In vitro Properties and Pathogenesis of A59/MHV4 Chimeric Mouse Hepatitis Viruses

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1. INTRODUCTION

Early in infection, the mouse hepatitis virus (MHV) envelope glycoprotein spike (S) binds a cell surface receptor glycoprotein and is postulated to undergo a conformational change to reveal a hydrophobic domain, promoting the fusion of the viral envelope and the cell membrane. The actual steps of MHV entry and their effects on pathogenesis are largely unknown. By binding truncated S1 peptides to receptor glycoproteins blotted on membranes, the putative receptor binding domain (RBD) was mapped to the N-terminal 330 amino acids of S (Kubo et al., 1994). In this study, we have generated two groups of chimeric recombinant viruses in an isogenic background in order to study the role of the RBD in pathogenesis.

MHV strains A59 and MHV-4 (JHM) provide a frame-work to study virus entry and pathogenesis. Although both strains replicate to similar levels in the brain, MHV-4 induces a more severe encephalitis in extent and intensity (Phillips et al., 1999). Interestingly, both seem to infect similar cell types. On the other hand, MHV-4 barely replicates in the liver and causes minimal hepatitis, whereas A59 replicates well and causes moderate to severe hepatitis. The outcome of infection is vastly different; the LD_{50} of MHV-4 is three logs lower than that of A59.

The Nidoviruses (Coronaviruses and Arteriviruses).
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2. RESULTS

2.1 Selection of Recombinant Viruses

All of the recombinant viruses, shown in Figure 1, contain either the A59, MHV-4, or an A59/MHV-4 chimeric spike, in the A59 background, and were selected independently from each other. All viruses grow in L2 cell cultures with similar kinetics and to similar extents.

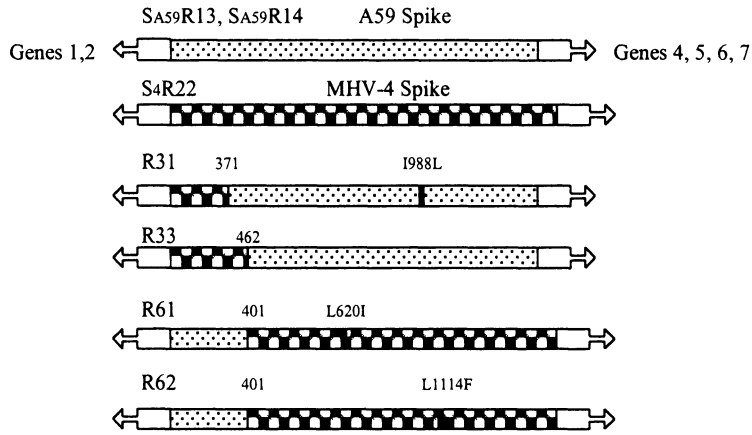

Figure 1. Schematic of the fully sequenced S genes of the Recombinant Viruses. Shown are the 5' end amino acid substitutions in the chimeric spikes and the secondary mutations. The methods for selection were described previously (Masters et al., 1994).

2.2 *In vitro* Characteristics

The recombinant viruses with chimeric spike glycoproteins are likely to exhibit *in vitro* characteristics which are different from the recombinant viruses with parental spike glycoproteins, resulting in altered functionality in virus entry and/or subsequent steps in pathogenesis. We have begun to identify these changes through a number of assays, including tissue culture assays, in order to correlate the *in vitro* properties to some functional role during infection.

Thermolability measures the loss of infectious virus over time when incubated at a given temperature. In our study, a possible mechanism is the spike glycoprotein's tendency to undergo a conformational change which impairs its function in virus entry and/or a subsequent step in the infectious cycle. Interestingly, all the chimeric recombinant viruses, except R33, are

highly stable compared to the recombinant viruses which contain the parental spike glycoproteins (Table 1).

Soluble receptor (sR) neutralization further characterizes the spike glycoprotein's ability to alter conformations, this time, in the presence of receptor glycoproteins. Replacing the S_4R22 RBD with the A59 RBD cause these chimeric recombinant viruses, R61 and R62 to resist neutralization. We do not see the same stabilizing shift when the S_{A59}R14 RBD is replaced with the MHV-4 RBD (Table 1).

Receptor-independent fusion is the phenomenon that certain infected cells, depending on the virus strain, can form syncytia with neighboring cells which do not express the cell surface receptor glycoprotein. These results parallel those of sR neutralization (Table 1).

Table 1. The stability of the viruses and virus-spike interactions

	Thermolability[a]	sR Neutralization[b]	R-indep. fusion[c]
SA59R14	2.7	0.4	-
R31	0.8	0.5	-
R33	2.3	1.3	-
S4R22	3.7	3.9	+
R61	1.2	0.4	-
R62	1.9	0.5	-

[a] Viral titres are taken after 30 minutes of incubation at pH 8.7 and 42°C. Both thermolability and sR Neutralization are presented here as the difference between the starting and the residual log titres.

[b] MHVR-Ig, constructed with domain 1 of CEACAM1[a] ligated to the immunoglobulin Fc domain (Gallagher 1997), is the soluble receptor (sR) used to neutralize viruses. The log titre after one hour of incubation at 37°C is subtracted from that incubated without receptors at 4°C.

[c] Infected L2 cells are overlaid onto BHK cell and syncytium observed after 24 hours.

2.3 Pathogenesis

We studied viral replication and virulence in C57Bl/6 mouse to determine whether or not the *in vitro* phenotypes attributed to S can correlate to or provide a mechanism for their pathogenesis (Table 2).

Table 2. Viral virulence and replication

		Replication [log(pfu/ml)]	
	LD50 [log(pfu)]	Brain	Liver
SA59R13	3.4	6.0	4.8
R31	>5.4	3.1	2.4
R33	5.1	4.8	2.9
S4R22	0.1	nd	nd
R61	>4.4	3.3	2.7
R62	>4.0	3.3	2.3

Methods are as described (Phillips et al., 1999), except that 5000 pfu were injected for replication.

S_4R22 replicates similarly to S_{A59}R13 in the brain and minimally in the liver (data not shown). The chimeric recombinant viruses are attenuated in both replication and virulence to varying degrees.

3. DISCUSSION

The RBD alone is not sufficient to confer the ability of the spike to undergo certain receptor-dependent or independent conformational changes. Thermolability, receptor-independent fusion, and soluble receptor neutralization all depend on mulitple regions of the spike.

Although the mechanism is unclear, it is interesting that the level of increased thermal stability of the chimeras, except R33, compared to the parental viruses, correlates with the level of *in vivo* attenuation.

The loss of S_4R22's ability for receptor-independent fusion and soluble receptor neutralization after the substitution of its MHV-4 spike RBD, in chimera R61 and R62, may result from a nonspecific disruption of the spike structure. Nevertheless, the fact that the loss of these *in vitro* properties correlate with *in vivo* attenuation leads us to hypothesize that these properties of S may be important for the pathogenesis of MHV-4.

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