesize that S1 deletion mutations increase the likelihood of a productive entry process, perhaps at the level of virus-receptor interaction.

Detailed studies of the binding between MHV particles and the cellular receptor can now be addressed because of numerous fundamental advances in coronavirology. The MHVR has been identified (65, 66), cloned, sequenced, and found to be identical to murine biliary glycoprotein (18). Through analysis of recombinant forms of the MHVR, the domain necessary for virus binding was identified (20). This domain is structurally homologous to an immunoglobulin (Ig) fold (5), thus suggesting that the Fab domains of an antibody could be replaced by virus-binding domains to generate a chimeric MHVR-Ig protein. Such a soluble recombinant MHVR was produced and used to study the interactions between the receptor and MHV particles.

MATERIALS AND METHODS

Cells and viruses. Monolayer cultures of murine 17 cl 1 fibroblasts (55) were grown in Dulbecco’s modified Eagle medium (DMEM) supplemented with 5% tryptose phosphate broth (Difco) and 5% heat-inactivated fetal calf serum (FCS; Gibco). 293 EBNA (InVitroGen, Inc.), HeLa tTA (32), and HeLa-MHVR (30) cells were grown in DMEM containing 10% FCS.

Murine coronaviruses were grown in 17cl1 cell cultures. To prepare radio-labeled virions, infected cells were incubated with labeling medium (methionine- and cysteine-free DMEM containing 1% dialyzed FCS; Gibco). 293 EBNA (InVitroGen, Inc.), HeLa tTA (32), and HeLa-MHVR (30) cells were grown in DMEM containing 10% FCS.

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Vaccinia virus-mediated S expression. To obtain a vaccinia virus recombinant encoding an S protein lacking S1 codons 446 to 598 (nucleotides 1336 to 1794; numbering according to that of Parker et al. [48]), RNA was isolated from JHM-infected 17 cl 1 cells at 12 h postinfection by the RNAzol B method (12) and then cDNA was prepared with random hexanucleotide primers and avian myeloblastosis virus reverse transcriptase (Promega). The resulting cDNA preparation was used as the template in PCR (37) with primers S1-1 and S1-2 (1). The purified 373-bp PCR DNA was digested with BspEI and MseI (New England Biolabs), resulting in a 201-bp restriction fragment which was used to replace the corresponding fragment (660-bp BspEI-MseI fragment excised from pTM1-S4) in pCEP4-S1 (30, 45). Recombinant plasmid pTM1-S8 was cloned and amplified in E. coli DH5 α (51) to confirm the absence of S1 nucleotides. pTM1-S constructs were recombined into the thymidine kinase (TK) gene of vaccinia virus (strain WR), and TK-recombinant viruses were selected by standard methodologies (42). TK− virus stocks were then screened for the synthesis of functional S proteins by inoculation onto HeLa-MHVR cells in conjunction with pVT7.3 (26).

RESULTS

Synthesis and analysis of the recombinant sMHVR-Ig. A recombinant MHVR that could be tethered at various densities to a solid phase would permit detailed investigation of the virion-receptor interaction. An MHVR with these capabilities was produced by covalently linking the NTD (virus-binding domain) of the MHVR (MHVR<sub>NTD</sub>) to the Fc domain of an antibody. To this end, a PCR cDNA clone of MHVR<sub>NTD</sub> (20) was ligated to a genomic fragment of human fetal liver DNA harboring exons for the hinge, C<sub>H2</sub>, and C<sub>H3</sub> of human IgG (21) and with the 10.4-kbp NotI-BamHI-linearized pCEP4 vector (InVitroGen, Inc.). The final plasmid (pCEP4-sMHVR-Ig) was cloned and amplified in E. coli DH5 α.

Conformation-specific receptor-binding assays. (i) In vitro binding. Beads of Sepharose-protein G (Pharmacia) were incubated with gentle agitation at 0.1% (vol/ vol) with DMEM–10% FCS containing serial dilutions of sMHVR-Ig medium for 2 h at 4°C. These incubation conditions resulted in quantitative adsorption of sMHVR-Ig to the beads. Sepharose beads were then pelleted by centrifuga-
100-kDa dimer under reducing and nonreducing conditions, respectively. The signals indicating the synthesis and secretion of sMHVR-Ig were compared to those generated by known masses of human IgG1, whose heavy chains have a slightly slower mobility than that of sMHVR-Ig. A comparison of band intensities revealed that the amount of sMHVR-Ig in 1 μl of conditioned medium was between 0.01 and 0.1 μg (Fig. 1B).

**Measurement of binding between the MHVR and MHV particles.** Immobilization of sMHVR-Ig via its Fc domain would be expected to display the virus-binding MHVR NTD in a fashion analogous to that of authentic MHVR on cell surfaces, thereby permitting in vitro measurements of binding between purified virus and the receptor. Thus, the medium from 293 EBNA transfectants was mixed with Sepharose-protein G beads to noncovalently link the sMHVR-Ig to a solid phase. Initially, serial 0.5-log10 dilutions of the sMHVR-Ig medium were prepared with 293 EBNA growth medium as diluent, and then Sepharose-protein G beads were added to 0.1% (vol/vol). This was intended to generate a series of bead preparations of various receptor densities. Additionally, a parallel series of control beads containing the human Ig Fc domain but lacking the MHVR NTD were prepared by incubation with dilutions of sCD30-Ig (17, 41a). sCD30-Ig was produced by 293 EBNA cells stably transfected with the pCEP4:sCD30-Ig construct.

The recombinant Fc-containing proteins that bound to the beads present in 0.1-ml volumes were identified by immunoblotting (Fig. 2A). All preparations contained bovine IgG, whose heavy chain was evident as a broad ca. 50-kDa band. This bovine IgG, which was derived from the FCS present in growth medium, bound strongly to Sepharose-protein G. More importantly, the expected increases in sMHVR-Ig (ca. 45 kDa) and sCD30-Ig (ca. 100 kDa due to the 359-residue CD30 ectodomain) were evident and the anticipated masses of recombinant protein per 0.1-ml bead suspensions were confirmed by comparing immunoblot signal intensities with those generated by known masses of human IgG1 (Fig. 2A).

To measure the virus-receptor interaction, these two series of Sepharose bead preparations were incubated overnight at 4°C with gradient-purified 35S-labelled MHV particles and the radioactivity adsorbed to pelleted beads was quantitated. The MHV strains chosen for these analyses were the prototype JHM (strain 4) and the tissue culture-adapted JHMX variant (43). These strains were selected primarily because JHMX is known to be a JHM derivative that lacks 153 residues within S1 (48, 59) and thus the role of this S1 deletion in receptor binding could be explored.

For both JHM and JHMX, the proportion of particles bound to beads after overnight incubation at 4°C increased with increasing density of immobilized sMHVR (Fig. 2B). Typically, JHM virion preparations contained a relatively low proportion of particles able to bind to sMHVR (maximum of 41% [Fig. 2]), while JHMX preparations exhibited significantly higher levels of binding. For both JHM and JHMX, increases in virion adsorption were not observed in conjunction with increasing densities of the control sCD30-Ig; indeed, the radioactivity associated with the entire Sepharose–CD30-Ig series was not significantly different from background levels of counts. Thus, the increases in virion adsorption with increasing sMHVR-Ig levels were specific to the sMHVR domain and did not result from binding to the human Ig Fc region, as might have been suggested by the reported affinity of the MHV S protein for Fc domains (47).

**Strain-specific elution of virus from the MHVR at 37°C.** In vitro interaction between virus and the receptor was stable at 4°C, but a shift to 37°C resulted in a remarkable strain-specific elution. This was discovered when Sepharose–sMHVR-Ig beads containing bound virions were shifted to 37°C for 1 h. The majority of the receptor-associated JHM eluted, while...
JHM remained with the Sepharose beads (Fig. 3). The specific failure of JHM to maintain a stable association with the receptor at a physiologic temperature and pH was evident throughout a 1.5-log10 range of receptor densities.

Radiolabelled virions that were bound to sMHVR-Ig at 4°C and then subsequently released at 37°C were collected and monitored for both radioactive content and infectivity (Fig. 4). In this experiment, high levels of 35S-labelled virions (10^5 cpm) were used; this allowed the measurement of virion elution from low-receptor-density beads. As expected, relatively low levels of JHM-specific radioactivity eluted from receptor-containing beads at 37°C (Fig. 4). While some infectious virus was present in these eluates, none appeared to be specifically due to elution from the receptor; as titers in eluates above receptor-containing beads were actually lower than those found above beads lacking the receptor. A far more interesting elution pattern was observed when JHM was examined (Fig. 4). For JHM, infective 35S-labelled virus was released from beads containing low receptor densities (0.1 to 1.0 μg of sMHVR-Ig per 0.1 ml beads); these eluates had titers higher than those of corresponding eluates from beads lacking sMHVR-Ig. However, the abundant amounts of 35S-labelled JHM released from beads with the highest receptor densities clearly lacked infectivity.

Initial attempts to identify biochemical distinctions between infectious JHM and noninfectious JHM released from high-density sMHVR beads involved the electrophoretic comparison of their 35S-labelled virion proteins (Fig. 5). In these electrophoretic profiles, the critical receptor-binding S proteins of JHM were evident as a thin ca. 100-kDa band of S1 and a more-diffuse ca. 90-kD aband of S2 (Fig. 5, lanes 1 through 5). Despite the incomplete separation of these S1 and S2 proteins, the profiles did show similar levels of S, nucleocapsid, and matrix proteins among input JHM virions (Fig. 5, lane 1) and virions bound to the receptor at 4°C (lane 3). More importantly, the side-by-side comparison of virion proteins that remained on receptor beads after 37°C exposure (Fig. 5, lane 5) with those that eluted (lane 4) showed that the peripheral S1 ligand was primarily associated with the receptor beads, while the majority of the integral membrane S2 fragment was in the eluted material. Together, these findings suggested that at least some of the JHM released from high-density sMHVR beads involved dissociation at the S1-S2 junction.

The S1 polypeptide of JHMX is 20% smaller than that of JHM; therefore, JHMX S1 and S2 chains coelectrophoresed (Fig. 5, lanes 6 through 10). Despite this situation, the electrophoretic profiles of the JHMX proteins verified that the vast majority of virions were not released from beads at 37°C (Fig. 5, lanes 9 and 10). These results were consistent with a more stable association of JHMX S1 and S2 chains.

**Strain-specific elution of virus from tissue culture cells producing the MHVR.** The detection of infectious JHM release from Sepharose beads containing a low density of immobilized sMHVR-Ig indicated that a similar release process might occur on the surface of a receptor-positive cell. This possibility was investigated by allowing 35S-labelled virions to bind overnight at 4°C to HeLa cells stably transfected with the complete MHVR gene (30). After this binding, HeLa-MHVR cells were rinsed extensively and the kinetics of virion elution into culture...
media was determined by scintillation spectrometry and plaque assays. The HeLa-MHVR cells employed in these experiments (clone 3) reproducibly bound a relatively small proportion of input virions, 19% ± 3.4% for JHM and 31.5% ± 1.3% for JHMX (x ± s;n = 3). As expected, these cell-associated 35S-labelled virions did not elute into media when the temperature was held at 4°C (Fig. 6A). However, parallel 37°C incubations caused the rapid release of 10 to 12% of JHM particles. In contrast, a much more limited elution of only 1 to 2% of JHMX particles was observed (Fig. 6A). Thus, these results correlated well with those obtained from in vitro binding assays.

The capacity of cells to adsorb 35S-labelled virions (19% for JHM) was roughly equivalent to that of Sepharose beads containing intermediate (0.3 to 1.0 µg per 0.1 ml) sMHVR densities (Fig. 2). Since the JHM virions released from beads with these receptor densities were found to be infective in plaque assays (Fig. 4), the elution of infective JHM from cells was anticipated. This was indeed observed. Infective JHM, but not JHMX, eluted specifically in response to 37°C exposure (Fig. 6B). JHM was found in culture media after 10 min at 37°C at 860 PFU/ml (Fig. 6B) and 405 cpm/ml (Fig. 6A). Thus, the specific infectivity of this eluted virus (2.1 PFU/cpm) was similar to the original input JHM (3.1 PFU/cpm); binding to cells and subsequent elution had little effect on virion infectivity. Prolonged (20- to 30-min) exposures at 37°C decreased JHM-specific infectivity (Fig. 6). This was not surprising; thermostability is a well-described characteristic of the JHM strain.

JHM S proteins lacking S1 residues 446 to 598 maintain a stable association with sMHVR-Ig, while complete JHM S proteins do not. To determine whether the 153-residue S1 deletion of JHMX is in fact the alteration responsible for the maintenance of receptor association, sMHVR-Ig was adsorbed to cells bearing a complete S protein or to parallel cell cultures bearing S proteins lacking residues 446 to 598 (designated $S_{ΔS1}$). To obtain S-expressing cells, vaccinia virus vectors capable of producing the two types of S protein were prepared as described in Materials and Methods. HeLa cells were then coinfected with vTF7.3 (26) and the respective vTM1-S or vTM1-$S_{ΔS1}$ vaccinia virus recombinants. S proteins were produced, as judged by syncytium-forming capacity and electrophoretic analysis of 35S-labelled intracellular proteins. Both the complete S protein and $S_{ΔS1}$ were powerfully syncytigenic in HeLa-MHVR cells, and the $S_{ΔS1}$ product had the relatively
rapidelectrophoreticmobilityexpectedforalargelS1deletion
data not shown). Thus, cultures in which cells displayed S
proteins that differed only in the presence or absence of S1
residues446 to 598 were established. At 4°C, sMHVR-Ig
 binds specifically to HeLa cells bearing either S or S
D
S1 (Fig. 7). sMHVR-Ig interaction with S
D
S1
differed from the parallel interaction with complete S protein
in two ways. First, cells displaying S
D
S1 retained a relatively
high proportion of sMHVR-Ig (Fig. 7). This was expected, as
JHMX virions were superior to JHM virions in adsorption to
dsMHVR-Ig (Fig. 2). Second, cells bearing S
D
S1 retained the
majority of sMHVR-Ig throughout a 1-h incubation at 37
°C (Fig. 7). This finding contrasted with the rapid and abundant
elution of sMHVR-Ig from cells bearing the complete S protein,
where over 50% of sMHVR-Ig was released from cells
after only 15 min at 37°C (Fig. 7). Thus, the deletion mutation
examined here was capable of significantly enhancing the S
protein’s ability to maintain association with the MHVR at
37°C.

DISCUSSION
To examine the effects of MHV S protein mutations on
receptor interaction, assays in which MHV could bind to its
receptor under defined in vitro conditions were developed.
Two different isolates of MHV were assayed, and a relation-
ship between a deletion mutation and stable receptor associa-
tion was revealed. This finding has implications for the evolu-
tion and pathogenesis of MHV infections.

MHV deletion mutants may be selectively amplified because
they maintain association with cellular receptors. Viral RNA-
dependent RNA replication results in spontaneous mutants
that often exhibit selective advantages over the parent virus
and therefore predominate after long-term growth. This is
exemplified in a remarkable way during MHV (strain JHM)
infections, where viable deletion mutations eliminating about
10% of S protein-encoding sequences are observed (3, 48).
Such a deletion event is thought to occur during elongation of
genomic RNA when the RNA-dependent polymerase makes a
rare template switch (35, 39). In tissue culture, the resulting

FIG. 6. Elution of JHM, but not JHMX, from HeLa-MHVR cells at 37°C. Purified 35S-labelled virions (JHM, 3.1 PFU/cpm; JHMX, 6.5 PFU/cpm) were diluted in DMEM–0.1% DFCs to 2 × 10^4 PFU/ml. Then 0.5-ml aliquots were added to 10^6 HeLa-MHVR cells and left for 14 h at 4°C. Unadsorbed virions were removed, cells were rinsed extensively, and then ice-cold DMEM–0.1% DFCs (0.5 ml per 10^6 cells) was added. A sample was taken and cell monolayers were dissolved in 0.5 ml of DFCs containing 1% NP-40 (0.5 ml per 10^6 cells). (A) After the indicated elution periods, the medium was removed and cell lysates were determined, the percentages of 35S present in media, as well as the counts of 35S per minute per milliliter of medium, were plotted as a function of elution time. (B) The infectivities associated with media were determined by plaque assays with HeLa-MHVR indicator cells. □, 4°C elution; □, 37°C elution.

FIG. 7. Effect of a deletion in S1 on the capacity of S proteins to maintain association with sMHVR-Ig. HeLa cells (10^6 per well) were infected with vaccinia virus recombinants lacking the S gene (no S) or encoding the complete MHV4 S protein (S) or the mutant form of MHV4 S protein in which S1 residues 446 to 598 are absent (S
D
S1). Twelve hours later, cells were chilled on ice and overlaid with ice-cold sMHVR-Ig (10 μg per well in 1 ml of DMEM–10% DFCs). After 2 h at 4°C, unbound sMHVR-Ig was removed and replaced with ice-cold DMEM–0.1% DFCs containing 5 μg of brefeldin A per ml (1 ml per well). At the indicated times after a shift to 37°C, supernatants were removed and cell monolayers were dissolved in DMEM–0.1% DFCs containing 0.5% NP-40 (1 ml per well). sMHVR-Ig in cell and supernatant fractions was precipitated with Sepharose-protein G and identified by Western immunoblotting as described in Materials and Methods. The amount of sample loaded per lane corresponds to the protein G-bound material from 0.5 ml of cell lysate or supernatant. IgG heavy chains due to a cross-reaction between the immunoblot detection reagent and FCS-derived bovine IgG appeared; these bands served as a sample loading control. Lane M, molecular size markers (in kilodaltons).
mutants typically grow to titers 10 to 100 times that of the parent JHM, which has a complete S protein (28, 49).

Multiple factors likely drive positive selection of S1 deletion variants. At least in some tissue culture lines, deletion variants are less cytocidal than is the parent JHM (28), thereby allowing for prolonged support of variant progeny. This report provides an additional fundamental explanation for the selection of deletion mutants. The variant tested here is superior to its parent in maintaining association with the MHVR. This increases the likelihood of productive cell entry by the variant.

This finding is consistent with elegant studies of DBT and 17 cl 1 cells persistently infected with MHV (strain A59). In these cultures, a selective process occurs during viral persistence that results in relatively virus-resistant cells containing dramatically reduced levels of the MHV R (52). Coevolution of virus also occurs, giving rise to variants able to maintain the ability to infect cells with the low-density MHVR (11). One might speculate that the variants within these persistently infected cultures contain numerous different S mutations, each of which enhances the stability of MHV association with the receptor. Indeed, that mutations other than S1 deletions might be important in this process is suggested by the results in Fig. 7. S proteins lacking S1 residues 446 to 598 interact more stably with the receptor than do complete S proteins, but even with this deletion, S proteins fail to quantitatively retain the receptor at 37°C. Presently unrecognized additional changes in S proteins that further contribute to the stable binding phenotype might include point mutations in the S protein which alter membrane fusion function (6, 29, 31) or overall S conformation (35).

Maintenance of virus-receptor interaction may be enhanced by a stable association between virion proteins S1 and S2. S1 residues bind to the MHVR (58), yet S1 fragments are known to be noncovalently associated with S2 and can in fact detach from virions under relatively mild conditions of slightly alkaline pH (8, 57). These findings suggest that some MHV particles which have bound the receptor via the S1 ligand quickly elute, leaving S1 bound to the receptor. The results presented here support this contention, as the JHM eluted from Sepharose-sMHVR beads was depleted of S1 (Fig. 5). In this study, convincing S1 depletion was identified among the noninfectious particles dissociated from high-density sMHVR beads. Conceivably, the extent of the S1 loss from these viruses leaves them with too little ligand to bind and infect the HeLa-MHVR cells used to monitor infectivity. Retention of S1 on low-density Sepharose-sMHVR beads and HeLa-MHVR cells is liable to be far less extensive, and therefore, infective virus can be observed in 37°C eluates (Fig. 4 and 6).

The restricted tropism of the JHM strain may be due to its inability to maintain association with the MHVR. The genes encoding receptors other than the primary MHVR have been identified by cDNA transfection of MHV-resistant, receptor-negative cell lines. cDNAs able to express a product which confers susceptibility to MHV infection are designated MHVR genes (10, 19, 46, 67, 68). Interestingly, these alternative MHVR transfectants often show a peculiar resistance to the MHV JHM strain. For example, a recently discovered brain-specific receptor (brain CEA) renders Cos7 cells susceptible to infection by MHV strains A59, 2, and 3, but not the JHM strain (10). Additionally, an allelic isoform of the primary MHVR, when present on the surface of murine SJL cells, confers sensitivity to infection by MHV strain A59, but not JHM (67). These findings have prompted suggestions that accessory factors other than the surface receptor are required specifically for JHM entry or that an additional as-yet-unidentified JHM-specific receptor(s) must exist (2). Additional receptors are indeed likely to be found as known receptors to date are members of a very large gene family (44). However, resistance to JHM in transfected and SJL cells might be due in large part to an unstable association between JHM and the receptor. This view is supported by the results of Pasick et al. (49), who found that SJL-derived glial cells were resistant to infection by JHM virus but were sensitive to infection by variants of JHM possessing deletions within S1. In preliminary comparative studies, MHV strain A59 has been found to exhibit extraordinarily high levels of thermostable binding to the MHVR (49a). Therefore, the likelihood of JHM elution from the cell surface, particularly from a cell surface with a low-affinity, alternative MHVR and/or low MHVR density, is higher than that of A59. This might render many cells unable to permit the successful penetration of the JHM strain in a 1-h 37°C incubation.

The kinetics of JHM dissemination in vivo may relate to the propensity of virus to elute from cell surface receptors. It is in the in vivo environment of the murine central nervous system (CNS) that JHM propagates more rapidly than does its deletion mutant offspring. JHM was in fact isolated after serial inoculations of infective brain homogenates through suckling mouse brain (63). After in vivo infection, in situ localization of MHV4 (JHM) RNA indicates a largely transneuronal spread through the CNS that is extremely rapid, regardless of whether the virus is administered intracerebrally (14) or intranasally (4). Relative to MHV4 (JHM), all other known MHV strains are neuroattenuated. Several studies have shown that attenuated MHV strains spread and infect the same neuroanatomic structures as does JHM but that they do so more slowly and with less dissemination of infection (23, 41). How might these kinetic differences be explained? One possibility is that rapid MHV4 elution from cell surfaces provides it with the ability to disseminate widely and rapidly in the CNS from individual sites of single-cell infection. On the other hand, MHV4 variants, many of which harbor deletions within S1, may remain localized to discrete foci due to the maintenance of receptor association.

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