Programmed Cell Death in MHV-Induced Demyelination

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1. INTRODUCTION

Coronavirus mouse hepatitis virus (MHV) strain A59 causes severe acute hepatitis, focal meningoencephalitis, and chronic demyelinating disease of the spinal cord. It serves as an experimental model for multiple sclerosis. MHV-2 causes acute hepatitis and meningitis. The pathogenesis of MHV-induced demyelination in mice is not clear and a potential mechanism of apoptosis was suggested. Previous studies showed apoptotic T cells, astrocytes, and oligodendrocytes in demyelinating areas following MHV-JHM infection (Barac-Latas *et al* 1997). However, the distribution of demyelination has been suggested to correlate better with macrophage infiltration than with the apoptotic cells (Wu and Perlman 1999). In the chronic demyelinating disease induced by Theiler's virus, apoptotic astrocytes were found in demyelinating lesions (Palma *et al* 1999). In experimental allergic encephalitis, the disease was much milder in mice lacking Fas-Fas ligand molecules, suggesting that apoptosis plays a role in the disease (Waldner *et al* 1997).

The Nidoviruses (Coronaviruses and Arteriviruses).
Edited by Ehud Lavi *et al.*, Kluwer Academic/Plenum Publishers, 2001.

2. METHODS AND MATERIALS

2.1 Viruses

MHV-A59 and MHV-2 were grown and titered on L2 cells. Viral titers were determined by standard plaque assays.

2.2 Mice

4-week-old male C57BL/6 and B6MRL-Fas-lpr (lpr) mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). C57BL/6 mice were injected intracerebrally (I.C.) with $2.5x10^3$, 25 and 5 PFU of MHV-A59, 25 and 5 PFU of MHV-2. B6MRL-Fas-lpr mice were injected I.C. with 25 and 5 PFU of MHV-A59. Mock-infected mice were injected I.C. with L2 cell lysate.

2.3 Histology

Mice were sacrificed on days 1,3,5,7,9,11,and 30 (2-3 mice per time point per virus, during the acute stage and 52 mice on day 30). Brain, spinal cord and liver were removed and placed in 10% normal buffered formalin and embedded in paraffin. Five μm thick sections were stained with LFB and H&E.

2.4 TUNEL

Detection of in situ DNA fragmentation was done with fluorescein *in situ* cell death detection kit (Boehringer Mannheim, Indianapolis, Indiana) as specified by the manufacturer.

2.5 Double labeling

Double labeling for TUNEL with viral antigen, and TUNEL with specific markers for astrocytes, oligodendrocytes, and macrophages was performed by immunohistochemistry. The analysis was performed by the avidin-biotin-phosphatase based technique (Biomeda, Foster City, CA) using fast-red or vector blue (Vector laboratories, Burlingame, CA) as a staining substrate and a 1:200 dilution of rabbit anti-MHV-A59 polyclonal antibody, 1:100 dilution of monoclonal glial fibrillary acidic protein (Lee *et al.* 1984),

1:500 carbonic anhydrase II (Cammer *et al* 1985) or 1:50 rat anti mouse F4/80 antigen (Serotec Inc., Raleigh, NC), respectively.

3. RESULTS

3.1 Acute Stage

TUNEL staining was detected in the brains and livers of mice infected with MHV-A59 and MHV-2. Extensive liver apoptosis was observed in both MHV-2 and MHV-A59 infections. Co-localization of A59 viral antigen and TUNEL staining was detected in hepatocytes. Apoptosis was found in the brain parenchyma of MHV-A59 infected mice and meningial apoptosis was found in both infections. The kinetics, intensity and pattern of apoptosis correlated with the inflammatory events. Mock-infected mice were apoptosis-negative in all tissues. No apoptosis was identified in the spinal cord during the acute stage.

3.2 Chronic stage

TUNEL staining was observed exclusively in the spinal cords with demyelinating lesions of 15 mice infected with MHV-A59. No apoptosis was detected in the spinal cords of 3 MHV-A59 infected mice without demyelination, 28 MHV-2 infected mice and 10 control mice. TUNEL staining was negative in the brains and the livers of all infected mice.

3.3 Fas knock-out mice

To assess the role of the apoptotic cascade in the demyelination process, lpr mice were infected with MHV-A59 virus. Two of 5 mice in both groups developed demyelination, however the extent of demyelination in the spinal cord was significantly less in the lpr mice compared to wt mice (10% and 34% demyelinating quadrants respectively).

3.4 Apoptotic cell types

Double labeling for TUNEL-positive nuclei and specific markers for astrocytes, oligodendrocytes and macrophages was observed. Quantification studies demonstrated 3-5% oligodendrocytes, 1-2% astrocytes and 70% macrophages, double stained.

4. DISCUSSION

Apoptosis was observed in mice following infection with MHV-A59 and MHV-2. The kinetics, intensity and pattern of the apoptotic staining correlated well with the distribution of inflammation. However, apoptosis was found in both inflammatory cells (macrophages and possibly lymphocytes), and parenchymal cells such as hepatocytes, oligodendrocytes and astrocytes.

Several findings suggest that apoptosis may play an active role in the process of tissue damage and demyelination in MHV infection. 1. The finding of apoptosis in hepatocytes, oligodendrocytes and astrocytes; 2. The high prevalence of apoptotic cells in the demyelinating lesions; 3. The absence of CNS apoptosis in mice infected with a non-demyelinating virus MHV-2 and control mice; 4. The significantly lower extent of demyelination in lpr mice. A recent study did not find any difference in the extent of demyelination between wt and lpr mice 13 days following JHM infection (Parra *et al* 2000). However, the different results may be due to the differences in strains of the virus or time points examined.

In conclusion, apoptosis may play an important role in both acute and chronic MHV disease. The relationship between apoptosis, inflammation and tissue damage is yet to be defined, but can possibly be defined through further dissection of the apoptotic cascade.

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