Mutation of the Immunodominant CD8+ Epitope in the MHV-4 Spike Protein

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1. INTRODUCTION

Our lab focuses on understanding the mechanism of murine coronavirus mouse hepatitis virus (MHV) pathogenesis in the central nervous system (CNS). MHV strains MHV-4 (JHM) and A59 induce acute encephalitis, followed by chronic demyelination in weanling C57Bl/6 (B6) mice; virus is cleared from the CNS by about 2 weeks post infection and demyelination occurs in the absence of infectious virus. We have previously used targeted recombination to select isogenic viruses differing only in the spike gene, expressing either the A59 spike (S_{A59}R16) or the MHV-4 spike (S_4R29) in the background of the A59 genome (Phillips et al., 1999). While the MHV-4 spike expressing virus (S4R29) is, like MHV-4, highly neurovirulent, the S_{A59}R16, like MHV-A59, is significantly less neurovirulent

Recently we have been interested in the role of the T cell response in acute pathogenesis and demyelination; we have begun to investigate the role of the immunodominant CD8+ T cell spike protein (H-2D^b) epitope S510-518 in pathogenesis of MHV in B6 mice. It is likely that response to this epitope may play a role in MHV pathogenesis for the following reasons. Pewe et al., (1996) demonstrated that in a suckling mouse model of MHV-JHM (MHV-4) infection, the development of demyelination requires the selection of CTL escape mutants to this epitope and resulting persistent viral infection; and 2) The highly neurovirulent MHV-4 spike gene expressing

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viruses and the less neurovirulent A59 spike gene expressing viruses differ in their CD8+ T cell epitomes. The immunodominant S510-518 epitope lies within a 52 amino acid hypervariable (HVR) domain that is present in the MHV-4 spike and absent in the A59 spike protein (Figure 1). Both spike proteins have the subdominant S598-605 epitope. (Perlman, 1998). Thus, in order to begin to understand the role of S510-518 in pathogenesis, we have selected viruses in which the S510-518 epitope has been inactivated within the MHV-4 spike protein.

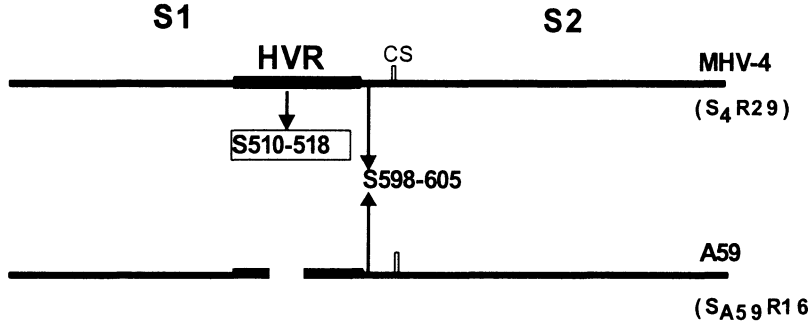

Figure 1. *MHV-4 and A59 spike genes.* S1 and S2 subunits and the cleavage site (CS) are indicated. The 52 amino acid deletion within the hypervariable region (HVR) of the A59 spike is shown relative to the MHV-4 spike. The CD8+ epitopes (arrows) and the CD4+ epitopes (open bars) within the spike protein are shown.

2. RESULTS AND DISCUSSION

Recombinant viruses with mutations in the immunodominant S510-518 CD8+ epitope, within the MHV-4 spike gene, were selected using targeted recombination (Kuo et al., 2000; Phillips et al., 1999). We selected two independent recombinants for each of two mutations, one a single amino acid substitution of the MHC class I anchor asparagine residue (N514S, recombinants S_4R141,143) and the other a two amino acid deletion replaced by one amino acid (NG to R; recombinants S_4R145,146) (see Table 1). The spike genes S4R141 and S4R143 were sequenced and demonstrated to have no additional amino acid substitutions. (The substitution in S4R141,145 was found in naturally selected CTL escape mutants isolated by Pewe et al., (1996) who showed that peptides with such mutations were not recognized by CD8+ T lymphocytes isolated from wild type virus infected mice.)

We compared the immune response to both CD8+ T cell epitopes in these mutants with control virus, S_4R29, which contains the wild type MHV-4 spike protein. We isolated spleen cells from animals infected with S_4R29 as well as S_4R141 and S_4R143; we then determined the percentage of CD8+ T cells that were positive also for intracellular IFNγ staining in

response to peptides containing the each of the CD8+T cell epitopes (Table 1). These data showed that the response to S510-518 is indeed not present in mice infected by either of S_4R141 or S_4R143. A similar level of cells specific for S598-605 was present in animals infected with S_4R29, each of the epitope mutants and MHV-A59 (Table 1 below). Thus A59 does indeed induce a CD8+ T cell response against S598-605, contrary to the report of Heemskirk et al., (1995) who did not detect this response in A59 infected animals, using a chromium release assay to detect cytolytic activity. Interestingly, there is a similar level of cells responding to S598-605 in the absence and presence of the S510-518 response, suggesting that this does not become a more dominant epitope in the absence of S510-518.

Table 1. Intracellular IFNγ assay of splenocytes to quantitate epitope specific CD8+ splenocytes. These intracellular IFNγ+ assays were carried out using splenocytes from infected animals 8 days after intraperitoneal inoculation with 10^4 PFU of virus. Splenocytes were incubated with epitope specific peptides and assayed as described by (Mural-Krishna et al., 1998). The values shown here are averages of two animals. This assay has been repeated twice with similar results.

VIRUS	% IFNγ+ cells (S510-518)	% IFNγ+ cells (S598-605)
S_4R29	20	4
S_4R141	0	8
S_4R143	0	6
S_{A59}R16	0	8

We have begun to examine the pathogenesis of these recombinant viruses with mutant S510-518 epitope. S_4R141,143,145,146 were slightly attenuated, although not dramatically so, as compared to S4R29. (The data are shown for S_4R141 and S_4R143 (Table 1); the data were similar for R145 and R146.) these small differences in LD_{50} were reproducible with all four mutants. Interestingly, at one month post infection, a significant percentage of the surviving mice that had been infected with mutant viruses were moderately to severely paralysed (more severe in the case of S_4R143), while all the surviving S_4R29 infected mice appeared normal. The extent of demyelination (the percentage of spinal cord quadrants with demyelination,) in these animals at one month post infection correlated with the severity of paralysis, with the average extent of demyelination being 62.5% for paralyzed mice and 10% for asymptomatic mice. Thus, these inactivating mutations in the CD8+ T cell epitope appear to shift the infection to be less lethal in the acute stage and result in more demyelination in the late stage. There was no detectable infectious virus in the brains or spinal cords of any of the surviving mice, whether paralyzed or asymptomatic when examined at one month post infection. Thus, the mutant viruses, like the S_4R29, are effectively cleared from the CNS.

Table 2. Pathogenesis of S510-518 mutant viruses. LD_{50} assays were carried out as previously described (Phillips et al., 1999). The LD_{50} assay was repeated twice; these are values from one representative experiment. Paralyzed mice were found among survivors at all doses of virus used (1-100 PFU). Demyelination was quantitated by counting spinal cord quadrants with demyelination and expressed here as the average value for the groups of paralyzed or normal mice for each virus, at one month post infection (Leparc-Goffart et al., 1997).

VIRUS	S510-518	LD_{50} (PFU)	Paralyzed/survivors
S_4R29	CSLWNGPHL	2.1	0/5
S_4R141	CSLWSGPHL	36	3/13 moderate
S_4R143	CSLWRPHL	12	5/10 severe
S_{A59}R16	Not present	3000	Not Done

We cannot distinguish as yet whether the higher levels of demyelination observed in the S510-518 mutant infected mice are due to the fact that more animals survive acute disease and are available to develop demyelination or that, in the absence of the S510-518 response, there is a change in the acute disease that results in more demyelination and paralysis. To address this question, we will investigate the cell types infected and the types of immune cells infiltrating the brain in S_4R29 and mutant infected animals.

3. CONCLUSION

Recombinant viruses containing inactivating mutations in the immuodominant S510-518 CD8+ T cells epitope were compared with parental S_4R29 virus containing the wild type MHV-4 spike in the background of the MHV-A59 genome. The S510-518 epitope mutants did not induce a response against S510-518 while maintaining a similar level of CD8+ T cell response to S598-605 as compared with parental S_4R29. The epitope mutants were slightly attenuated during acute infection but displayed higher levels of paralysis and demyelination compared with S4R29.

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