Cutting Edge: The T Cell Chemoattractant IFN-Inducible Protein 10 Is Essential in Host Defense Against Viral-Induced Neurologic Disease

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The contribution of the T cell chemoattractant chemokine IFN-inducible protein 10 (IP-10) in host defense following viral infection of the CNS was examined. IP-10 is expressed by astrocytes during acute encephalomyelitis in mouse hepatitis virus-infected mice, and the majority of T lymphocytes infiltrating into the CNS expressed the IP-10 receptor CXCR3. Treatment of mice with anti-IP-10 antiserum led to increased mortality and delayed viral clearance from the CNS as compared with control mice. Further, administration of anti-IP-10 led to a >70% reduction (p < 0.001) in CD4+ and CD8+ T lymphocyte infiltration into the CNS, which correlated with decreased (p < 0.01) levels of IFN-γ. These data indicate that IP-10 functions as a sentinel molecule in host defense and is essential in the development of a protective Th1 response against viral infection of the CNS. The Journal of Immunology, 2000, 165: 2327–2330.

Chemoattractant IFN-Inducible Protein 10 Is Essential in Host Defense Against Viral-Induced Neurologic Disease

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Materials and Methods

Virus and mice

The MHV strain V5A13.1 (referred to henceforth as MHV) was derived from wild-type MHV-4 as previously described (39). Age matched (5–7 wk), male wild-type C57BL/6 mice (H-2b background) were used for studies described. Mice were purchased from Harlan Sprague-Dawley Laboratories (San Diego, CA). Mice were injected intracranially with 10 PFU MHV suspended in 30 μl sterile saline (2). Control (sham) animals were injected with sterile saline alone. Animals were sacrificed at days 7 and 10 postinfection (p.i.), at which point brains and spinal cords were removed. One-half of each brain was used for plaque assay on the DBT astrocytoma cell line to determine viral burden (2). The remaining half of each brain was used for either RNA isolation, FACs analysis, or ELISA.

Ab preparation and treatment of mice

The generation of rabbit polyclonal antisera specific for mouse IP-10 has previously been described (28). This reagent has previously been shown to be specific for IP-10 and does not cross-react with other known chemokines (28). MHV-infected mice were divided into two groups and treated with either normal rabbit serum (NRS) or anti-IP-10. Mice were injected i.p. with 0.5 ml anti-IP-10 antisera or NRS on days 0, 2, 5, 7, and 9 p.i. and sacrificed at days 7 and 10 p.i.

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3 Abbreviations used in this paper: IP-10, IFN-inducible protein 10; NRS, normal rabbit serum; RPA, ribonuclease protection assay; p.i., postinfection; MHV, mouse hepatitis virus.

C hemokines have been the subject of numerous studies to define the functional contributions of these molecules in inflammation (for review, see Ref. 1). Growing evidence indicates that chemokine expression represents a pivotal point in host defense by initiating specific inflammatory events that lead to leukocyte activation, extravasation, migration, and ultimately clearance of foreign Ag (2–4). IFN-inducible protein 10 (IP-10) is a major ELR (glutamic acid-leucine-arginine) CXC chemokine that has been shown to be a potent chemoattractant for activated T cells and NK cells by binding to the receptor CXCR3 (5–8). Both human and mouse IP-10 expression is inducible by type I and II IFNs following treatment of a wide variety of cell types (9–18). Functionally, IP-10 is thought to contribute to various inflammatory pathologies by attracting leukocytes to sites of infection or injury (19–27). In addition, IP-10 exhibits antitumor properties by inhibiting angiogenesis and has recently been shown to exhibit an antiviral role (4, 28, 29).

IP-10 is expressed early within the CNS in response to infection with a wide variety of viruses (30–37). Expression often represents a dominant and localized response, suggesting that IP-10 acts as a sentinel molecule in host defense by serving to initiate and maintain an inflammatory response (38). However, the contributions of IP-10 in response to viral infection of the CNS have not been fully evaluated. To assess functional significance, IP-10 activity was selectively neutralized by administration of anti-IP-10 antisera to mice infected with the neurotropic coronavirus mouse hepatitis virus (MHV). The results presented indicate that IP-10 is an essential component in host defense by coordinating the trafficking of Th1 T lymphocytes into the CNS in response to viral infection.

Confocal microscopy

Primary Abs (diluted in PBS containing 5% normal horse serum) used for dual fluorescent detection of cellular Ags were as follows: rat anti-mouse CD4 (PharMingen, San Diego, CA) at 1:100, rat anti-mouse CD8 (PharMingen) at 1:50, and goat anti-mouse CXCR3 (Santa Cruz Biotechnology, Santa Cruz, CA) at 1:50. For CD4 and CD8 primary Abs, a TRITC-conjugated secondary Ab was used (1:50; Sigma, St. Louis, MO). For CXCR3 primary Ab, a FITC-conjugated secondary Ab was used (1:50; Zymed, South San Francisco, CA). Staining was performed on 8-μm frozen sections fixed in acetone for 10 min at −20°C. Dual-stained slides were then subjected to confocal microscopy using a Bio-Rad MRC UV laser-scanning confocal microscope (Bio-Rad, Richmond, CA).

T cell isolation and flow cytometry

Cells were obtained from brains of anti-IP-10- or NRS-treated MHV-infected mice at 7 days p.i. using a previously described protocol (2). FITC-conjugated rat anti-mouse CD4 and CD8 were used to detect infiltrating CD4+ and CD8+ T cells (PharMingen). As a control, an isotype-matched FITC-conjugated Ab was used. Cells were incubated with Abs for 30 min at 4°C, washed, fixed in 1% paraformaldehyde, and analyzed on a FACStar (Becton Dickinson, Mountain View, CA).

Ribonuclease protection assay (RPA)

Total RNA was extracted from the brains of NRS- or anti-IP-10-treated mice at day 7 p.i. using TRIzol reagent (2, 37). Cytokine transcripts were analyzed using a multitemplate probe set (mCK3; PharMingen). RPA analysis was performed using 15 μg of total RNA using a previously described protocol (2, 37).

IFN-γ ELISA

IFN-γ levels were quantified using the Quantikine M mouse IFN-γ immunoassay kit (R&D Systems, Minneapolis, MN) using brains of mice obtained at day 7 p.i. using previously described protocols (2).

Statistical analysis

All data were analyzed by performing the Mann-Whitney Rank Sum test using Sigma Stat 2.0 software. Values of p ≤ 0.05 were considered significant.

Results and Discussion

To determine the functional significance of IP-10 expression following viral infection of the CNS, mice were infected with a neurotropic strain of MHV, a positive-strand RNA virus. Intracranial infection of mice with MHV results in an acute encephalomyelitis followed by chronic neurologic disease in susceptible strains of mice (40, 41). The acute stage of disease is represented by widespread viral infection of neurons and glial cells, whereas the chronic stage is characterized by viral persistence in astrocytes and oligodendrocytes accompanied by mononuclear cell infiltration and myelin destruction (40, 41). IP-10 is expressed very early (day 1 p.i.) within the CNS following MHV infection and remains the predominant chemokine expressed during the acute phase of disease (37). IP-10 activity was selectively inhibited through i.p. administration of rabbit polyclonal anti-IP-10 antisera. Such treatment led to an increase in mortality with <5% of anti-IP-10-treated mice surviving until day 12 p.i. (Fig. 1). In marked contrast, ~50% of NRS-treated control mice survived MHV infection (Fig. 1). Correlating with increased mortality was a pronounced decrease in the ability of anti-IP-10-treated mice to clear virus from the CNS as compared with NRS-treated mice. Surviving anti-IP-10-treated mice displayed a 2-log increase in viral titers in the brain as compared with titers present in NRS-treated mice at day 10 p.i. (Table 1).

Numerous studies have shown that CD4+ and CD8+ T cells are important in clearing MHV from the CNS (2, 42–44). Therefore, IP-10 expression may be important in host defense by attracting T lymphocytes into the CNS in response to viral infection. In support of this is the demonstration that the majority of infiltrating CD4+ and CD8+ T lymphocytes express the IP-10 receptor, CXCR3. CD4+ and CD8+ T lymphocytes expressing CXCR3 were present within the meninges as well as the parenchyma, indicating these cells were able to migrate into the brain (Fig. 2A). Flow cytometric analysis revealed that anti-IP-10 treatment of MHV-infected mice resulted in a significant decrease (p ≤ 0.001) in both CD4+ (82.3% decrease) and CD8+ (70.4% decrease) infiltration as compared with infected mice treated with NRS (Fig. 2B). Both anti-IP-10- and NRS-treated mice displayed comparable levels of monocyte/macrophage infiltration, suggesting that IP-10 does not attract these cells into the CNS following viral infection (data not shown).

One potential mechanism by which infiltrating T lymphocytes contribute in host defense against MHV infection of the CNS is through the release of the antiviral cytokine IFN-γ (42, 45, 46). To determine whether the decrease in T lymphocyte infiltration observed in anti-IP-10-treated mice correlated with decreased IFN-γ expression, IFN-γ mRNA and protein levels within the brains of anti-IP-10- and NRS-treated mice were determined by RPA and ELISA, respectively. The data shown in Fig. 3A indicates that neutralization of IP-10 resulted in decreased (p ≤ 0.05) mRNA transcripts for IFN-γ as compared with transcript levels present within the brains of NRS-treated mice. Correlating with the decrease in IFN-γ mRNA transcript levels was an ~80% decrease in IFN-γ protein levels (NRS, 386 ± 56.7 pg/ml, n = 5; anti-IP-10, 72 ± 51 pg/ml, n = 5; p ≤ 0.01) at day 7 as compared with levels found in control mice treated with NRS (Fig. 3B). Although the levels of IFN-γ mRNA transcripts in anti-IP-10-treated mice were slightly higher than would be predicted based on the IFN-γ ELISA data, this is most likely due to mouse-to-mouse variation and sensitivity in the RPA and not the result of IP-10 modulating IFN-γ mRNA translation.

The data presented in this report have demonstrated that early and prominent expression of IP-10 within the CNS following MHV infection is important in initiating and maintaining a protective Th1 immune response characterized by high-level

![Figure 1. Increased mortality in anti-IP-10-treated mice. Mice were infected intracranially with 10 PFU MHV and treated i.p. with either anti-IP-10 or NRS. By 12 days p.i., ~50% of NRS-treated mice survived the infection, whereas <5% of anti-IP-10-treated mice survived. Anti-IP-10, n = 27; NRS, n = 27.](http://www.jimmunol.org/)

**Table I. Delayed viral clearance from the CNS in anti-IP-10-treated mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day p.i.</th>
<th>n</th>
<th>Titer (PFU/g tissue, log_{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRS</td>
<td>7</td>
<td>3</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>Anti-IP-10</td>
<td>10</td>
<td>12</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Anti-IP-10</td>
<td>7</td>
<td>4</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>Anti-IP-10</td>
<td>10</td>
<td>15</td>
<td>4.3 ± 0.8</td>
</tr>
</tbody>
</table>

*No. of mice examined. Data presented represents three independent experiments.
production of the antiviral cytokine IFN-\(\gamma\). IP-10 is prominently expressed within the CNS of mice following infection with other viruses such as lymphocytic choriomeningitis virus (34) and Theiler’s virus (35, 36). Therefore, based upon the data presented in this manuscript, it is not unreasonable to suggest that IP-10 may play a similar role in host defense by promoting T cell infiltration into the CNS following viral infection. However, no data on the role of IP-10 in host defense within these models or others is available. Similar to IP-10, the non-ELR CXC chemokine Mig (monokine induced by IFN-\(\gamma\)) is induced by IFN-\(\gamma\) and has been shown to exert a chemotactic effect upon T lymphocytes by binding to CXCR3 (6). Studies are currently in progress to evaluate the contributions of Mig to T lymphocyte infiltration into the CNS following MHV infection.

In addition to being expressed during the acute stage of MHV infection, IP-10 is expressed during chronic stages of disease almost exclusively within areas of viral persistence undergoing de-myelination (37). A recent study has demonstrated that CD4\(^+\) T lymphocytes are essential in driving de-myelination in mice persistently infected with MHV (2). Collectively, these observations indicate that early expression of IP-10 is beneficial through attracting Th\(^1\) T lymphocytes into the CNS that participate in viral clearance. However, chronic expression of IP-10 may ultimately be detrimental by recruiting CD4\(^+\) T cells to sites of MHV persistence, which then contribute to demyelination through the release of additional chemokines such as RANTES (2). Indeed, treatment of MHV-infected mice with anti-RANTES antisera results in a significant decrease in the severity of de-myelination by reducing macrophage infiltration (2). The data presented within this study also indicates that targeting IP-10 may offer a unique target for interventional therapies for the treatment of neuroinflammatory disorders in which IP-10 is expressed and considered to contribute to neurologic disease such as multiple sclerosis (47, 48).
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References


