

## Chapter C1

# HISTOPATHOLOGY IN CORONAVIRUS-INDUCED DEMYELINATION

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**Abstract:** The experimental model system of coronavirus mouse hepatitis virus (MHV) induced demyelination in 4-6 week old C57Bl/6 or Balb/c mice exhibits a biphasic disease and two distinct forms of virus-induced demyelination. During the acute phase of the disease MHV infection causes acute encephalitis, and some strains of virus cause also hepatitis. Infection with the JHM strain of MHV causes severe panencephalitis, whereas MHV-A59 causes mild to moderate encephalitis involving specific limbic and limbic related areas of the brain and brain stem. The target cells are neurons and glia including oligodendrocytes. Demyelination during the acute stage is due to cytolytic infection of oligodendrocytes. After two weeks, the disease process enters a chronic stage of immune-mediated demyelination, in the presence of high levels of anti-viral antibodies and persistent low levels viral RNA in glial cells, without detectable levels of infectious virus or viral antigens.

**Key words:** Demyelination, mouse hepatitis virus (MHV), coronaviruses, nidoviruses, histopathology, pathology, pathogenesis, autoimmunity.

## INTRODUCTION

The mechanism of demyelination in the model system of MHV-induced demyelination has been the subject of intense controversy. In the early years following its original description, the MHV model has been considered a perfect example of virus-induced demyelination due to direct cytolytic effect on oligodendrocytes, as opposed to EAE and the Theiler's virus induced encephalomyelitis model, which were considered the prototypes of autoimmune demyelination. In recent years, evidence was presented in favor of an immune-mediated mechanism in MHV-induced demyelination. However, examining the strong evidence that supports each mechanism it

appears that the only explanation for to this discrepancy is that the two mechanisms co-exist in the same model. While the early descriptions referred to the cytolytic mechanism of acute infection, the later evidence described the mechanism that dominates the chronic disease. In the following chapter the histopathological features of MHV disease of the brain will be described for the two stages of the disease.

## **MHV INDUCED ACUTE ENCEPHALITIS**

Some strains of MHV are purely hepatotropic (e.g. MHV-2) (Hirano et al. 1981), some are primarily neurotropic (e.g. JHM ) (Robb et al. 1979), while others (MHV-A59, MHV-S and MHV-3) are both hepatotropic and neurotropic (Virelizier et al. 1975; Barthold and Smith 1983; Lavi et al. 1986). MHV infection has been extensively used as a model system for viral persistence and for acute and chronic neurologic diseases (Weiner 1973; Knobler et al. 1981; Stohman and Weiner 1981; Sorensen et al. 1982; Wege et al. 1982; Barthold and Smith 1983; Buchmeier et al. 1984; Lavi et al. 1984; Perlman et al. 1990). The experimental models of neurotropic MHVs exhibit a bi-phasic disease. Acute meningoencephalitis (with or without hepatitis) is the major pathologic process in the first two weeks following inoculation. Subsequently, subacute and chronic diseases develop, which can be either an inflammatory demyelinating disease (in JHM and A59) or vasculitis (in MHV-3).

MHV enters and spreads into the brain primarily by hematogenous spread. This conclusion is based on the fact that injection of virus into one hemisphere produces a simultaneous appearance of viral antigen in both hemispheres at the same time. When the route of infection is by intracerebral injection virus quickly enters the blood circulation and re-enters the brain, and other target organs such as liver, by hematogenous spread. However, following intranasal inoculation of mice, virus can be traced propagating from the olfactory system into limbic system structures and their connections in the brain stem. Thus interneuronal transport has been suggested as an additional mode of spread within neuronal cells during acute encephalitis (Barthold 1988; Lavi et al. 1988; Barnett et al. 1993). In addition, the fact that the virus travels from cerebral hemispheres to brainstem and then to spinal cord (Perlman et al. 1990) suggests an interneuronal transport.

During the acute phase of the disease the JHM strain of MHV causes severe panencephalitis, involving the telencephalon, diencephalon, brain stem, cerebellum and spinal cord. In contrast to the pantropic property of wt JHM, MHV-A59 and certain mutants of JHM produce a limited CNS disease restricted to specific locations of predilection. These include the olfactory

and limbic systems, and certain basal nuclei, which are physiologically connected with the limbic system.

Acute MHV encephalitis causes a lytic infection of cells including neurons, astrocytes and oligodendrocytes (Lampert et al. 1973). This finding raised the speculation that demyelination is caused only by direct cytolytic effect of the virus on oligodendrocytes.

#### THE MHV DEMYELINATING DISEASE

The first description of the ability of MHV to cause demyelination was in 1949. Cheever and co-workers isolated JHM, a strain of MHV, from the brains of mice with hind leg paralysis (Cheever et al. 1949). A detailed histological analysis by Bailey et al revealed disseminated encephalomyelitis and areas of demyelination with sparing of axons in the brain and spinal cord (Bailey et al. 1949). Twenty-four years later, Leslie Weiner studied in more details the pathogenesis of demyelination induced by JHM (Weiner 1973). He found that the development of demyelination was a function of the age of mice, the dose of virus and the route of inoculation. The study was focused on demyelination during the 7-15 days post infection. There was evidence of viral antigen but no evidence of immunopathology. Immunosuppressive treatment did not abolish demyelination and taking all this information together the study concluded that JHM-induced demyelination was due to a cytopathic effect of the virus on oligodendrocytes. In the same year Lampert and co-workers came to the same conclusion. They studied by electronmicroscopy the ultrastructural pathology of JHM-induced acute demyelination, 3-6 days after infection. They found acute encephalomyelitis with patchy demyelination in the brainstem and spinal cord. They also found viral particles consistent with the appearance of coronaviruses in the cytoplasm of oligodendrocytes. The obvious conclusion from that study was that JHM replicated in oligodendrocytes and killed some of them causing demyelination.

Both JHM and A59 then cause subacute and chronic inflammatory demyelination in the brain, but mainly in the spinal cord (Stohlman and Weiner 1981; Lavi et al. 1984). Propagation of virus from the initial site of infection in the brain to the spinal cord occurs by transport of the virus in neurons and astrocytes (Sun and Perlman 1995). Astrocytes in particular may play an important role in this process by secreting cytokines and producing iNOS (Sun et al. 1995). Perivascular mononuclear (lymphocytic/macrophage) inflammatory infiltration of meninges and Virchow-Robin spaces is seen adjacent to areas of destruction of myelin, denuded, but otherwise intact axons, and macrophages containing myelin debris are seen in various areas of white matter, especially in the spinal

cords of infected animals (Lampert et al. 1973; Weiner 1973; Fleury et al. 1980; Lavi et al. 1984). Recurrent demyelination, remyelination, regeneration of oligodendrocytes and increased myelin basic protein gene expression have been demonstrated in various MHV model systems (Herndon et al. 1975; Herndon et al. 1977; Kristensson et al. 1986). These features parallel many of the pathologic findings seen in multiple sclerosis in contrast to the monophasic viral or post viral human demyelinating diseases such as acute disseminated encephalomyelitis (ADEM), and progressive multifocal leukoencephalopathy (PML).

Several laboratories including our own showed evidence of persistent coronavirus infection in both glial cell cultures and in animals. Persistent virions were demonstrated in chronic infection with ts mutant of JHM (Knobler et al. 1982), and persistence of viral genome was found following infection with MHV-A59 (Lavi et al. 1984). Persistent infection of glial cultures with MHV-A59 was used to demonstrate induction of MHC class I expression on astrocytes and oligodendrocytes, mediated by a soluble factor (Suzumura et al. 1986; Suzumura et al. 1988; Lavi et al. 1989). MHC class II induction mediated by viral particles has been demonstrated in glial cell infection with JHM (Massa et al. 1986). In the last decade the role of an immune mediated pathogenesis as the major mechanism of chronic MHV-induced demyelinating disease has been suggested based on indirect evidence. Adoptive transfer of demyelination with T cells from JHM-infected rats and in-vitro sensitivity to myelin basic protein suggested the possibility that MHV induced demyelination can be at least in part an immune-mediated, EAE-like disease (Watanabe et al. 1984). Immunosuppression of mice infected with JHM decreased the incidence of demyelination suggesting that the chronic demyelinating disease is immune-mediated (Wang et al. 1990).

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