Chapter C11

APOPTOSIS IN MHV-INDUCED DEMYELINATION

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Abstract: Mouse hepatitis virus (MHV) induced encephalitis, hepatitis and chronic demyelinating disease involves inflammatory and parenchymal cell death. TUNEL assays and electron microscopy reveal that brain parenchymal cells such as neurons, astrocytes, microglia/macrophages and oligodendrocytes are undergoing apoptosis during acute and chronic infection with a neurotropic strain MHV-A59. Although apoptosis is a normal phenomenon in helping the turnover of inflammatory cells, apoptosis of brain parenchymal cells may have significant implications for the pathogenesis of viral-induced demyelination.

Key words: apoptosis, astrocytes, microglia, macrophages, coronavirus, demyelination

INTRODUCTION

Coronavirus mouse hepatitis virus (MHV) strain A59 causes severe acute hepatitis, focal meningoencephalitis, and chronic demyelinating disease. It serves as an experimental model for multiple sclerosis (MS). A closely related strain (MHV-2) causes acute hepatitis and meningitis without encephalitis or demyelination. The pathogenesis of MHV-induced demyelination in mice is not completely understood and a potential mechanism of apoptosis was suggested. Previous studies showed apoptotic T cells, astrocytes, and oligodendrocytes in demyelinating areas following infection with JHM, another neurotropic-demyelinating strain of MHV (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 (EAE), 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).

APOPTOSIS IN MHV-INDUCED DISEASE

To study apoptosis in MHV-A59 disease we used the experimental model system of MHV-A59 and MHV-2 infection in 4-week-old male C57BL/6 and B6MRL-Fas-lpr (lpr) mice (Schwartz, Fu et al. 2001; Schwartz, Fu et al. 2002). 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. 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. 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. 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.

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.

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.

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).

Recently mice were infected with MHV-A59 and simultaneously implanted with subcutaneous ALZET osmotic pumps that were filled with a pan-caspase inhibitor Z-Val-Ala-D1-Asp-fluromethylketone (Z-VAD-fmk). This treatment has been previously shown to be effective in the treatment of apoptosis in EAE and also reduce the amount of demyelination. The treatment was given continuously at a dose of 1 mg per day, at a delivery rate of 1microliter per hour, for 30 days, beginning 1 day prior to the infection with the virus. While encephalitis was not different between the treated and untreated mice, demyelination was significantly reduced in the treated mice. In 4 mice receiving A59 and anti-pan-caspase treatment demyelination was found in only 3/76 spinal cord quadrants (3.94%), whereas 6 untreated A59-infected mice in the same experiment had 18/120 demyelinating quadrants of spinal cord (15%), and mice receiving only treatment without infection had no demyelination. Although these studies are preliminary due to small number of animals, they may support an important role for apoptosis in autoimmune demyelination as in EAE and Theiler's virus induced demyelination.

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.

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,

neurons and astrocytes. The involvement of parenchymal cells in CNS apoptosis and the location and temporal relationship between apoptosis and pathology (either encephalitis or demyelination), may indicate that apoptosis is indeed a significant factor in the pathogenesis of the disease and not a merely epiphenomenon. This conclusion is also supported by the reduced demyelination in animals lacking the Fas-lpr pro-apoptotic molecule and mice receiving prolonged pan-caspase inhibitor treatment. Since these treatments affect both inflammatory cells and parenchymal cells the result may be the balance between two opposing trends. A recent report documented the ability of oligodendeocytes to undergo apoptosis in culture upon infection with MHV-A59 (Liu, Cai et al. 2003). This in vitro model greatly supports the hypothesis that death of oligodendrocytes and demyelination due to MHV are caused at least in part by apoptosis. A previous 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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