MHV Neuropathogenesis: The Study of Chimeric S Genes and Mutations in the Hypervariable Region

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1. INTRODUCTION

Mouse hepatitis virus (MHV) is studied as a model system for both acute and chronic virus-induced neurologic disease. Infection of susceptible mice with MHV results in a potentially fatal acute encephalomyelitis, and survivors of this acute disease often go on to develop a chronic demyelinating disease. Using targeted recombination we have demonstrated that the MHV spike (S) glycoprotein is a major determinant of the severity of the acute disease (Phillips et al., 1999). Isogenic recombinant viruses differing exclusively in the spike gene were found to differ dramatically in their neurovirulence. Recombinants containing the spike of the highly neurovirulent MHV-4 virus were highly neurovirulent while recombinants containing the spike of the mildly neurovirulent MHV-A59 virus exhibited a similarly mild degree of neurovirulence.

The MHV spike, found on the virion envelope and on the plasma membrane of infected cells, plays a major role in viral entry and the immune response to infection. The S protein can be divided both structurally and functionally into two subunits, S1 and S2. S1, thought to form the globular head of the spike, is responsible for binding to the viral receptor, and S2, thought to form the stalk of the spike, mediates membrane fusion. Within the S1 subunit there is a region termed the

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hypervariable region (HVR) that exhibits a great deal of diversity among viral strains. A number of studies have associated mutations or deletion in the HVR with alterations in pathogenesis, however, the specific role of this region in pathogenesis is not well understood (Dalziel et al., 1986, Fazakerley et al., 1992, Fleming et al., 1986, Parker et al., 1989, Wege et al., 1988).

Despite the dramatic difference in virulence conferred by the MHV-4 and MHV-A59 spike proteins they are highly homologous. Overall they share more than 92% amino acid identity. The most striking difference between the two spikes is that the MHV-A59 spike contains a deletion of 52 amino acids in the HVR relative to MHV-4. To examine the role of various regions of the MHV-4 spike gene in neurovirulence, we generated a series of recombinnt viruses containing exchanges between the MHV-4 and MHV-A59 spike genes. Using these recombinant viruses containing chimeric spike genes we were able to address the importance of specific regions of the spike in neuroviulence and viral replication in the brain.

2. RECOMBINANTS WITH CHIMERIC MHV-4/MHV-A59 SPIKE GENES

To determine if either the S1 or the S2 subunit of the MHV-4 S protein could confer an increase in neurovirulence we generated recombinant viruses that contained exchanges between the MHV-4 and MHV-A59 subunits. Chimeric spikes were constructed by introducing a silent mutation creating an EcoRV restriction site at codon 775 (MHV-4 sequence). Using targeted recombination we then generated recombinant viruses containing either the S1 of MHV-4 and the S2 of MHV-A59, named $S1_4$R70 or R71, or the S1 of MHV-A59 and the S2 of MHV-4, named $S2_4$R81 and R82 (Kuo et al., 2000). The members of each pair of recombinants exhibited similar phenotypes in vitro and in vivo, and the data from only one member of each pair is shown. As controls we also generated recombinants containing the MHV-4 spike gene, S_4R29, and the MHV-A59 spike gene, S_{A59}R16. To verify that the selected recombinants could replicate efficiently in cell culture we performed growth curves on L2 cell monolayers. The virus titers (16 hours post infection) shown in Table 1, demonstrate that all of the recombinants exhibited efficient replication. To examine the virulence of these recombinants we determined intracranial LD_{50}s in C57Bl/6 mice. The results are shown in Table 1. As expected, S_4R29 was extremely neurovirulent and S_{A59}R16 was only mildly neurovirulent. The recombinants with chimeric spike genes, however, were highly attenuated

for virulence. To determine if the decrease in virulence was attributable to decreased efficiency of replication in the brain, we examined the virus titers in the brain following intracranial inoculation with 10 PFU of virus. The virus titers at the peak of virus replication, day 5 post infection, are shown in Table 1. Both the $S1_4$ and the $S2_4$ recombinants exhibited ineffficient replication in the brain. Thus despite their ability to function well in cell culture, it appeared that recombinants with chimeric spike genes between MHV-4 and MHV-A59 replicated inefficiently in vivo. Moreover, this suggested that in vivo homotypic S1/S2 interactions are required for efficient replication and high neuroviulence.

Table 1. In vivo and in vitro properties of recombinant viruses containing exchanges between the S1 and S2 subunits of MHV-4 and MHV-A59.

Recombinant virus	Log (LD_{50})	Peak Brain Virus Titers (Log(PFU/g))[a]	Virus Replication in Cell Culture (Log(PFU/mL))
S_4R29	0.6	4.8	4.0
$S1_4R70$	>4.7	3.2	5.3
$S2_4R81$	>3.9	2.5	5.0
$S_{A59}R16$	3.4	4.9	6.4

[a] Virus replication on day 5 post intracranial inoculation, n= 6 animals.

[b] Virus replication on L2 cell monolayers at 16 hours post infection, MOI 1.8 PFU/cell .

3. RECOMBINANTS WITH ALTERATIONS IN THE HYPERVARIABLE REGION

Various mutations and deletions in the MHV hypervariable region have been associated with neuroattenuation. To specifically examine the role of the HVR in pathogenesis and neurovirulences we generated a series of recombinant viruses. To determine if the MHV-4 HVR was necessary to confer an increase in neurovirulence, we deleted the MHV-4 HVR. Based on a previously identified neutralizing monoclonal antibody escape mutant (Parker et al., 1989), we generated $S_4\Delta$HVR160 and R161, which contained a 142 amino acid deletion in the HVR (Δ434-575). In addition, we replaced a segment of the MHV-4 HVR (488-600) with the corresponding region from the MHV-A59 HVR (488-548), S_4HV-A59R131 and R133. To determine if the MHV-4 HVR was sufficient to alter the neurovirulence of a recombinant virus, we generated S_{A59}HV-4R51 and R52 in which a region of the MHV-A59 HVR (412-562) was replaced with the corresponding region from the MHV-4 HVR (412-614). Each pair of viruses exhibited similar properties in vitro and in vivo and the data is presented for only one member of each pair. As shown in

Table 2, all of the recombinants exhibited efficient replication in cell culture. When we examined the intracranial virulence of these recombinants, however, we found that alterations in the HVR had a profound affect on pathogenesis. Deletion or replacement of the MHV-4 HVR resulted in a dramatic decrease in virulence as demonstrated by $S_4\Delta$HVR160 and S_4HV-A59R131. The MHV-4 HVR was not sufficient, however, to confer an increase in neurovirulence as demonstrated by the similar virulence of S_{A59}HV-4R51 and S_{A59}R16. Furthermore, these data suggest that the MHV-A59 spike can tolerate manipulations within the HVR with little affect on neurovirulence.

Table 2. In vivo and in vitro properties of recombinant viruses with alterations in the spike hypervariable region.

Recombinant Virus	Log(LD_{50})	Peak Brain Virus Titers (Log(PFU/g)) [a]	Virus Replication in Cell Culture (Log(PFU/mL))[b]
S_4R29	0.6	4.8	4.8
$S_4\Delta$HVR160	>4.1	2.3	4.8
S_4HV-A59R131	>4.0	2.4	5.8
S_{A59}HV-4R51	3.7	3.4	7.0
S_{A59}R16	3.4	4.9	6.7

[a] Virus replication on day 5 post intracranial inoculation, n=4-6 animals.

[b] Virus replication on L2 cell monolayers at 16 hours post infection. MOI 0.4 PFU/cell.

4. CONCLUSION

The MHV spike is a major determinant of neurovirulence. Using targeted RNA recombination, we have now begun to examine the role of specific regions of the spike in neuropathogenesis. From our studies with recombinants containing exchanges between the MHV-4 and MHV-A59 spike it appears that multiple regions of the MHV-4 spike are required to confer increased neurovirulence. Despite efficient replication in vitro the recombinants containing chimeric spike genes exhibited attenuated replication and virulence in vivo suggesting that homotypic S1/S2 interactions are required for an efficient in vivo infection. Furthermore, alterations in the HVR revealed that the MHV-4 hypervariable region was necessary but not sufficient to confer an increase in neurovirulence.

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