Demyelination Determinants in the S Gene of MHV

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1. INTRODUCTION

Demyelination is the hallmark pathologic process of several viral-induced, and inflammatory, immune-mediated diseases of the central nervous system (Allen and Bradkin 1993). Several experimental animal model systems are used to study demyelination, including MHV-induced demyelination in mice (Stohlman *et al* 1981; Wege *et al* 1982; Lavi *et al* 1984; Lavi *et al* 1984b; Lavi and Weiss 1989; Houtman *et al* 1996; Lavi *et al* 1999). Previous studies with this model suggested that the S protein and gene may be important for pathogenesis since the spike (S) protein participates in many functions related to virus-host interactions (Dalziel *et al* 1986). Thus we wanted to explore the possibility that the S gene contains molecular determinants of demyelination. We used targeted RNA recombination as previously described (Peng *et al* 1995; Leparc-Goffart *et al* 1998; Phillips *et al* 1999) to insert the S gene of a non-demyelinating virus (MHV-2) into the background of a demyelinating virus (MHV-A59). We then studied the pathogenesis of the new recombinant viruses.

2. GENERATION OF RECOMBINANT VIRUSES

We first cloned the S gene of MHV-2 into a pGEM-T[(a)] Vector. We then removed the MHV-2 S gene from the pGEM-T[(a)] vector by digestion with AvrII and SbfI. The S gene of MHV-2 was then gel purified and subcloned

into the corresponding site in pMH54 (Kuo *et al* 2000), which contains the entire 3' end of MHV-A59 downstream to the HE gene. The new plasmid was labeled pMHV2. We sequenced the S gene portion of pMHV2 in order to verify that it contained the exact sequence of the MHV-2 S gene. We then carried out targeted RNA recombination between Alb4 (a temperature sensitive MHV-A59, obtained from Dr. Paul Masters, containing an 87 nucleotide deletion in the N gene) and synthetic capped RNAs transcribed from pMHV2. MHV-2 S gene recombinant viruses were selected by antibody neutralization treatment. The released viruses were treated with A2.1 and A2.3, anti-S monoclonal antibodies (obtained from Dr. John Fleming) specific for the S protein of MHV-A59. The antibody treatment neutralized the parent viruses Alb4 and MHV-A59, but not MHV-2 or recombinant viruses containing the S gene derived from MHV-2. Viruses were then identified by small plaque morphology. The presence of AvrII and SbfI restriction sites was then confirmed in putative recombinant viruses and selected recombinants were plaque purified two additional times. The identity of the S gene in the selected recombinant viruses and the absence of additional mutations were confirmed by sequencing. The new recombinant viruses were labelled Penn98-1 and Penn98-2.

3. PATHOGENESIS OF THE RECOMBINANT VIRUSES

3.1 Virulence

The LD_{50} experiments revealed that the virulence of both Penn98-1 and Penn98-2 was higher than that of both parental viruses, but was closer to that of MHV-2 (5 PFU = 1 LD50). Penn 98-1 and Penn 98-2 replicated efficiently in both brain and liver. The kinetics of replication was closer to that of MHV-2, suggesting that the S gene contains determinants of virulence and hepatotropism.

3.2. Pathology

Histopathological studies revealed that Penn98-1 and Penn98-2 produced acute meningoencephalitis similar to MHV-A59. Brain pathology included focal acute encephalitis, which was characterized by inflammatory mononuclear cell infiltrates, predominantly lymphocytes. Microglial proliferation, microglial nodules, and neuronophagia were also identified. Areas of involvement included the regions of the brain typically susceptible to MHV-A59 infection. Liver pathology of moderate to severe hepatitis

following Penn98-1 and Penn98-2 infection was characterized by multiple foci of necrosis throughout the liver. Each area of necrosis consisted of degenerating hepatocytes, polymorphonuclear and lymphocytic inflammatory infiltrates, and cellular debris. The extent and distribution of the hepatitis caused by 5 PFU of Penn98-1 and Penn98-2 was similar to the hepatic changes produced by 1000 PFU of MHV-2 and was more severe than the changes produced by 5000 PFU of MHV-A59.

3.2 Immunohistochemistry

Viral antigen was analyzed by immunohistochemistry on tissue sections obtained from mice during acute infection with the recombinant viruses (Penn98-1, Penn98-2) and was compared to sections obtained from mice infected with MHV-A59 and MHV-2. In MHV-A59 infected mice, viral antigen was distributed in focal areas of the brain parenchyma concomitant with the distribution of inflammatory infiltrates. In MHV-2 infected mice, viral antigen was detected in the meninges, choroid plexus, and ependymal cells. There was no viral antigen detected in neurons. The involvement of glial cells was minimal and restricted to the subependymal location. In Penn98-1 and Penn98-2 infected mice, the distribution of viral antigen was similar to that seen in MHV-A59 infection.

3.3 Demyelination

The ability of recombinant viruses Penn 98-1 and Penn 98-2 to induce demyelination, was examined and compared to wild type recombinant wtR13 and wild type viruses MHV-A59 and MHV-2. Penn 98-1 and Penn 98-2 did not produce demyelination in any of the 7 mice injected with each virus. Wild type recombinant virus wtR13, containing an S gene derived from A59, produced demyelination in 100% of the mice (5/5), similar to wild type MHV-A59. All three viruses (wtR13 and Penn98-1 and Penn 98-2) were given at the same dose (5 PFUs). The recombinant virus wtR13, when given at 2500 PFUs, produced larger demyelinating lesions than at 5PFU, but with both doses of virus 100% of the mice produced demyelination.

3.4 Viral persistence

In order to investigate whether differences in the ability of viruses to cause demyelination were associated with differences in viral persistence, we amplified viral RNA from livers, brains and spinal cords of mice infected with demyelinating and non-demyelinating viruses. During the acute infection, PCR products with a band size consistent with MHV RNA

(601bp), were detected at 5 days post infection, in all mice infected with each one of the viruses. Viral RNA was detected in all 3 anatomic locations examined (liver, brain and spinal cord). However, at 30 days post infection, PCR products corresponding to viral RNA were detected only in the MHV-A59 infected spinal cords, but not in the liver or brain of the same mice. There were no detectable PCR products in the livers, brains and spinal cords of mice infected with MHV-2 and another non-demyelinating virus, Penn 97-1, or in organs of control uninfected mice. Using a second pair of primers, RT-PCR amplified a fragment of the predicted size of 147 bp only in the sample of MHV-A59 infected spinal cord, but not in the liver and brain of the same mouse. The spinal cord had the most abundant viral transcript during the chronic phase. This is consistent with previous reports suggesting that the spinal cord is the major site of viral persistence during chronic infection with JHM and MHV-A59. Mice infected with non-demyelinating viruses MHV-2 and Penn97-1, and uninfected controls, were negative.

4. CONCLUSIONS

Using targeted RNA recombination, the present study provides direct evidence that a molecular determinant of demyelination maps to the S gene of MHV. However, we cannot rule out that demyelination may depend on the integrity of additional, non-S determinants, within the viral genome. Viral persistence appears to be an important factor and may even be a prerequisite for MHV–induced demyelination. However, based on the studies presented here, viral persistence per se is insufficient to induce demyelination. The findings presented here pave the way for further studies to investigate in more detail the potential role of the viral envelope S glycoproteins in autoimmunity and demyelination. These studies are potentially relevant to other forms of demyelination including MS.

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