Neurovirulence for Mice of Soluble Receptor-Resistant Mutants of Murine Coronavirus JHMV

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1. INTRODUCTION

Soluble receptor-resistant (srr) mutants isolated from a highly neurotropic mouse hepatitis virus (MHV), JHMV cl-2 strain, have a single mutation in either S1 or S2 subunit of spike (S) protein (Saeki et al., 1997). These mutants were shown to grow and induce syncytia in cultured cells as efficiently as wild type (wt) virus (Saeki et al., 1997). Since it has been reported that the S protein is a key determinant in the viral virulence for animals (Dalziel et al., 1986; Fleming et al., 1986; Philips et al., 1999), we have attempted to see whether the mutations observed in srr mutants affect the neurovirulence of JHMV.

2. MATERIALS AND METHODS

2.1 Virus and animals

Neurotropic MHV JHMV cl-2 strain (wt) (Taguchi et al, 1985) and the soluble receptor-resistant (srr) mutants, srr7, srr11 and srr18, derived from cl-2 (Saeki et al., 1997) were used. Srr7, srr11 and srr18 have a mutation at amino acid position 1114 (Leu to Phe), 65 (Leu to His) and 1163 (Cys to

The Nidoviruses (Coronaviruses and Arteriviruses).

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Phe), respectively. These viruses were propagated and plaque-assayed as previously reported (Taguchi et al., 1981). Four-week-old male ICR mice, 21 to 25g body weights, proven free from the infection of MHV and other murine pathogens, were purchased from Charles River Japan. They were inoculated intracerebrally (i.c.) with various doses of wt or *srr* mutants in 50 µl phosphate buffered saline, pH 7.2 (PBS) and fed with commercial pellets and water *ad libitum* before and after infection.

3. RESULTS

3.1 Neurovirulence of wt and *srr* mutants for ICR mice as examined by survival time and LD₅₀

Mice were inoculated i.c. with various doses of wt and *srr* mutants and the mortality was daily checked for 9 days after infection. All mice inoculated with 3×10^3 PFU of wt virus and *srr* mutants died within 9 days postinfection (p.i.), though the mice inoculated with *srr7* and *srr11* survived longer than those infected with wt or *srr18*. Upon 3×10^2 and 3×10^1 PFU inoculation, all mice infected with wt and *srr18* died, while some mice infected with *srr11* and *srr7* survived over the observation period. The LD₅₀s examined on day 9 p.i. were 3 PFU, 95 PFU, 5 PFU and 3 PFU for wt virus, *srr7*, *srr11* and *srr18*, respectively. These results indicate that *srr7* and *srr11* have reduced neurovirulence for ICR mice and *srr18* is similar to wt virus.

Clinically, mice infected with wt virus developed central nervous system (CNS) symptoms, such as hypersensitivity to physical stimulants, trembling and finally paralysis, from second to third day p. i. Mice infected with *srr7* or *srr11* mutants started displaying such CNS symptoms from day 5 or later and showed mild and prolonged CNS symptoms. Some of those mice suffering CNS symptoms such as hind leg paralysis survived over the observation period.

3.2 Viral growth in the brain and spinal cord

Viral growth in the brain and spinal cord was examined after inoculation with 10^3 PFU of wt virus, *srr7* and *srr11* mutants. On day 2 p.i. the wt virus titer in the brain was about 1 and 2.5 log₁₀ PFU/g higher than those of *srr11* and *srr7*, respectively. Wt virus reached a plateau, 6.2 log₁₀ PFU/g on day 2 p.i., while *srr* mutants failed to attain such a high titer even when they attained the plateau on day 4 p.i. Especially, *srr7* grew inefficiently in both

brain and spinal cord relative to the wt virus. Also, similar difference was detected in the virus titer in the liver between wt-infected mice and those infected with *srr* mutants.

3.3 Histopathological examination

We have compared the histopathological changes of the mice inoculated with 10^3 PFU of the least virulent *srr7* and those with wt virus. On day 2 p.i., there was minimal neuropathological lesions in the mice. Mild infiltration in the meningeal space and small necrotic foci scattered in parenchyma were found in the most of the infected animals. In spite of the variations among individuals, the necrosis and inflammatory changes were slightly more intensive in the wt-infected mice than in those infected with *srr7*. In *srr7* infected mice, the clusters of small vacuolation were formed in the prefrontal region and thalamus (Fig. 1A). The vacuolating lesion became obvious as a huge spongiform lesion on day 5 or later of infection (Fig. 1B). Immunohistochemical analysis also revealed a slight difference in the localization and the intensity of viral antigens. In the brain of wt-infected mice, neurons containing viral antigen were detected in the wide area of the cortex (Fig. 1C), while the distribution of the antigen was restricted in the white matter of periventricular area in the brain of some of the *srr7*-infected mice (Fig. 1D). A significant difference in pathology between wt and *srr7* infection was the occurrence of apoptosis in the parenchyma, which was detected by a tunnel method. Large number of apoptotic cells were observed in the periventricular and the deep cortex of the *srr7*-infected mice, while only a small number of apoptotic cells were encountered in the brain parenchyma of the wt-infected mice.

Figure 1. Vacuolating degeneration observed on day 2 p.i. in *srr7* infected mouse (A). A large spongiform change in basal nucleus 8 days after *srr* infection (B). Viral antigens detected in the brain of wt infected (C) and *srr* infected (D) mice on day 2 p.i.

4. CONCLUSION

Three *srr* mutants derived from a highly neurotropic JHMV have a single amino acid mutation in the S protein. To see whether these amino acids are involved in the neurovirulence, we have examined their neuropathogenicity for ICR mice. *Srr7* and *srr11* caused alleviated neurological diseases relative to wt virus in terms of the LD50 and survival time, while *srr18* showed virulence not significantly different from wt virus. The growth of *srr7* and *srr11* with reduced virulence was 1 to 2 log₁₀ PFU/g lower than that of wt virus in the brain and spinal cords. Histopathological changes in the brain of *srr7*-infected mice were slightly less remarkable relative to those caused by wt virus. Vacuolation was prominent in the brain of *srr7*-infected mice on day 5 or later of infection. Many apoptic cells were found in *srr7*-infected brain while it was less evident in wt-infected mouse brain. These results suggest that the amino acids at positions 1114 and 65 mutated in *srr7* and *srr11* S proteins, respectively, influence the neurovirulence of JHMV.

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