Inefficient Infection of Soluble Receptor-Resistant Mutants of Murine Coronavirus in Cells Expressing MHVR2 Receptor

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1. INTRODUCTION

We have isolated several soluble receptor-resistant (srr) mutants of mouse hepatitis virus (MHV) strain JHMV cl-2, which are resistant to neutralization with the soluble receptor CEACAM1[a] (MHVR1) derived from MHV-susceptible BALB/c mice (Saeki et al., 1997). We report here that srr mutants infected cultured cells expressing MHVR1 as efficiently as wt virus, yet failed to efficiently infect cells expressing CEACAM1[b] (MHVR2) derived from MHV-resistant SJL mice. Our results suggest that inefficient infection by srr mutants in these cells is due to defects in viral entry into cells.

2. MATERIALS AND METHODS

2.1 Viruses and cells

MHV strain JHMV cl-2 (wt cl-2) (Taguchi et al., 1985) and srr mutants (srr7, srr11, srr18) (Saeki et al., 1997) were used. Srr11 has an amino acid change in the S1 subunit at position 65 (Leu to His). Srr 7 and 18 have a

The Nidoviruses (Coronaviruses and Arteriviruses).
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change in the S2 subunit at position 1114 (Leu to Phe) and 1163 (Cys to Phe) respectively. BHK-21 (BHK), BHK-R1 and BHK-R2 cell lines were used for these studies. BHK-R1 and BHK-R2 cells constitutively express the MHVR1 and MHVR2, respectively (Saeki et al., 1997).

2.2 Virus Overlay Protein Blot Assay (VOPBA)

The virus overlay protein blot assay (VOPBA) was used to detect the binding of the S proteins to the receptor protein, as described previously (Saeki et al., 1997). A cross-linker BS^3 [Bis(sulfosuccinimidyl)substrate] was used to form crosslinks between S protein and MHVR2.

2.3 Construction of expression vectors containing the S genes and their expression by vTF7.3

DNA encoding the wt S protein was amplified by RT-PCR from the original wt S gene and ligated into the pTarget vector in the downstream the T7 promoter to create pTar-cl-2S. To make srr S constructs, we have substituted the segment that contains the srr mutation for the corresponding segment of pTar-cl-2S. The vectors containing various S genes were expressed in BHK-R1 and BHK-R2 cells using vTF7.3 (Fuerst et al., 1986).

3. RESULTS AND DISCUSSION

MHV JHMV and its srr mutants, srr7, srr11 and srr18, grew and induced syncytia equally well in BHK-R1. In contrast, srr syncytia formations (Figure 1) and growth (data not shown) were drastically (srr7 and srr11) and moderately (srr18) reduced relative to wild type (wt) virus in BHK-R2.

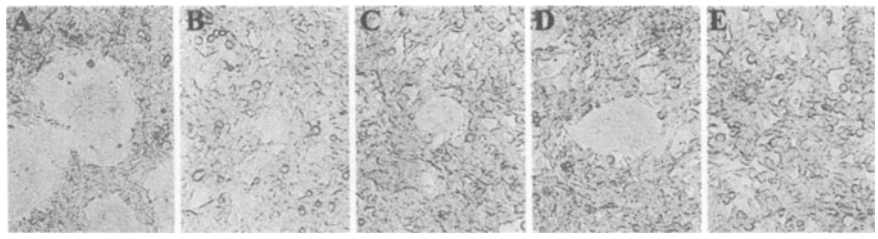

Figure 1. Cytopathic effects of wt and srr mutants in BHK-R2 cells.
BHK-R2 cells infected with wt cl-2(A), srr7(B), srr11(C), srr18(D) at an MOI=0.1, or mock(E) were photographed under the light microscope at 18h after infection.

Since the difference in infection in BHK-R2 cells could be related to their interaction with MHVR2, we examined the direct binding of these viruses to MHVR2 by a VOPBA in the presence of cross-linker, BS^3. There was no difference between srr7, srr18 and wt in their ability to bind to BHK-R2. The binding ability of srr11 was weaker relative to the wt ability. However, these binding features were very similar to those observed in the binding to MHVR1; srr7 and srr18 bound to MHVR1 in a same efficiency as wt, yet srr11 did less efficiently. (Figure 2).

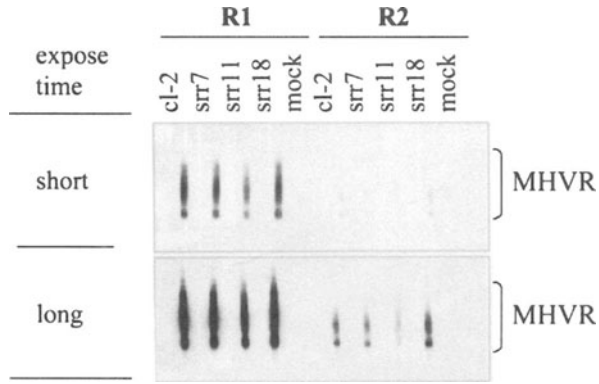

Figure 2. Analysis of virus-binding to the MHVR1 and MHVR2 by VOPBA.
Same amounts of S protein of wt cl-2 or srr mutants were incubated with the soluble MHVR1 and MHVR2 transferred to the membrane paper in the presence of cross-linker, BS^3. The binding of viruses were evaluated with the S1 specific monoclonal antibody No.7 conjugated with biotin and HRPO labeled avidin by enhanced chemiluminescence. X-ray film was exposed for 2 min (long) or 15 sec (short).

Table 1. Fusion activity of transfected S proteins of wt and srr in BHK-R1 and BHK-R2 cells.

S protein	Fusion index (%)		Reduction (%) [b]
	BHK-R1	BHK-R2	
cl-2	93± 4[a]	74±14	21
srr7	87±11	15± 9**	83
srr11	87±10	6± 8**	93
srr18	90± 8	27± 3**	70

[a] Mean fusion indices ± SD. Fused cells and nuclei were counted in four different fields, and fusion indices [1-(cells /nuclei)] ×100 were calculated.
[b] Relative reduction of fusion index in BHK-R2 compared to the value for BHK-R1.
** ($P < 0.01$), Significant reduction by Student's t test compared with the cl-2 fusion index in BHK-R2 cells.

Since the above data suggest that srr mutants bind to both MHVR1 and MHVR2 with similar efficiency as wt did, we have examined their entry into these cells. As a marker of viral entry potential, the fusogenicity of these srr

S proteins was examined. As shown in Table 1, wt and srr mutants induced syncytia with similar efficiencies in BHK-R1 cells. The fusion index of BHK-R2 cells, however, was remarkably low when srr S proteins, but not wt S proteins, were expressed. The finding that srr7 and srr11 fusion activity was reduced to a greater degree than that of srr18 or wt is compatible with the degree of their lesser efficiency of infection in BHK-R2 cells.

From the results obtained, we have concluded that srr mutants can enter into BHK-R1 cells more efficiectly than into BHK-R2 cells, though there is little difference in the binding of srr mutants to MHVR1 and to MHVR2. However, wt virus bound and entered into BHK-R1 and BHK-R2 cells to the same extent.

S proteins of srr mutants may become fusogenic when bound to a high-affinity receptor, but not when bound to a low-affinity receptor, while the wt S protein becomes fusogenic even when bound to a low-affinity receptor. It is likely that binding to either a high- or low-affinity receptor causes the conformational changes of wt S protein which result in virus-cell membrane fusion. Srr mutants, however, might not undergo such conformational changes after binding to the low-affinity receptor because of the mutations in their S protein. Studies are currently in progress to search the conformational changes of S protein.

4. CONCLUSION

Srr mutants infected BHK-R1 as efficiently as wt, while their infection to BHK-R2 was less efficient than wt virus. The inefficient infection was not due to their low affinity to MHVR2, but due to the low fusogenicity. These results suggest that the srr inefficient infection in BHK-R2 cells results from an impaired entry into cells.

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