Analysis of CNS Inflammatory Responses to MHV

Role of spike determinants in initiating chemokine and cytokine responses

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1. INTRODUCTION

Development of demyelination in the MHV system is associated with inflammatory cytokine responses in the brain. It is likely that these responses reflect the influx of T cells into the brain following infection. Previous work has described a central role for CD4+ T cells in viral clearance and subsequent demyelination (Lane, Liu et al. 1999; Lane, Liu et al. 2000; Wu, Dandekar et al. 2000). While CD8+ T cells are necessary for viral clearance, their involvement in demyelination is less defined (Castro, Evans et al. 1994; Stevenson, Belz et al. 1999). Glial cells have also been implicated in the disease process, contributing to cytokine production in the central nervous system (Sun, Grzybicki et al. 1995; Lane, Asensio et al. 1998).

We were interested in examining how the host immune response against an extensively demyelinating virus (MHV-A59) and a highly encephalitic virus (JHM/MHV-4) might contribute to disease outcome. Furthermore, previous studies have emphasized the importance of the spike glycoprotein as a determinant of viral pathogenesis (Phillips, Chua et al. 1999) and as a target for immune responses (Bergmann, Yao et al. 1996). To determine how the JHM spike protein participates in regulating host responses seen upon JHM infection, immune responses against a recombinant virus containing the JHM spike protein in the context of a MHV-A59-like genome (Phillips, Chua et al. 1999) were examined.

The Nidoviruses (Coronaviruses and Arteriviruses).
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2. JHM INFECTION INDUCES IL-1 AND IL-6 MESSENGER RNA EXPRESSION

MHV-A59 and JHM (MHV-4) are neurotropic MHV strains that differ in pathology. Infections with high doses of the less neurovirulent virus MHV-A59 result in acute encephalitis and extensive demyelination (Lavi, Gilden et al. 1986). In contrast, mice infected with a low dose of the highly neurovirulent JHM die within a week from acute encephalitis (Dalziel, Lampert et al. 1986). To investigate how host immune responses induced by these viruses might participate in disease outcome, mice were infected with doses of JHM (10 PFU) and MHV-A59 (1000 PFU) that produced similar degrees of acute encephalitis. Isolation of cells from the brains of mice infected with JHM consistently resulted in recovery of 1.7 fold more cells during acute encephalitis (day 7 post infection) compared to MHV-A59 infected mice. However, CD8 T cells were decreased approximately 3 fold upon JHM infection as compared to MHV-A59 as determined by flow cytometry. CD4 T cell numbers were also reduced but to a lesser degree. This translated into approximately 1.4 percent of the total cells isolated upon JHM infection being T cells; by comparison with MHV-A59 infection about 5.1% of the total cells were T cells. Thus, the increase of total cells isolated from the brains of JHM infected mice did not reflect an overall increase in recruitment of T cells from the periphery.

The disparity in T cell recruitment was not reflected in a difference in RANTES mRNA regulation as determined by RNA protection assay (RPA) analysis (Figure 1). A two-fold increase in MIP-1α, MIP-3 and IP-10 mRNA expression relative to MHV-A59 infection was evident suggesting that JHM infection may result in a greater influx of other peripheral mononuclear cells, such as macrophages. This possibility will be explored by cell surface staining with macrophage markers.

Differential responses seen by RPA analysis of cytokine transcription also implicated non-lymphocyte populations, possibly resident glial cells in participating in disease outcome. JHM and MHV-A59 infection produced similar TNFα, TGFβ1, TGFβ3 transcription (Table 1). This indicated that while these cytokines played a critical role in the immune response against MHV infection, they probably did not contribute to the differences in neurovirulence between JHM and MHV-A59. A striking difference was, however, observed in IFNα/β, IL-6 and IL-1β mRNA message (Table1). JHM infection resulted in strong expression of IFNα/β, IL-6 and IL-1β mRNA's; whereas, MHV-A59 did not. IL-1 and IL-6 are pro-inflammatory cytokines that can act synergistically in the periphery and in the central nervous system. High levels of IL-6 in the brain were shown to contribute to astrogliosis (Campbell, Abraham et al. 1993; Campbell, Stalder et al. 1998). More importantly, TNFα, IL-6 and IL-1β production was previously attributed to glial cells in JHM models of acute encephalitis and demyelination (Sun, Grzybicki et al. 1995).

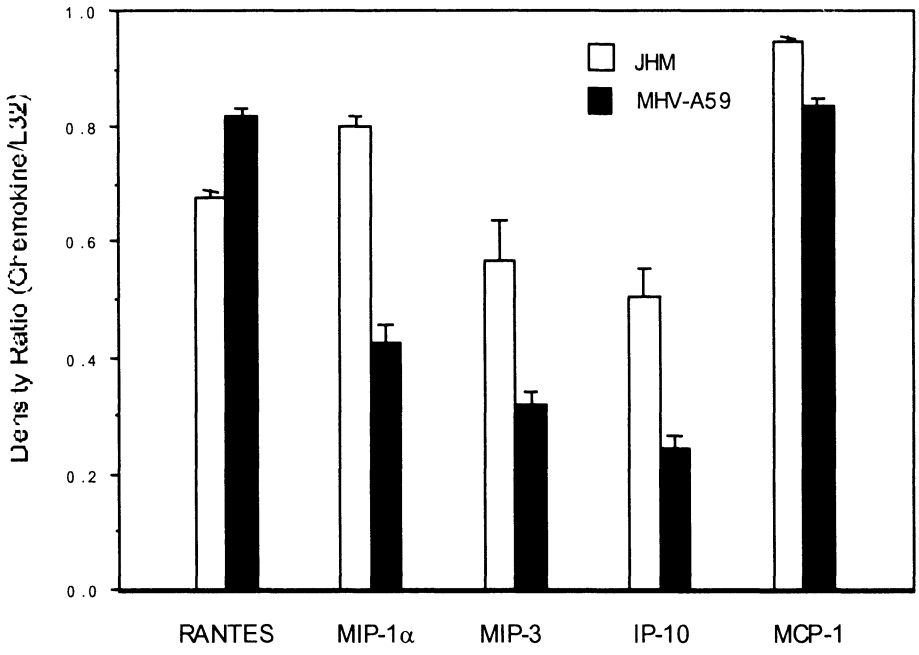

Figure 1. Chemokine mRNA expression following JHM and MHV-A59 infection. Mice were injected intracranially with either 10 PFU of JHM or 1000 PFU of MHV-A59. Brains were harvested on day 7. Total RNA was isolated and analyzed by RNase protection assay as previously described (Stalder and Campbell 1994). Means of 4 mice ± SE are shown.

Table 1. JHM infection induces greater expression of IL-1 and IL-6 mRNA[a].

Virus[b]	LTB	TNFα	IL-1	IL-6	IFNγ	IFNα/β	TGFβ1	TGFβ3
JHM	0.11	0.39	0.40	0.36	0.16	0.49	0.32	0.47
MHV-A59	0.13	0.31	0.15	0.10	0.25	0.11	0.25	0.38

a. Means of 4 mice shown representing band density of cytokine normalized against the corresponding band density of the housekeeping gene. Standard error < ± 0.07.

b. Mice were injected intracranially. Brains were harvested on day 7 and total RNA was isolated.

Astrocytes were also shown to synthesize chemokines after in vitro infection with V5A13.1 (a JHM variant) suggesting that astrocytes play an important role in participating in immunity against MHV infection (Lane, Asensio et al. 1998). As such, they could influence the direction of innate and adaptive immune activity within the brain. Clearly, differences in how viruses affect chemokine and cytokine responses by these cells may be a crucial early event in determining the pathogenesis of infection.

3. CYTOKINE PRODUCTION MAY BE REGULATED BY SPIKE DETERMINANTS

It is known that specific viral proteins can directly affect immune responses and pathology (Yoshimoto, Rosenfeld et al. 1991; Hoshino, Jones et al. 1998). The MHV spike glycoprotein has been established as an important target for adaptive immune responses. It contains neutralizing antibody and T cell epitopes, some of which are shared between JHM and MHV-A59 (Castro, Evans et al. 1994; Bergmann, Yao et al. 1996; Xue and Perlman 1997). However, differing spike determinants appear critical to the variation in disease outcome seen between JHM and MHV-A59. Using MHV-A59 variant chimeric viruses that differed only in their spike protein, the variation in the severity of acute encephalitis between JHM and MHV-A59 was attributed to differences in this glycoprotein (Phillips, Chua et al. 1999). Specifically, the S4R22 chimera with a MHV-A59-like background and the JHM spike protein displayed a neurovirulence more reflective of JHM than MHV-A59.

Infection with S4R22 produced an increased number of total cells and T cells as compared to JHM or MHV-A59 infections, most likely reflecting coordinated MHV-A59 background and JHM spike activity. While the significance has not been established definitively, it was interesting that S4R22 infection resulted in a similar percentage of infiltrating T cells as MHV-A59. Preliminary evaluation of the chemokine responses elicited by S4R22 infection did not reveal any marked differences (Table 2). Examination of cytokine responses also indicated similar TNFα, TGFβ1, TGFβ3 transcription. However, in this study S4R22 infection resulted in IL-6 and IFNα/β mRNA responses similar to those seen in MHV-A59 infection suggesting that the background, non-spike, genes of these viruses may participate in the induction of these cytokines. This is not surprising in light of the presence of known CD4 T cell epitopes on the M protein and potential for others within the large remaining uncharacterized genome. In contrast, IL-1 expression from these animals reflected that of JHM, perhaps indicating that spike protein interactions can trigger this cytokine. The presence of IL-1 may be significant in that it has been suggested to alter the antigen presenting capacity of astrocytes (Williams, Dooley et al. 1995). The possibility that the neurovirulence of JHM may be in part due to astrocyte responses against its spike and non-spike genes is currently being investigated.

Table 2. Cytokine and chemokine mRNA expression following S4R22 infection[a, b].

Chemokine	RANTES	MIP-1α	MIP-3	IP-10	MCP-1
	0.83	0.54	0.43	0.28	0.98
Cytokine	TNFα	IL-1	IL-6	IFNα/β	TGFβ1
	0.38	0.36	0.13	0.14	0.37

a. Means of 2 mice shown representing band density of virus normalized against the corresponding band density of the housekeeping gene. Standard error $< \pm 0.07$.
b. Mice were injected intracranially. Brains were harvested on day 7 and total RNA was isolated.

4. CONCLUSION

Our studies with the virulent JHM, the mildly neurovirulent MHV-A59 and the recombinant S4R22 (containing the JHM spike gene on a MHV-A59-like background) suggested that differential chemokine and cytokine responses to infection may participate in the disease outcome. Most prominently, IL-1 and IL-6 up regulation was associated with high neurovirulence. We are currently investigating how early sentinel responses of glial cells to these viruses might impact IL-6 and IL-1 production and alter subsequent disease.

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