SPIKE GENE DETERMINANTS OF MOUSE HEPATITIS VIRUS HOST RANGE EXPANSION

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1. INTRODUCTION

The emergence of new viruses is a poorly understood process, but one demanding increased attention in the wake of HIV, hantaviruses, avian influenza virus, and the SARS-CoV. We are using mouse hepatitis virus (MHV) as a model to explore potential mechanisms that mediate coronavirus cross-species transmission. These previously published models include a persistent infection system[1, 2] and a mixed infection system.[3] Both systems resulted in MHV variants with extended host range. Our current efforts involve characterizing the genetic determinants of the expanded host range phenotype and receptor usage of these variants as compared with the parental viruses.

2. RESULTS AND DISCUSSION

The persistent infection model produced variant V51 with an expanded host range that includes human and hamster cell lines, cell lines that the parental A59 strain is unable to productively infect. Sequencing of the Spike (S) gene revealed the presence of 13 amino acid mutations, seven in the S1 cleavage subunit and six in the S2 subunit. The mixed infection model resulted in variant C4, again with extended host range to human, hamster, and primate cell lines. Sequencing revealed 17 amino acid mutations in the S gene when compared with the A59 and JHM strains that initiated the co-infection. Ten changes are located in the S1 subunit while the remaining seven are in the S2 subunit. As a result of the co-infection, the C4 S gene is a chimera of the A59 and JHM spike genes, with the amino-terminal 85% of S derived from JHM and the remainder from A59. Interestingly, there are only two overlap mutations between our two model systems and a single overlap mutation with an additional persistent infection model characterized by Schickli et al.[4] The first overlap involves a codon deletion in C4 versus an amino acid change in V51 at position 939, while the second manifests as an amino acid change at position 949 in both V51 and C4 resulting in different amino acids.

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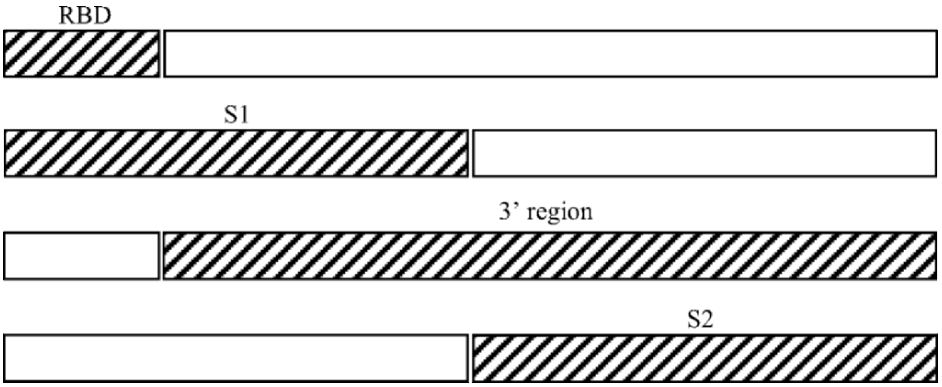

Figure 1. S gene recombinants produced for this study. The hatched regions represent S regions derived from host range isolates, while the unhatched regions are contributed by A59 from the targeted RNA recombination system, or a chimeric S gene derived from A59 and JHM for C4. From top to bottom, the recombinants depicted are: RBD recombinant, S1 recombinant, 3' region recombinant, and S2 recombinant.

To further characterize the impact of the identified mutations on the host range phenotype, we utilized the targeted RNA recombination system[5] and No See'm restriction site technology[6] to allow for precise insertion of S gene pieces from host range variants into the A59 genetic background. Isolated recombinants contained either the full length V51 or C4 S glycoproteins (Full S Recombinant) or specific domains of the molecules incorporated into the appropriate parental S gene. Recombinants include: (1) the receptor binding domain consisting of the amino terminal 330 residues (RBD recombinant), (2) the S1 cleavage subunit (S1 recombinant), (3) all residues but the receptor binding domain (3' region recombinant), and (4) the S2 cleavage subunit (S2 recombinant) (Figure 1). As the co-infection model resulted in a chimeric S gene (as noted above), recombinants containing pieces from C4 were paired with S gene regions from a prototype chimera S gene made with JHM and A59. All recombinants are viable, although the C4 3' region recombinant required multiple passages before CPE and appreciable titers could be observed. It has not been evaluated experimentally to date.

Growth curve analysis on human cell lines HepG2 and MCF7 indicates that the determinants for host range in both model systems are encoded in the S2 region, with the phenotype presenting most clearly in the V51 recombinants. Recombinants containing the S2 region alone from V51 or C4 are capable of productively replicating in HepG2 or MCF7 cell lines, respectively. Table 1 lists 48-hour titers supporting this observation. Additional cell lines from a wider array of species will be examined to determine if this trend holds or is unique to expansion into these human cell lines. Analysis of the location of the mutations in V51 S2 places two mutations at the 5' terminus of the recognized HR1 region[7] and two within the previously identified PEP3 region[8, 9] (a putative fusion domain). S2 mutations in C4 are not as tightly clustered as in V51, however one mutation is located in PEP3 (the overlap deletion versus residue change discussed earlier) and a second is located in HR1 (the common position change also discussed earlier). This unusual clustering of mutations is being more closely examined to determine its impact on host range expansion.

Table 1. Titers on HepG2 cells (V51 recombinants) and MCF7 cells (C4 recombinants).

Recombinant virus	48 hour titer (pfu/mL)	
	V51	C4
Full S gene recombinant	$1.7x10^6$	$3.45x10^4$
RBD recombinant	$6.5x10^2$	ND[1]
S1 recombinant	$6.5x10^2$	$1.5x10^2$
3' Region recombinant	$1.35x10^7$	Data not available
S2 recombinant	$5.35x10^6$	$1.3x10^4$
A59 Control	$2.5x10^2$	ND

[1] Indicates that no plaques were detected.

Receptor usage of C4, V51, and various recombinants was examined utilizing swine ST cells (refractory to infection by MHV and variants in their natural state) that express CEACAM1a, the well characterized high affinity receptor for MHV. The original V51 isolate, the Full S recombinant, the S2 recombinant, and the 3' region recombinant are all unable to productively infect ST-CEACAM1a cells. The RBD and S1 recombinants are capable of a productive infection, with 24-hour titers similar to that of A59 and are sensitive to CC1 blockade (a monoclonal antibody specific for the MHV binding domain on CEACAM1a). The situation is somewhat reversed on murine DBT cells, which express CEACAM1a along with CEACAM1b and CEACAM2. On DBT cells, the RBD and S1 recombinants are hampered by CC1 in a fashion similar to A59 while S2 and 3' region recombinants are fairly resistant to CC1 blockade. The C4 Full S recombinant and original C4 isolate are unable to productively infect ST-CEACAM1a cells, suggesting they may have lost the ability to use this receptor to initiate infection. These same viruses are resistant to blockade on DBT cells. Data on C4 derived domain recombinants are unavailable at this time. Table 2 summarizes these results.

Table 2. CEACAM1a receptor usage on ST-CEACAM1a and DBT cells in the presence of blocking antibody CC1 (24-hour titer, pfu/mL).

		ST-CEACAM1a		**DBT**	
	Virus	**Untreated**	**CC1 treated**	**Untreated**	**CC1 treated**
V51	A59 Control	$3.85x10^5$	ND[1]	$5.25x10^6$	ND
	Original isolate	$1x10^2$	ND	Data not available	
	Full S recombinant	$1x10^2$	ND	$1.39x10^7$	$5.35x10^5$
	S1 recombinant	$3.05x10^5$	ND	$3.65x10^7$	$2x10^2$
	S2 recombinant	$2x10^2$	ND	$4.15x10^7$	$6.3x10^5$
	RBD recombinant	$1.11x10^5$	ND	$4.54x10^7$	$5.5x10^2$
	3' Region recombinant	$7x10^2$	ND	$2.03x10^7$	$8.65x10^5$
C4	A59 Control	$1.4x10^7$	ND	$1x10^7$	$4.5x10^4$
	Full S recombinant	ND	ND	$2x10^7$	$5.1x10^7$
	Original isolate	$3.0x10^3$	ND	$1.5x10^7$	$4.7x10^7$
	JHM control	$4.05x10^6$	ND	Data not available	

[1] Indicates that no plaques were detected.

Although a definitive group of mutations leading to MHV host range expansion has not been examined, the data presented here expand traditional concepts of MHV S protein biology. Previous work has identified the receptor binding domain as an important determinant in MHV host range expansion[10, 11] as well as virulence and tissue tropism. We have demonstrated that the receptor binding domain does not appear to be a genetic factor in the expansion process into the human cell lines tested as part of this study. All host range determinants are encoded in the S2 region of the protein, suggesting the possibility for additional functions of S2 such as receptor binding (to an as yet unidentified receptor) and/or initiation of viral entry through mechanisms not traditionally associated with coronaviruses (processes such as endocytosis or phagocytosis).

3. REFERENCES

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