The CXC Chemokines IP-10 and Mig are Essential in Host Defense Following Infection with a Neurotropic Coronavirus

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1. INTRODUCTION

Chemokines represent an ever-growing family of secreted proteins that function as potent mediators of inflammation (for review, see Luster, 1998). These molecules have been classified depending on the number and spacing of the first two conserved amino terminal cysteine residues into the C, CC, CXC, and CX_3C family. Studies have shown that chemokines target specific leukocyte populations during periods of inflammation (Luster, 1998; Lane et al., 2000; Biddison et al., 1998; Kolb et al., 1999). In addition, chemokines have been shown to be prominently expressed following viral infection of the CNS (Lane et al., 1998; Cheret et al., 1997, Asensio and Campbell, 1997; Hoffman et al., 1999). However, the functional significance of chemokine expression within this environment has not been fully defined.

1.1 The MHV model of CNS disease

Mouse hepatitis virus (MHV) is a positive strand RNA virus that is a member of the *Cornaviridae* family. Infection of susceptible strains of mice with MHV results in an acute encephalomyelitis followed by chronic

The Nidoviruses (Coronaviruses and Arteriviruses).
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neurological disease. The acute stage is characterized by wide spread viral infection of neurons and glial cells (Houtman and Fleming, 1996; Buchmeier and Lane, 1999). Inflammatory $CD4^+$ and $CD8^+$ T cells as well as the antiviral cytokine IFN-γ are essential to host defense against MHV-induced neurological disease (Lane et al., 1997, 2000; Parra et al., 1999; Hoffman et al., 1999).

1.2 The CXC chemokines IP-10 and Mig

Intracranial infection of mice with MHV results in a dramatic increase in chemokine expression within the CNS (Lane et al., 1998). The present study focuses on the chemokines interferon inducible protein-10 (IP-10) and monokine induced by interferon gamma (Mig) which are prominently expressed during the acute stage of disease (Lane et al., 1998). IP-10 and Mig are non-ELR CXC chemokines (chemokines lacking the glutamic acid-leucine-arginine motif in the amino terminus) which have been shown to have a chemotactic effect on T cells by binding to the chemokine receptor CXCR3 (Biddison et al., 1998; Loetsher et al., 1996; Farber, 1997; Piali et al., 1998). The present study was undertaken to investigate the contribution of IP-10 and Mig in host defense against MHV infection of the CNS.

2. IP-10 AND MIG IN HOST DEFENSE

IP-10 and Mig were selectively inhibited through intraperitoneal administration of rabbit polyclonal anti-IP-10 or anti-Mig antisera to MHV-infected mice. Control mice were treated with normal rabbit serum (NRS). Approximately 50% of control animals survived up to 12 days post infection and successfully cleared infectious virus (2.1 ± 0.2 $\log_{10}$ pfu/g tissue n=14). In contrast, mice treated with either anti-IP-10 or anti-Mig showed a decreased ability to clear virus (anti-IP-10: 5.7 ± 0.3 $\log_{10}$ pfu/g tissue n=15, anti-Mig: 5.4 ± -0.3 $\log_{10}$ pfu/g tissue n=4) which corresponded to an increase in mortality (Figure 1). These data indicate that both IP-10 and Mig play an essential role in host defense by contributing to viral clearance.

Previous studies have shown that both $CD4^+$ and $CD8^+$ T cells are essential for optimal host defense following viral infection (2). Mice treated with either anti-IP-10 or anti-Mig displayed a significant reduction in both $CD4^+$ and $CD8^+$ T cell infiltration when compared to NRS treated control animals (Figure 2) indicating that both IP-10 and Mig function in host defense through attraction of T cells which participate in viral clearance.

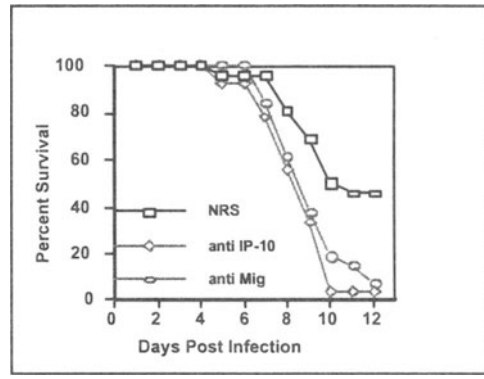

Figure 1. MHV infected mice treated with either anti-IP-10 or anti-Mig display increased mortality when compared to infected mice treated with NRS.

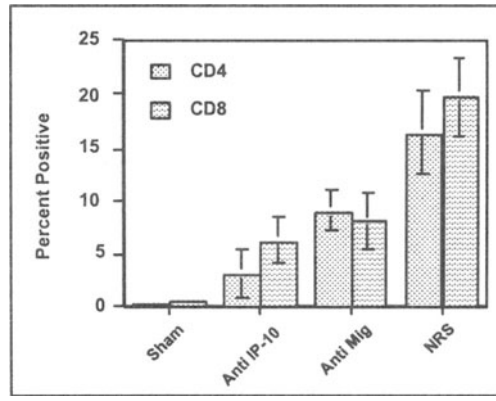

Figure 2. Mice treated with either anti-IP-10 or anti-Mig have decreased levels of CD4 and CD8 infiltration into the CNS as compared to NRS treated control animals. (82.4% and 70% for anti-IP-10 mice $P \leq 0.001$ and 45.4% and 60.2% for anti-Mig $P \leq 0.005$).

One mechanism by which T cells contribute to viral clearance is through release of the anti-viral cytokine IFN-γ (11,12). Examination of IFN-γ transcript levels in the CNS by ribonuclease protection assay revealed that anti-IP-10 and anti-Mig treated mice displayed a significant decrease ($P<0.05$) (Data not shown) in transcript levels as compared to levels present in NRS control mice. Corresponding with the decrease in IFN-γ transcript levels was a significant decrease ($P \leq 0.05$) of IFN-γ protein levels in anti-IP-10 and anti-Mig treated mice (Figure 3).

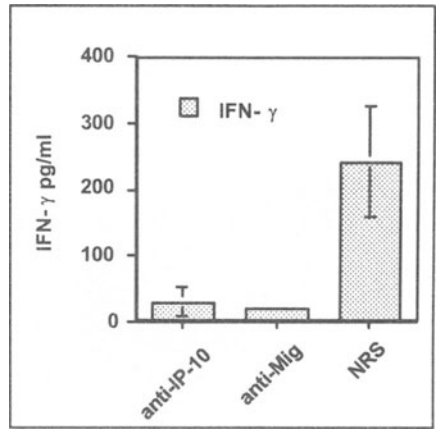

Figure 3. IFN-γ protein levels are significantly decreased in anti-IP-10 and anti-Mig treated mice. ($P<0.05$)

3. CONCLUSION

Neutralization of IP-10 and Mig activity results in i) increased mortality, ii) delayed viral clearance, and iii) inhibition of a protective Th1 response characterized by infiltrating T cells and IFN-γ expression. Collectively, these data indicate that both IP-10 and Mig are essential in contributing to host defense following MHV infection of the CNS. Moreover, IP-10 has been demonstrated to be expressed within the CNS following infection with other viruses such as lymphocytic choriomeningitis virus (LCMV) and Theiler's virus suggesting that IP-10 exerts a protective effect in these models (Hoffman et al., 1999; Theil et al., 2000).

In addition to contributing to host defense during the acute stage of MHV infection, IP-10 is expressed during the chronic stage of disease almost exclusively within areas undergoing demyelination (Lane et al., 1998). A recent study has implicated a role for $CD4^+$ T cells in driving demyelination in persistently infected mice (Lane et al., 2000). Taken together, this suggests that persistent viral infection results in chronic IP-10 expression which serves to attract $CD4^+$ T cells to sites of MHV persistence. $CD4^+$ T cells contribute to demyelination by attracting macrophages which ultimately results in myelin destruction. Therefore, regulation of T cell entry into the CNS by targeting molecules such as IP-10 and Mig may represent promising targets for therapeutic interventions of human CNS inflammatory diseases.

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