Analysis of CNS Inflammatory Responses to MHV
Role of spike determinants in initiating chemokine and cytokine responses

JULIA D. REMPEL AND MICHAEL J. BUCHMEIER
The Scripps Research Institute, La Jolla, CA.

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.
JHM INFECTION INDUCES IL-1 AND IL-6Messenger 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).