

# The Pathogenesis of Murine Coronavirus Infection of the Central Nervous System

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**ABSTRACT:** Mouse hepatitis virus (MHV) is a positive-strand RNA virus that causes an acute encephalomyelitis that later resolves into a chronic fulminating demyelinating disease. Cytokine production, chemokine secretion, and immune cell infiltration into the central nervous system are critical to control viral replication during acute infection. Despite potent antiviral T-lymphocyte activity, sterile immunity is not achieved, and MHV chronically persists within oligodendrocytes. Continued infiltration and activation of the immune system, a result of the lingering viral antigen and RNA within oligodendrocytes, lead directly to the development of an immune-mediated demyelination that bears remarkable similarities, both clinically and histologically, to the human demyelinating disease multiple sclerosis. MHV offers a unique model system for studying host defense during acute viral infection and immune-mediated demyelination during chronic infection.

**KEY WORDS:** host defense, viral infection, multiple sclerosis, demyelination

## I. MOUSE HEPATITIS VIRUS

Mouse hepatitis virus (MHV) is a positive-strand RNA virus and a member of the family Coronaviridae, representing a significant and ubiquitous group of viral pathogens that infect both humans and animals, causing respiratory, gastrointestinal, and neurologic diseases. MHV, a group II coronavirus, is a natural pathogen of mice, normally infecting the liver, gastrointestinal tract, and central nervous system (CNS), and causing a wide range of diseases, including hepatitis, gastroenteritis, and acute and chronic encephalomyelitis.<sup>1–3</sup> MHV pathogenesis is dependent upon several factors, including viral strain, mouse background, and inoculation route.<sup>4</sup> Structurally, MHV is com-

prised of three main proteins: the nucleocapsid (N, 60 kDa), which forms a helical complex with the genome; the membrane protein (M, 25 kDa), which associates with the nucleocapsid and aids in envelope formation and budding; and the extracellular spike glycoprotein (S, 180 kDa), which associates with the membrane protein and controls host cell receptor recognition and fusion.<sup>2–4</sup> MHV spike glycoprotein recognizes the host cell receptor CEACAM-1 (carcinoembryonic antigen-cell adhesion molecule-1)<sup>5,6</sup> and dictates host pathogenesis and immune responses.<sup>7–9</sup>

Intracranial inoculation of susceptible strains of mice with neuro-adapted strains of MHV induces an acute encephalomyelitis that evolves into a chronic fulminating demyelinating disease.<sup>10</sup>

## ABBREVIATIONS

**MHV**, mouse hepatitis virus; **CNS**, central nervous system; **MS**, multiple sclerosis; **NK**, natural killer; **MMP**, matrix-metalloproteinase; **TNF**, tumor necrosis factor; **IFN**, interferon; **IL**, interleukin; **EAE**, experimental autoimmune encephalomyelitis

Received: 6/16/09; Accepted: 9/4/09

During acute encephalomyelitis, MHV infection stimulates the production of pro-inflammatory cytokines and chemokines that activate and attract the antiviral arms of the immune system.<sup>11-13</sup> Antiviral effector T lymphocytes are absolutely required for controlling viral replication via IFN- $\gamma$  secretion or cytolytic activity.<sup>14,15</sup> Eventually, MHV is cleared below detectable levels, but sterile immunity is not achieved. The majority of mice that survive the initial acute infection develop an immune-mediated chronic demyelinating disease, characterized by viral persistence within white matter tracts of the spinal cord and continued T lymphocyte and macrophage infiltration.<sup>16-19</sup> Numerous clinical and histological similarities exist between MHV-induced demyelination and the human demyelinating disease multiple sclerosis (MS), making the MHV model system relevant for evaluating the underlying mechanisms associated with disease and repair. Moreover, given that the etiology of MS remains enigmatic,<sup>20-23</sup> and infectious agents such as viruses have been considered possible environmental triggering agents,<sup>20,24-27</sup> the application of viral models of demyelination offers unique and important insight into the potential mechanisms that trigger and maintain immune-mediated demyelination. This review will provide a succinct overview of mechanisms associated both with host defense and disease progression in response to MHV infection of the CNS.

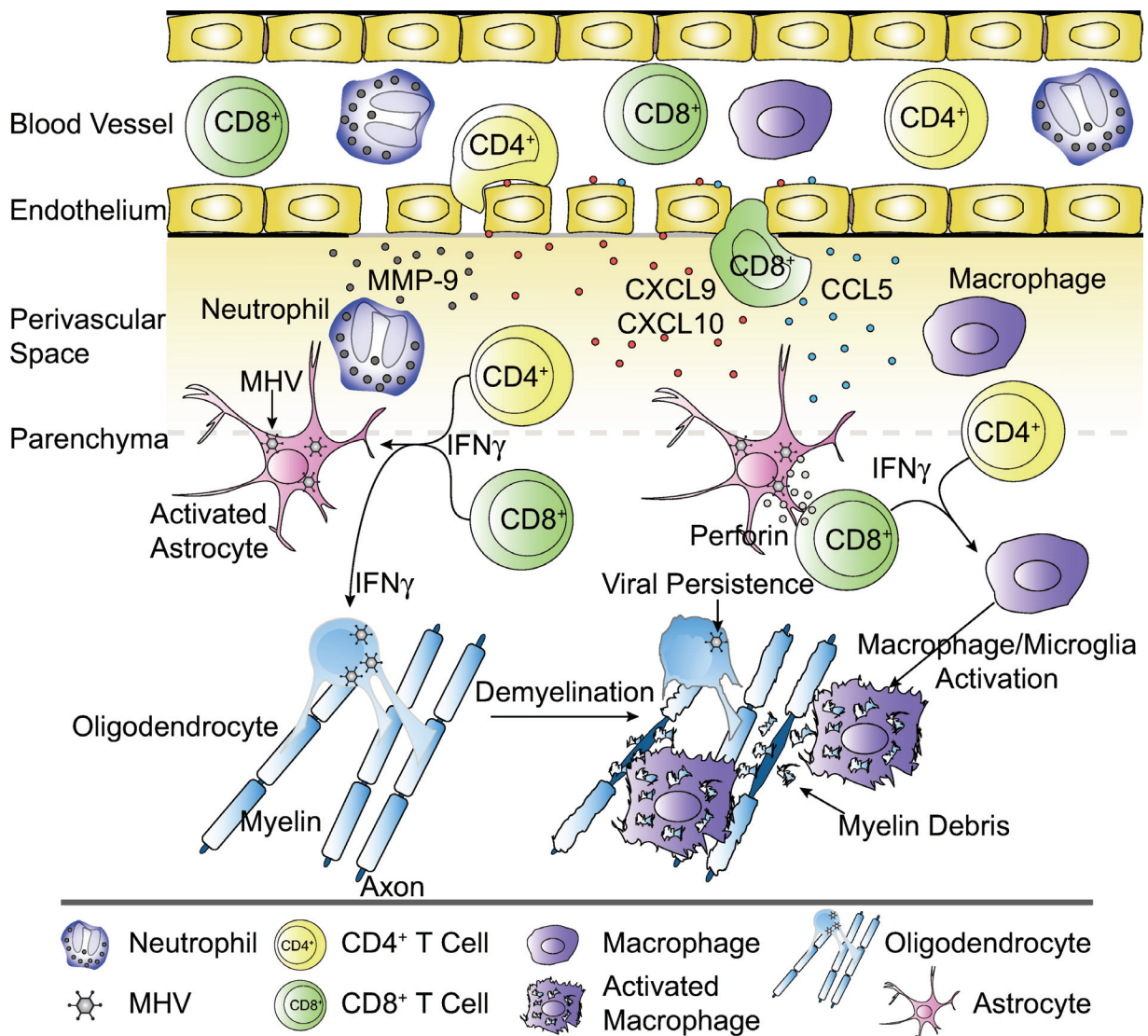
## II. ACUTE MHV-INDUCED ENCEPHALOMYELITIS

Following intracranial infection, MHV replicates first within the ependymal cells of the lateral ventricles before spreading throughout the parenchyma, targeting astrocytes, oligodendrocytes, and microglia<sup>28</sup> (Fig. 1). Neurons are spared within immunocompetent mice inoculated with neuro-attenuated strains of MHV.<sup>29-31</sup> Following infection, MHV also trafficks to the spinal cord, spreading through the cerebral spinal fluid and similarly infecting the local ependyma before disseminating throughout the parenchyma.<sup>28</sup> MHV infection of the CNS manifests significant early up-regulation of inflammatory cytokines, chemokines, and matrix-metalloproteinases (MMPs), all of which serve to initiate, attract, and support a robust host antiviral response.<sup>11-13,32-37</sup>

Type I interferons (IFN- $\alpha$  and IFN- $\beta$ ), IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, and tumor necrosis factor-alpha (TNF $\alpha$ ) are secreted following MHV infection.<sup>12,13,32-34</sup> Protective roles for the type I interferons during MHV infection have been well described. Exogenous treatment of either IFN- $\alpha$  or IFN- $\beta$  limits MHV replication and dissemination within the CNS,<sup>38,39</sup> while mice deficient in IFN- $\alpha/\beta$ -receptor quickly succumb to MHV infection.<sup>40</sup> However, the mechanisms of type I IFN in *in vivo* protection are complicated because MHV is resistant to IFN- $\beta$  treatment *in vitro*.<sup>41</sup> Additionally, evidence suggests that MHV can shield its viral RNA genome from host pattern recognition receptors and therefore prevent IFN- $\beta$  induction.<sup>42,43</sup> Nevertheless, type I IFNs are clearly protective *in vivo*, and may help to regulate innate and adaptive immune responses by enhancing MHC I expression.<sup>44</sup>

Neutrophils, natural killer (NK) cells, and macrophages are the primary innate immune cells recruited into the CNS immediately following MHV infection.<sup>45,46</sup> Neutrophils are detectable within the CNS by day 1 post-infection and peak between days 3 to 5,<sup>45,47</sup> responding to chemotactic signals through the chemokine receptor CXCR2.<sup>47</sup> Neutrophils are primarily responsible for degrading the blood-brain barrier through MMP secretion, facilitating extracellular matrix and basement lamina degradation and subsequent leukocyte migration<sup>48,49</sup> (Fig. 1). Although neutrophils secrete MMP-9,<sup>36,49</sup> they are not the sole source of MMPs within the CNS, as MMP-3 and MMP-12 are derived from resident glia.<sup>35</sup> Nevertheless, neutrophils are indispensable for proper antiviral responses, because their depletion prevents leukocyte entry into the CNS, thus limiting effective control of viral replication and spread.<sup>49</sup>

Similar to neutrophils, NK cells rapidly and transiently infiltrate into the CNS following MHV infection, peaking at day 5 post-infection.<sup>46</sup> Overexpression of viral-derived CXCL10 in immune-deficient mice enhanced NK cell infiltration and reduced viral burden,<sup>50</sup> suggesting that NK cells may contribute to controlling viral replication. However, depletion of NK cells in immune-compromised mice did not enhance viral burden,<sup>51</sup> and, moreover, the absence of NK cells within the CNS of immune-competent mice did not influence viral clearance or pathogenesis,<sup>46</sup>



**FIGURE 1.** MHV pathogenesis. Following intracranial infection of susceptible mice, MHV replicates within astrocytes and oligodendrocytes. Among the earliest immune cells responding to infection, neutrophils are recruited into the CNS and serve to degrade the blood-brain barrier through the release of MMPs, including MMP9, thus facilitating subsequent immune cell entry. Activated astrocytes secrete the T cell and macrophage chemoattractants CXCL9, CXCL10, and CCL5, directing virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells and macrophages into the CNS. Direct cytolytic activity by CD8<sup>+</sup> T cells, mediated through perforin and granzyme B secretion, aids in viral clearance from infected astrocytes. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells also secrete IFN $\gamma$ , activating macrophages and microglia and promoting viral control within infected oligodendrocytes. Viral clearance from oligodendrocytes is incomplete, and MHV antigen and/or RNA persist within oligodendrocytes. Chronic MHV persistence drives continued CXCL10/CCL5 secretion and T-cell/macrophage infiltration within the CNS, leading to the development of immune-mediated demyelination. Activated macrophages/microglia present within the white matter digest myelin debris and serve to further enhance demyelination.

indicating that NK cells probably play little to no role in viral clearance under normal circumstances of MHV infection.

Inflammatory macrophages are first detectable by day 2 post-infection<sup>45</sup> and, unlike the

transitory neutrophils and NK cells, continue to accumulate within the CNS during the course of infection. Macrophage infiltration is dependent upon numerous chemokine-signaling pathways, including CCR2/CCL2,<sup>52,53</sup> CCL3,<sup>54</sup> and CCL5/

CCR5.<sup>55-57</sup> Similar to NK cells, macrophages do not appear to perform any direct antiviral activity within the CNS, because depletion of macrophages or neutralization of CCL5 during acute MHV infection does not enhance viral burden.<sup>57,58</sup>

Both myeloid- (CD11b<sup>+</sup> CD11c<sup>+</sup>) and lymphoid (CD11b<sup>-</sup> CD11c<sup>+</sup>)-derived dendritic cells are detectable within the CNS by day 2 post-infection,<sup>59</sup> although the chemotactic signals controlling their infiltration has not been fully explored. Migration of myeloid dendritic cells to the draining lymph nodes is dependent in part on CCL3 expression.<sup>59</sup> Moreover, CCL3 deficiency reduces lymph node dendritic cell activation and skews T<sub>H</sub>1 anti-MHV responses.<sup>59</sup>

Early following MHV infection, virus-specific T cells are detectable within the local lymph nodes and spleen and subsequently migrate into the CNS<sup>60</sup> (Fig. 1). Protective immunity and antiviral responses conform to a T<sub>H</sub>1 phenotype, which is broadly characterized by vigorous IFN- $\gamma$  secretion and cytolytic activity.<sup>14,15,61</sup> Virus-specific T-cell generation is independent of IL-12 and/or IL-23, as viral clearance is unaffected following antibody neutralization of IL-23 and IL-12/23<sup>62</sup> or genetic deletion of IL-12.<sup>63</sup> T cells isolated from the CNS are CXCR3-reactive,<sup>64</sup> and their migration into the CNS is dependent upon the CXCR3 ligands CXCL9 and CXCL10.<sup>64-67</sup> Neutralization of CCL5 during infection also abrogates CD4<sup>+</sup> and CD8<sup>+</sup> T-cell infiltration<sup>57</sup>; however, CCR5-deficient CD8<sup>+</sup> T cells adoptively transferred into MHV-infected RAG1<sup>-/-</sup> recipients have no problem trafficking into the CNS,<sup>68</sup> while transferred CCR5-deficient CD4<sup>+</sup> T cells do not efficiently enter the CNS.<sup>69</sup> Virus-specific CD8<sup>+</sup> T cells are the main cytolytic effector cell within the CNS and begin to accumulate by 5 days post-infection.<sup>16,60</sup> CD8<sup>+</sup> T cells are essential to controlling MHV replication,<sup>57,61</sup> and their accumulation within the CNS is concurrent with viral clearance from resident glia,<sup>61,70,71</sup> CD8<sup>+</sup> T cells isolated from the CNS are cytolytic *ex vivo*,<sup>71,72</sup> secreting IFN- $\gamma$  and lytic molecules, including granzyme B and perforin.<sup>17</sup> *In vivo*, perforin-mediated cytolysis eliminates MHV from astrocytes and microglia<sup>14</sup> and IFN- $\gamma$  controls MHV replication within oligodendroglia.<sup>15,73</sup> Recent evidence has also demonstrated that NKG2D signaling within the CNS enhances antiviral CD8<sup>+</sup> cytotoxic activity.<sup>72</sup>

Virus-specific CD4<sup>+</sup> T cells function in a supporting role for CD8<sup>+</sup> T cells, and they are also critical in controlling MHV replication.<sup>57,74</sup> *In vivo*, CD4<sup>+</sup> T cells secrete IFN- $\gamma$ , facilitating viral clearance from oligodendroglia,<sup>15,73</sup> up-regulating MHC class II expression on microglia<sup>61</sup> and MHC class I expression on oligodendroglia,<sup>75</sup> and thus enhancing immune cell activity within the CNS.<sup>61,76</sup> CD8<sup>+</sup> cytotoxicity and survival within the CNS is heavily dependent upon the presence of CD4<sup>+</sup> T cells.<sup>77,78</sup> How CD4<sup>+</sup> T cells support and enhance CD8<sup>+</sup> T-cell activity is unknown, however, it is assumed to be a secreted factor, because CD4<sup>+</sup> T cells are spatially restricted near the vasculature, instead of migrating throughout the parenchyma like CD8<sup>+</sup> T cells, possibly as a result of CD4<sup>+</sup> T-cell TIMP-1 expression.<sup>35</sup>

Antibody-secreting cells are detectable within the CNS of MHV infected mice by 5 days post-infection, and neutralizing antibody is detectable within the serum by 10 days post-infection.<sup>79</sup> However, B cells do not participate in viral clearance during acute infection,<sup>80,81</sup> rather, MHV-specific antibodies prevent viral recrudescence in chronically infected mice.<sup>80-82</sup>

### III. CHRONIC MHV-INDUCED DEMYELINATION

After 2 weeks of MHV infection, viral loads within the brain are reduced to below-detectable levels by plaque assay. However, sterile immunity is not achieved, and viral antigen and/or RNA are detectable within oligodendrocytes up to a year post-infection<sup>11,83</sup> (Fig. 1). Mechanisms contributing to viral persistence may include antigenic escape variants<sup>84</sup> and the generation of RNA quasispecies, although with regard to the later, the observed mutations are random and neither indicate specific immune pressure nor aid in escape from CD4<sup>+</sup> or CD8<sup>+</sup> surveillance.<sup>85</sup> More recently, CD8<sup>+</sup> T-cell exhaustion has been proposed to be a mechanism of MHV persistence. During chronic MHV infection, oligodendrocytes prominently express B7-H1 concurrently with infiltrating virus-specific CD8<sup>+</sup> T cells that express PD-1. In the absence of B7-H1, MHV is cleared faster from the CNS, confirming that B7-H1/PD-1 signaling inhibits CD8<sup>+</sup> antiviral activity *in vivo*.<sup>86</sup>



Mice that survive acute MHV infection develop a chronic immune-mediated demyelinating disease. Infected mice first demonstrate signs of ascending demyelination during acute infection, which ranges from limp tails to partial and complete hind limb paralysis. Analysis of the spinal cords of chronically infected mice confirms that the loss of myelin integrity is associated with the continued presence of both viral antigen and inflammatory immune cells<sup>87</sup> and not the apoptotic or necrotic death of myelinating oligodendrocytes.<sup>88</sup> No role for endogenous complement or antibody-mediated demyelination has been documented,<sup>89</sup> although exogenous auto-antibodies can exacerbate demyelination independent of complement during chronic infection.<sup>90</sup> Nevertheless, the immunopathology observed during chronic MHV infection resembles what is observed in the majority of active MS lesions,<sup>10,91</sup> making chronic MHV infection an excellent model to study mechanisms of pathogenesis and potential treatments.

Concomitant with the absence of detectable infectious virus, total immune infiltration into the CNS wanes by 2 weeks post-infection, yet virus-specific T cells and macrophages remain within the CNS for up to 3 months.<sup>16-19</sup> Unlike in other models of CNS demyelination<sup>92-94</sup> and in MS,<sup>95-97</sup> autoreactive T cells to defined myelin epitopes are not considered important in contributing to disease, indicating that chronic demyelination is mainly driven by antiviral responses and not epitope spreading.

While both CD4<sup>+</sup> and CD8<sup>+</sup> T cells remain CXCR3<sup>+</sup> during chronic infection,<sup>64</sup> only CD4<sup>+</sup> T cells appear to rely upon CXCL10 for antiviral trafficking into the CNS; CD8<sup>+</sup> T-cell infiltration remains unaffected during CXCL10 neutralization.<sup>19</sup> Notably, CCL5 neutralization abrogates both CD4<sup>+</sup> and CD8<sup>+</sup> T-cell accumulation during chronic infection,<sup>56</sup> indicating differential chemokine usage between the T-cell subsets.<sup>98</sup>

The main effectors of demyelination during chronic MHV infection are T cells and macrophages (Fig. 1). Both CD4<sup>+</sup> and CD8<sup>+</sup> T cells are important to the pathogenesis of chronic demyelination, although to differing degrees. Mice deficient in adaptive immune systems<sup>57,74,88</sup> or CD4<sup>+</sup> T cells<sup>57</sup> do not readily demyelinate, regardless of their ability to clear virus. Moreover, adoptive transfer of CD4<sup>+</sup> T cells into infected

RAG1-deficient hosts is sufficient to initiate demyelination.<sup>57</sup> CD4<sup>+</sup> T cells also enhance demyelination by attracting macrophages through CCL5 secretion.<sup>57</sup> Although it was reported that CD8<sup>-/-</sup> mice exhibit muted demyelination during chronic MHV infection,<sup>57</sup> IFN- $\gamma$ -dependent demyelination was observed following the transfer of CD8<sup>+</sup> T cells into RAG-1<sup>-/-</sup> mice,<sup>74,99,100</sup> providing evidence that CD8<sup>+</sup> T cells are capable of initiating and potentiating demyelination.

Although the exact mechanisms of demyelination have not been fully characterized, T-lymphocyte-secreted inflammatory cytokines, including IFN- $\gamma$  and TNF- $\alpha$ , persist within the brain<sup>13</sup> and/or spinal cord<sup>19,72</sup> up to 4 weeks post-infection, even though infectious virus is no longer detectable. CD8<sup>+</sup> cytolytic activity is muted during chronic infection, presumably as a result of decreasing viral antigen<sup>17,71</sup>; however, these cells still retain their capacity to secrete IFN- $\gamma$ .<sup>70</sup>

Within chronically MHV infected mice, apoptosis has been observed to be associated with areas of pathological damage.<sup>101</sup> However, no causal link between apoptosis and demyelination has been established, especially since RAG1<sup>-/-</sup> and wild-type mice display similar patterns of apoptosis, while only wild-type mice readily demyelinate.<sup>88</sup> Moreover, demyelination is observed during chronic MHV infection within mice that lack IFN- $\gamma$ R1 upon oligodendroglia, indicating that additional mechanisms for damage in addition to IFN- $\gamma$  certainly exist.

Nevertheless, IFN- $\gamma$  is directly harmful to both oligodendrocytes and oligodendrocyte precursor cells, reducing cell viability and inducing apoptosis, and, in some cases, necrosis.<sup>102-110</sup> IFN- $\gamma$  can also indirectly induce microglia/macrophage secretion of TNF- $\alpha$  and nitric oxide, triggering oligodendrocyte cell death.<sup>111,112</sup> Moreover, IFN- $\gamma$  overexpression during development results in widespread hypomyelination and oligodendrocyte loss,<sup>104,113-115</sup> while IFN- $\gamma$  overexpression abrogates remyelination and recovery during cuprizone-induced demyelination or peak experimental autoimmune encephalomyelitis (EAE) disease.<sup>116</sup> Within active MS lesions, IFN- $\gamma$  is detectable by immunohistochemistry and is associated with oligodendrocyte apoptosis at the leading edges of the lesion.<sup>105</sup> Moreover, IFN- $\gamma$  treatment of MS patients exacerbates disease,<sup>117</sup> whereas IFN- $\gamma$  neutralization reduces disease disability.<sup>118</sup> Interestingly, within

the spinal cords of chronically infected mice that have been treated with neutralizing antibodies for CXCL10, IFN- $\gamma$  mRNA levels are reduced, and this is associated with reduced demyelination and enhanced remyelination.<sup>19</sup>

As with other demyelinating diseases,<sup>119,120</sup> ultrastructural analysis of MHV-induced demyelinating lesions reveal myelin-laden macrophages stripping and engulfing myelin<sup>121</sup> (Fig. 1). During chronic infection, macrophages are spatially associated within demyelinating white matter lesions of the spinal cord and are critical to demyelination. Neutralization of the potent macrophage chemokine CCL5 during chronic infection diminishes macrophage infiltration into the CNS and is associated with reduced demyelination.<sup>56,57</sup> Moreover, genetic silencing of CCR5, the chemokine receptor for CCL5, also prevents widespread demyelination, even in the absence of viral clearance.<sup>55</sup> Adoptive transfer of MHV-immunized splenocytes into RAG1<sup>-/-</sup> recipients resulted in rapid demyelination, and this was associated with the widespread recruitment of activated macrophages to regions of pathology.<sup>88</sup> These observations are consistent with other models of demyelination, including EAE<sup>122,123</sup> and cuprizone-induced demyelination<sup>124</sup>; reactive macrophages have also been described within demyelinating MS plaques.<sup>125</sup>

Although the main effectors of demyelination are certainly T cells and macrophages, this does not preclude the possibility that MHV may directly participate in damage, especially since oligodendrocytes are the main reservoir of MHV during chronic infection.<sup>73,126</sup> In some MS lesions, oligodendrocyte apoptosis has also been observed<sup>127,128</sup>; however, the exact role of apoptosis in MS pathogenesis and pathology is unresolved.<sup>129</sup> *In vitro*, cultured murine oligodendrocytes are susceptible to MHV-induced apoptosis through FAS-spike glycoprotein interactions.<sup>130-133</sup> Moreover, the HIV protein Tat<sup>134</sup> and the JC virus protein agnoprotein<sup>135</sup> also enhance oligodendrocyte apoptosis *in vitro*. However, *in vivo* oligodendrocyte apoptosis during chronic MHV infection is not readily observed, and the presence of viral antigen does not appear to predispose an oligodendrocyte to apoptosis.<sup>88</sup> Therefore, it is likely that protective mechanisms exist during chronic infection that

protect oligodendrocytes from MHV, IFN- $\gamma$ , and other apoptotic inducers.

Endogenous remyelination has been observed within chronic MHV demyelinating lesions.<sup>136-138</sup> Moreover, remyelination and actively proliferating oligodendrocytes have been observed within MS lesions, indicating that repair can occur concurrently with acute or chronic inflammatory events.<sup>139,140</sup> *In vitro*, growth factors and cytokines including IGF (insulin-like growth factor), CNTF (ciliary neurotrophic factor), LIF (leukemia inhibitory factor), NT3 (neurotrophin-3), and PDGF (platelet-derived growth factor) promote oligodendrocyte survival.<sup>141-144</sup> Additionally, the cytokine IL-11, which has been detected on reactive astrocytes within MS lesions<sup>145</sup> and in MHV-infected astrocytes,<sup>146</sup> has recently been demonstrated to enhance oligodendrocyte survival *in vitro*.<sup>145</sup> Studies by Kilpatrick et al.<sup>147-149</sup> have further demonstrated a potent role for LIF in limiting demyelination during EAE by enhancing oligodendrocyte survival *in vivo*. Taken together, these data indicate the complex protective and damaging inflammatory environment that exists within demyelinating lesions.

#### IV. CONCLUSIONS

This review highlights MHV as a model system for viral-induced neurologic disease. Specifically, MHV offers a platform for differentially studying the underlying mechanisms that dictate host defense during acute viral infection and later contribute to demyelination during chronic viral persistence. Notably, the pathology observed during chronic MHV demyelination closely parallels the damage observed in MS patients. Recent documented inconsistencies between EAE and MS,<sup>117,150,151</sup> in which protective treatments in EAE exacerbate or have no effect on MS patients, underscore the necessity for the broader application of diverse demyelinating models that can complement each other and lead to a greater understanding of the fundamental processes that lead to demyelination and the development of MS.

## ACKNOWLEDGMENTS

This work was supported by National Multiple Sclerosis Society grant no. 3278 and National Institutes of Health grant no. NS41249 to T.E.L. M.P.H. was supported by NIH grant no. T32 AI-060573.

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