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Distinct roles for IP-10/CXCL10 in three animal models, Theiler's virus infection, EAE, and MHV infection, for multiple sclerosis: implication of differing roles for IP-10

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Theiler's murine encephalomyelitis virus (TMEV) causes demyelination with inflammation of the central nervous system (CNS) in mice and is used as an animal model for multiple sclerosis (MS). Interferon-γ inducible protein-10 kDa (IP-10) is a CXC chemokine and a chemoattractant for CXCR3$^+$ T cells. IP-10 mRNA is expressed in the CNS during TMEV infection. However, administration of anti-IP-10 serum caused no difference in clinical signs, inflammation, demyelination, virus persistence or anti-virus antibody response in TMEV infection, while levels of virus specific and autoreactive lymphoproliferation increased. This likely reflects a difference in the pathogenesis of TMEV infection from that of two other animal models for MS, mouse hepatitis virus infection and experimental allergic encephalomyelitis (EAE), where blocking of IP-10 resulted in clinical and histological improvement with suppression of antigen specific lymphoproliferation. In this review, we compare and contrast the roles of IP-10 between the three animal models for MS, and discuss the relevance to MS patients with different clinical courses.
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Introduction

Chemokines and chemokine receptors: differential chemokine receptor expression between Th1 and Th2 cells

Chemokines, chemotactic cytokines, are a group of small ($\sim$8–14 kDa) structurally related molecules that selectively attract leukocyte subsets into tissues; some chemokines act specifically toward neutrophils and others toward monocytes or T cells.[1] Chemokines are subdivided into four groups depending on the positions of conserved cysteine residues within the protein. The groupings are termed the CXC (α-chemokine), CC (β-chemokine), C (γ-chemokine), and CX_3C (δ-chemokine) chemokines.[1] Functionally, chemokines can be divided into two categories. Inflammatory chemokines are induced or strongly upregulated in peripheral tissues by inflammation, while immune (system) chemokines or constitutive chemokines fulfil housekeeping functions and may be involved in constitutive leukocyte trafficking.[2] Chemokines bind to proteoglycans on the surface of endothelial cells of blood vessels. Leukocytes interact with these chemokines through receptors; this triggers integrin activation, leading to firm adhesion.[3,4] Chemokines produced by a variety of tissue cells drive leukocyte migration through tissue to the target microenvironment.

Selected chemokine receptors are expressed predominantly on leukocyte cell types, including Th1 and Th2 cells. In humans, Th1 cells have been demonstrated to express CXCR3 (CD183, receptor for IP-10) and CCR5 (CD195, receptor for MIP-1α, MIP-1β, and RANTES), whereas Th2 cells express CCR3, CCR4 and CCR8. Although the differential expression of chemokine receptors on Th subsets has also been suggested for mice,[5,6] it is not well defined compared with those of human subsets.[2,7]

Theiler's murine encephalomyelitis virus infection: three distinct CNS diseases

Theiler's murine encephalomyelitis virus (TMEV) belongs to the family *Picornaviridae*, the genus *Cardiovirus*. TMEV subgroups have been demonstrated to cause biologically distinct clinical diseases in mice.[8,9] The TO subgroup of TMEV, including Daniels (DA) and BeAn viruses, causes a biphasic disease in mice.[10] During the acute phase, one week after infection, virus infects neurons in the gray matter of the brain and causes an intense mononuclear cell (MNC) infiltration (acute polioencephalomyelitis). Although inflammation in the gray matter subsides about two weeks after infection in susceptible mouse strains, such as SJL/J mice, the mice remain persistently infected. Infected mice usually develop a spastic paralysis one month after infection

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(chronic phase). During the chronic phase, virus infects glial cells and macrophages in the white matter of the spinal cord. Neuropathology during the chronic phase of TMEV infection is similar to that of multiple sclerosis (MS) where demyelination is accompanied by perivascular and meningeal inflammation composed of $CD4^+$ and $CD8^+$ T cells and macrophages. In contrast to DA or BeAn viruses, GDVII virus (GDVII subgroup) is a highly neurovirulent strain and causes an acute fatal polioencephalomyelitis, in which active viral replication in neurons is accompanied by neuronal apoptosis and microglial proliferation with minimal T cell infiltration.[11,12] H101 virus is a variant of DA virus and causes pachymeningitis, which leads to communicating hydrocephalus in surviving mice.[13] H101 virus can neither infect neuronal cells nor cause MNC infiltration in the central nervous system (CNS) parenchyma; infiltrates are restricted to meninges. Although we do not know the precise mechanisms in which activated T cells are recruited and remain in the CNS in TMEV infection, the role of chemokines has recently been investigated.

Chemokine expression is not necessarily associated with TMEV-induced disease

Hoffman *et al.*[14] first demonstrated chemokine expression in the CNS of susceptible SJL/J mice infected with the BeAn strain of TMEV. They showed that MIP-1α, MIP-1β, RANTES, MCP-1, C10 and IP-10 mRNAs were specifically expressed in the spinal cord, but not in the spleen or lymph nodes of infected mice. In addition, Murray *et al.*[15] demonstrated that IP-10, RANTES and MCP-1 mRNA were expressed predominantly in the brains in both resistant B10.M and susceptible B10 mice, five days after DA virus infection (acute phase). All chemokine expression subsided by day 21, but was re-expressed on day 45 (chronic phase) in only the spinal cord of susceptible mice. These studies suggest that there is coordinated regulation and regionally restricted expression of chemokines in the biphasic disease induced by TO subgroup viruses of TMEV.

On the other hand, Ransohoff *et al.*[16] compared the levels of mRNAs encoding chemokines MCP-1, RANTES and IP-10 in susceptible PL/J and resistant C57BL/6 mice that possessed or lacked either $CD4^+$ or $CD8^+$ cells ($CD4^{-/-}$ or $CD8^{-/-}$ mice) during the chronic phase of DA virus infection. In this study, chemokine expression did not correlate with a susceptible or resistant genotype, demyelination or $CD4^+$ and $CD8^+$ T cell infiltration. Therefore, neither $CD4^+$ nor $CD8^+$ T cells appeared to regulate the expression of these chemokines. Here, the authors suggested a fundamental difference with regard to chemokine induction between TMEV infection and experimental allergic encephalomyelitis (EAE), an experimental autoimmune animal model for MS. In EAE, the cytokine products of leukocytes could regulate chemokine expression. In TMEV infection, chemokine expression might be independent of the adaptive immune response; chemokine expression could be driven by innate CNS immune responses to infection, including those of astrocytes and microglia. In support of the idea that the astrocytes, rather than T cells, are potential sources of chemokines during TMEV infection, one study found that *in vitro* treatment of astrocytes, oligodendrocytes and microglia with either live or UV-inactivated TMEV led to the expression of mRNAs for several chemokines including IP-10.[17]

Previously, we compared cytokine and chemokine mRNA expression between three distinct TMEV infections: DA virus, GDVII virus and H101 virus infections.[18] Using an RNase protection assay (RPA), we detected the same pattern of chemokine (RANTES, MCP-1, IP-10, MIP-1α, MIP-1β and MIP-2) mRNA expression in the CNS during all three infections. Since the difference in the number and distribution of MNC infiltrates is a main histological feature distinguishing the three TMEV infections, similar chemokine patterns suggest that resident cells rather than MNC infiltrate produce chemokines. In addition, chemokine expression was identical during the acute and chronic phases of DA virus infection. Therefore, chemokine expression appears not to be a main determinant driving disease progression in DA virus infection. This conclusion is concordant with those reported by Ransohoff *et al.*[16]

Role of IP-10/CXCL10 in MS and TMEV infection

Upregulation of IP-10 and its receptor CXCR3 in MS

Among the chemokines that were detected in TMEV infection, IP-10 is of particular interest. IP-10, interferon (IFN)-γ inducible protein 10 kDa/CXC chemokine ligand (CXCL) 10/cytokine responsive gene 2 (Crg-2), is a non-ELR (glutamic acid–leucine–arginine) CXC chemokine. IP-10 is inducible by IFN-α/β and IFN-γ and is a potent chemoattractant for activated T cells and NK cells by binding to the CXCR3 receptor. CXCR3 has been shown to be differentially expressed on lymphocytes and is expressed at much higher levels on Th1 cells than on Th2 cells.[5,19]

The role of IP-10 has been investigated in MS. Within demyelinating lesions[20] or in the surrounding parenchyma,[21] IP-10 has been detected in astrocytes, which were identified by immunohistochemistry with glial fibrillary acidic protein (GFAP)[22] or morphologically.[23] Macrophages and T cells are also potential sources of IP-10. Simpson *et al.*[21] reported that macrophages expressed IP-10 within plaques. *In vitro*, IP-10 was expressed by $CD14^+$ monocytes after stimulation, but not by unstimulated monocytes, while no significant difference in the percentage of $IP\text{-}10^+$ monocytes was seen between controls and MS patients.[24] Myelin specific $CD8^+$ T cell lines derived from MS patients were shown to synthesize IP-10 and chemoattract myelin specific $CD4^+$ T cell lines.[25]

A significant increase in IP-10 levels was also detected in the cerebrospinal fluid (CSF).[20,26,27] Franciotta *et al.*[28] demonstrated that serum and CSF IP-10 levels were significantly higher in acute MS patients versus patients with stable MS. However, IP-10 levels did not correlate with clinical signs. In addition, neither methylprednisolone nor IFN-β1a therapy altered IP-10 levels. Sørensen *et*

al.[26] also showed that the CSF concentration of IP-10 did not change after methylprednisolone treatment, which suppressed both clinical and magnetic resonance imaging (MRI) measures of disease activity.

Expression of the receptor for IP-10, CXCR3, has also been demonstrated in the CNS of MS patients. $CXCR3^+$ cells were found to comprise 5–25% of perivascular MNCs in MS lesions,[20] and $CXCR3^+$ cells were closely related to IP-10 expression.[22] Balashov *et al.*[23] also showed a few $CXCR3^+$ lymphocytes were present in the brain. On the other hand, Simpson *et al.*[21] found CXCR3 expression not only in T cells, but also in astrocytes within the MS plaque.

An increase in the percentage of $CXCR3^+$ cells in the CSF compared with that in peripheral blood has been reported in MS.[22] Here, CXCR3 is proposed to mediate recruitment and/or retention of MNCs in the CNS. The enrichment of $CXCR3^+$ cells in the CSF seemed to be independent of underlying CNS pathology. Teleshova *et al.*[29] found that percentages of $CXCR3^+$ T cells in the CSF were higher in MS than in other noninflammatory neurological diseases, but similar to levels in inflammatory neurological diseases, suggesting the changes observed in MS were not MS-specific, but reflect CNS inflammation.

On the other hand, another group found no differences in percentages of $CXCR3^+$ T cells either in the CSF or in the blood between MS and noninflammatory neurological diseases.[22,30] This is in agreement with reports in which no alteration was found in percentages of $CXCR3^+$ T cells either in blood or in CSF between a relapse period and a remission period in relapsing–remitting (RR)-MS.[31] In the blood of MS patients, Sørensen *et al.*[22] showed that the number of $CXCR3^+$ cells did not increase in the blood, while Balashov *et al.*[23] demonstrated that the number of $CXCR3^+$ T cells increased in the circulation of both RR and progressive MS patients.

Neutralization of IP-10 does not alter the clinical or pathological outcome of TMEV infection

Here, we investigated the role of IP-10 *in vivo* during TMEV infection using anti-IP-10 serum.[32,33] Anti-IP-10 sera can neutralize IP-10 and block its association with CXCR3 on Th1 cells and NK cells. Both of these cell types have been suggested to play important roles in TMEV infection. Th1 cells are one of the candidate effector cells that cause demyelination by delayed type hypersensitivity (DTH) responses and epitope spreading during the chronic phase of TMEV infection.[34] NK cells, on the other hand, have been shown to protect from polioencephalomyelitis during the acute phase of TMEV infection in resistant C57BL/10 mice.[35] Here, IP-10 can play two different roles (protection versus immunopathology): 1) a protective role by enhancement of NK cell activity; and 2) an immunopathological role by contributing to inflammatory responses. The former has been demonstrated in an animal model for vaccinia virus infection, where IP-10 appeared to enhance the cytolytic activity of NK cells, contributing to virus clearance *in vivo*.[36] In this model, nude mice inoculated with vaccinia virus encoding IP-10 resolved the infection successfully, whereas mice given a similar dose of control vaccinia virus died. On the other hand, IP-10 seems to play an immunopathological role in two animal models for MS: murine hepatitis virus (MHV) infection[33] and EAE,[37] where administration of anti-IP-10 antibody has been shown to suppress clinical signs of demyelinating diseases. Therefore, we sought to determine the role of IP-10 in TMEV infection.

Rabbit polyclonal antibody to IP-10 was produced by Biosynthesis (Lewisville, TX) using a synthetic peptide selected from the IP-10 protein sequence (CIHIDDGPVRMRAIGK) coupled to carrier protein KLH.[38] This anti-IP-10 serum has been demonstrated to neutralize IP-10 *in vivo* in MHV infection.[32,33] SJL/J mice were infected intracerebrally with 2×10^5 plaque forming units (PFU) of the DA virus. During the acute phase, (days zero, two, four and six post infection), or during the chronic phase (days 24, 27, 29, and 31 post infection), groups of mice were injected intraperitoneally (i.p.) with 0.5 mL of either normal rabbit serum (NRS) or anti-IP-10 serum (total of four injections per mouse). One group of mice was infected with the DA virus without serum injection.

To assess the severity of clinical signs in TMEV-infected mice, weight change and righting reflex alterations were monitored. A decrease in weight gain is associated with TMEV-induced disease, as is a loss of righting reflex, which is indicated by an increase in the righting reflex score.[39,40] No difference in the clinical signs between anti-IP-10-injected, NRS-injected, and no injection groups was noted, whether anti-IP-10 was given during the acute phase or the chronic phase (data not shown). Five weeks after infection, histological analysis[41,42] revealed perivascular MNC infiltration and demyelination with meningitis in the spinal cord in all groups of mice (Figure 1). Lesions were seen in the white matter, most frequently in the anterior funiculus, ventral root exit zone and lateral funiculus. There was no difference in lesion distribution among the groups. No significant differences in the extent of meningitis, perivascular cuffing, demyelination and overall pathology among the groups were noted (Figure 1, data not shown). By immunohistochemistry,[39] viral antigen positive cells were found associated with white matter demyelinating lesions in the spinal cord, and were similar in number and distribution within the CNS of animals from all groups (Figure 1).

Neutralization of IP-10 enhances TMEV-specific and autoreactive lymphoproliferation, but not virus-specific antibody responses

We also compared humoral and cellular immune responses to TMEV between mice treated with NRS or anti-IP-10 serum during the acute or chronic phase of infection. Using an enzyme-linked immunosorbent assay (ELISA), we titrated serum anti-TMEV antibodies, as described previously.[39] By day 35, all groups of mice developed significant TMEV antibody responses. Mean TMEV antibody titers ($\log_2$) during the acute phase were 9.6 ± 0.5 with NRS injections and 10.6 ± 0.5 with anti-IP-10 serum injections, while those during the chronic phase were 10.6 ± 0.5 with NRS injections and 10 ± 0.7 with anti-IP10 serum injections. There was no statistical difference between the groups ($P > 0.05$, ANOVA).

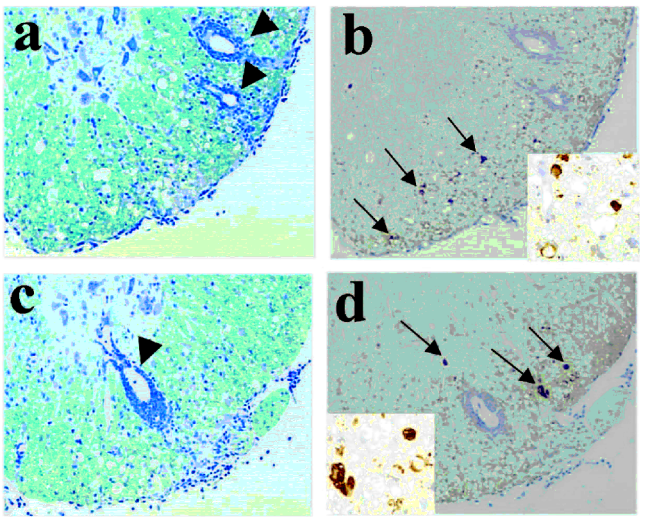

Figure 1 *Anti-IP-10 serum administration did not affect neuropathology or virus persistence. Mice were given either NRS (a, b) or anti-IP-10 serum (c, d) during the chronic phase of TMEV infection. (a, c) Using Luxol fast blue staining, we detected perivascular cuffing (arrow heads) and demyelination with meningitis at the ventral root exit zone of the thoracic segment of the spinal cord in both control mice (a) and mice treated with anti-IP-10 serum (c). (b, d) Consecutive sections immunostained with antiserum against TMEV showed a similar number and distribution of virus antigen positive cells between control mice (b) and mice injected with anti-IP-10 serum (d). Magnification* $\times$ *60, inset* $\times$ *250.*

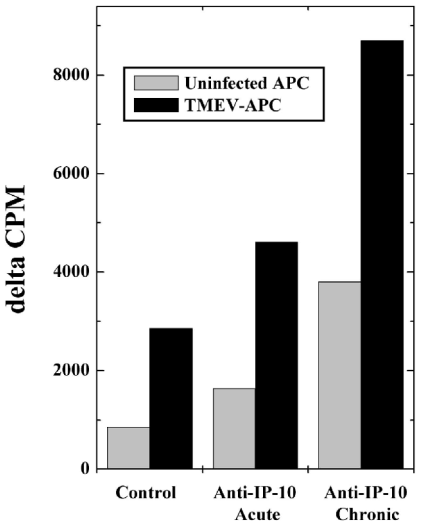

Figure 2 *Anti-IP-10 serum treatment during the chronic phase showed the highest TMEV specific and autoreactive lymphoproliferation among TMEV-infected mice. TMEV-infected mice were treated with anti-IP-10 serum during either the acute phase (middle) or the chronic phase (right). Control TMEV-infected mice received no serum or NRS (left). MNCs were isolated from two to three spleens in each group and stimulated with uninfected antigen presenting cells (APC) or TMEV-infected APC (TMEV-APC) for five days. All cultures were performed in triplicate. Results are representative of two independent experiments.*

On day 35, MNCs were isolated from spleens of infected mice. MNCs were stimulated with irradiated TMEV-infected spleen cells (TMEV-APC). Since TMEV-infected mice develop autoreactive immune responses in the spleen,[43] MNCs were also stimulated with irradiated uninfected syngenic spleen cells (uninfected APC). Cells were cultured for five days.[44] Mice injected with anti-IP-10 serum either during acute phase or chronic phase showed higher lymphoproliferation against TMEV-APC and against uninfected APC, compared with infected mice which received no serum or NRS (Figure 2). The group treated with anti-IP-10 serum during the chronic phase showed higher lymphoproliferation than that of the group receiving anti-IP-10 serum during the acute phase. This suggests that administration of anti-IP-10 serum, to some extent, can induce immunomodulation *in vivo* in TMEV infection.

Role of IP-10/CXCL10 in MHV infection and EAE

Neutralization of IP-10 reduces MNC infiltration in MHV-induced demyelinating disease

In the study described in the previous section, we did not find significant differences in clinical signs, neuropathology, virus persistence or anti-viral antibody responses between control mice and mice administered anti-IP-10 serum. This is in contrast to other animal models of MS, where neutralization of IP-10 has been shown to suppress clinical disease (Table 1).

Intracerebral infection of mice with MHV results in an acute encephalomyelitis followed by a chronic demyelinating disease. Following MHV infection, IP-10 is expressed during both acute and chronic phases of infection. Anti-IP-10 serum treatment during the chronic phase of MHV strain J2.2-V-1 infection reduced clinical signs, demyelination, and T cell and macrophage infiltration in MHV infected brains.[33] The anti-IP-10 serum in these experiments was the same as that used in the TMEV experiments described in the previous section. In contrast, treatment with anti-IP-10 sera during the acute phase led to increased mortality due to a failure in viral clearance from the CNS of mice infected with the MHV strain V5A13.1.[32,45] Since reduction of both $CD4^+$ and $CD8^+$ T cell infiltrates coincided with decreased levels of IFN-γ in the brains of mice treated with anti-IP-10, early expression of IP-10 may play a beneficial role in host defense by attracting Th1 lymphocytes into the CNS that contribute to viral clearance. These results using neutralizing antibody were further supported by a recent study using IP-10-deficient mice ($IP\text{-}10^{-/-}$) infected with MHV strain J2.2-V-1.[46] MHV-infected $IP\text{-}10^{-/-}$ mice had an impaired ability to control viral replication, which was associated with reduced levels of inflammation and demyelination in the CNS.

IP-10 modulates EAE clinically and histologically

In EAE, Ransohoff *et al.*[47] first demonstrated expression of IP-10 mRNA in the CNS of SJL/J mice sensitized with myelin proteolipid protein $(PLP)_{139-151}$ peptide. IP-10 mRNA expression in the liver in this EAE model was

Table 1 Blocking of IP-10 in animal models for MS

Model	Treatment	Blocking period	Clinical	Inflammation	Demyelination	Lympho-proliferation*	Reference
Active EAE	intrathecal antisense	5–6 days after EAE induction	improve	no change	N.E.	N.E.	51
Passive EAE	anti-IP-10 antibody	days 0 and 2	improve	decrease	N.E.	no change or decrease**	37
Active EAE	DNA vaccine	before or after EAE induction	improve	decrease	N.E.	Th2 deviation	53
MHV	anti-IP-10 antibody	12–19 days after infection	improve	decrease	decrease	N.E.	33
MHV	IP-10$^{-/-}$ mice	entire period	N.E.	decrease	decrease	decrease	46
TMEV	anti-IP-10 antibody	acute or chronic	**no change**	no change	**no change**	**increase**	Present study

N.E., not examined.
* Myelin antigen specific lymphoproliferation in EAE, and virus antigen specific lymphoproliferation in MHV or TMEV infection.
** No change in antigen-specific IFN-γ production. The authors also described that antigen (PLP)-specific lymphoproliferation was unchanged, although figure 6(C) of Fife *et al.*[37] showed mild suppression of PLP-specific lymphoproliferation in anti-IP-10 treated group.

also reported by Ransohoff *et al.*[47] but not by others.[6] The amount of chemokine mRNA had diminished to control levels by day 21 despite the presence of inflammation.[47] Godiska *et al.*[48] also demonstrated expression of IP-10 mRNA in spinal cords during the acute, remission and relapsing phases not only in actively induced EAE but also in adoptive transfer EAE induced by a $PLP_{139-151}$ specific T cell line. When the $PLP_{139-151}$ specific T cell line was stimulated *in vitro* with $PLP_{139-151}$ peptide, no IP-10 mRNA was detected, but MIP-1α, MIP-1β, TCA3 and RANTES mRNAs were found. In other studies, protection from PLP-induced EAE by treatment with an altered peptide ligand was accompanied by reduced levels of IP-10 and its receptor CXCR3.[6] IP-10 or its mRNA expression in the CNS were also detected in C57BL/6 and Sv129 X C57BL/6 mice sensitized with myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ peptide,[49,50] Lewis rats sensitized with myelin basic protein (MBP),[51] and macaque monkeys inoculated with human brain white matter.[52]

The role of IP-10 in EAE has also been investigated by modulating IP-10 *in vivo*. Wojcik *et al.*[51] showed that intrathecal infusion of antisense oligonucleotides against IP-10 reduced clinical signs in Lewis rats with MBP-induced EAE without concomitant alteration of inflammation in the spinal cord. Similarly, Fife *et al.*[37] found that administration of anti-IP-10 antibody decreased clinical and histological disease in SJL/J mice receiving $PLP_{139-151}$ specific T cells.

DNA immunization has been used not only as a way of targeted delivery of gene products, but also as a way to elicit an immune response against the gene product encoded by the construct.[44] Wildbaum *et al.*[53] reported that administration of plasmid DNA encoding IP-10 suppressed EAE with production of anti-IP-10 antibodies and alteration of myelin antigen specific lymphoproliferation from Th1 to Th2. The suppression was seen not only when the naked DNA was given prior to induction of EAE, but also six to seven days after EAE induction, or 15–17 days after EAE induction (effector phase). Neutralization of IP-10 by anti-IP-10 antibodies produced by DNA vaccination seemed to play the pivotal role, since these antibodies inhibited the development of disease when passively transferred to other EAE animals.

Discussion

Distinct roles of IP-10 and CXCR3$^+$ T cells could contribute to differences in efficacy of IP-10 neutralization among animal models for MS

Table 1 illustrates the differences in the effect of IP-10 blocking studies between three animal models for MS. While blocking of IP-10 resulted in clinical or histological improvement with suppression of antigen specific lymphoproliferation in both EAE and MHV models, TMEV infected mice showed no alteration in CNS disease, clinically or histologically, and increased levels of lymphoproliferation.

The failure to ameliorate clinical and pathological disease by anti-IP-10 serum treatment in TMEV infection compared with EAE and MHV infection could be due to a difference in the role CXCR3$^+$ cells play, particularly Th1 cells and NK cells, in each model. In EAE, CD4$^+$ Th1 cells are known to initiate demyelinating disease, while the role of Th1 cells in TMEV infection is still controversial.[9] Further, a relative lack of NK cells in SJL/J mice,[54] which were used in our experiments, suggests that NK cells might not be the major target of IP-10 in models using this strain of mouse.

Another factor that could influence the efficacy of the anti-IP-10 treatment is where, in the body, the antiserum blocks the interaction between IP-10 and its receptor. In some EAE models, IP-10 expression has been demonstrated in secondary lymphoid organs, which would help in the recruitment and activation of encephalitogenic T cells to these sites. However, no IP-10 expression has been detected in lymphoid organs in TMEV infection.[14,18] This might explain why neutralization of IP-10 resulted in an

increase in antigen specific and autoreactive T cell responses in TMEV infection, but not in EAE.

Although we do not know exactly where IP-10 and its receptor interact in the CNS, the difference in the location of the interaction among animal models for MS might be important in using anti-IP-10 as a therapeutic agent. Theoretically, anti-IP-10 antibody could block IP-10 not only on endothelial cells but also within the brain parenchyma, if there is a disruption of the blood–brain barrier around regions producing IP-10. IP-10 has been shown to bind not only to its receptor CXCR3 but also to heparan sulfate proteoglycans (HSPG).[55] In the CNS, the majority of the proteoglycans contain either chondroitin sulfate or heparan sulfate side chains. Some are constituents of the extracellular matrix and others are bound to the cell surface.[56] Therefore, while $CXCR3^+$ encephalitogenic T cells can bind to IP-10 displayed by HSPG on endothelial cells during transendothelial migration into the CNS, $CXCR3^+$ T cells might also bind to IP-10 presented by HSPG on neural cells and extracellular matrix, helping encephalitogenic T cells remain in the CNS. In this scenario, i.p. injection of anti-IP-10 antibody could effectively block the chemokine presented on endothelial cells and prevent activation of integrin on encephalitogenic cells, thereby blocking invasion of MNCs into the CNS. It may not be, however, effective enough to block the interaction between IP-10 and $CXCR3^+$ T cells in the CNS parenchyma. Although breakdown of the blood–brain barrier has been reported in inflammatory lesions in the CNS, it may not be significant enough for anti-chemokine antibody to penetrate and function in the CNS parenchyma. Here, the differences in the preservation of blood–brain barrier between animal models could also influence the efficacy of treatment with the antibody.

Involvement of other ligands for the CXCR3 receptor

IP-10 is structurally and functionally related to two other chemokines: monokine induced by IFN-γ (Mig/CXCL9) and IFN-inducible T cell α chemoattractant (I-TAC/CXCL11). IP-10, Mig, and I-TAC are all induced by IFN-γ in a wide variety of cell types and act through the chemokine receptor CXCR3. I-TAC expression was seen in the CNS of mice with MOG_{35-55}-induced EAE.[57] Thus, Mig and I-TAC might substitute for the actions of IP-10 in TMEV-infected mice treated with anti-IP-10 sera. However, while these three ligands activate the same receptor, they have been reported to exhibit an unique expression pattern *in vivo*, and experiments using neutralizing antibodies and gene targeted mice support the concept that these three chemokines may have nonredundant functions *in vivo*.[46] In MHV infection, treatment with anti-IP-10 sera, but not with anti-Mig sera, reduces demyelination,[33] while both IP-10 and Mig contribute to viral clearance.[45] A mouse CC chemokine CCL21 [secondary lymphoid cytokine (SLC)/6Ckine/Exodus-2/thymus-derived chemotactic agent (TCA)4] has also been identified as a ligand for CXCR3, and is suggested to have a functional involvement in mouse EAE.[58] However, human CCL21 is not a ligand for the CXCR3 receptor.[59]

Roles of IP-10 and $CXCR3^+$ T cells may differ among MS patients with difference clinical courses

The difference in efficacy of IP-10 modulation among animal models for MS was discussed above. This difference may reflect the potentially different roles of IP-10 in individual patients with MS, since MS has been suggested to be multifactorial and has clinical and histological subtypes. One confounding factor is that different strains of mice, C57BL/6J, BALB/cJ and SJL/J, have been shown to differ in IP-10 mRNA expression in spleens, even when immunologically naïve.[60] In addition, one model of EAE involves no CNS expression of IP-10. In IFN-γ knockout BALB/c mice, IP-10 was not expressed in the CNS when EAE was induced with two subcutaneous injections of bovine MBP.[61] Thus, the production of IP-10 in the CNS and its role in EAE development has been proposed to differ among various subtypes of EAE.

The spectrum of clinical disease of MS is diverse. The National Multiple Sclerosis Society (USA) has defined four clinical courses of MS by an international consensus among clinicians involved in MS clinical research and care: RR, primary progressive (PP), secondary progressive (SP), and progressive relapsing (PR).[62] Since the clinical, epidemiological and pathological findings in PP-MS are notably different from those described for RR-MS, the question arises whether PP-MS and RR-MS are two distinct disease entities.[63] Using an ELISA, Scarpini *et al.*[64] demonstrated that IP-10 levels were significantly elevated in CSF and sera from RR and SP, but not from PP-MS patients. Similarly, Jalonen *et al.*[65] detected IP-10 mRNA from peripheral blood MNCs in one of 11 patients with SP-MS and two of 17 patients with RR-MS, but none of the seven patients with PP-MS, using RPA. In contrast, by ELISA, Mahad *et al.*[27] demonstrated that the concentration of IP-10 was significantly greater in patients with RR-MS compared with SP-MS. These studies suggest that roles of IP-10 may differ among MS patients with different clinical courses. On the other hand, one study found no differences in IP-10 levels between MS patients with different clinical courses, probably due to the fact that IP-10 expression in patients of one clinical form was highly variable.[66]

The same situation is apparent in the analysis of the number of $CXCR3^+$ cells in the blood from MS patients. Sørensen and Sellebjerg[67] showed that IFN-β1b treatment did not alter the percentage of $CXCR3^+$ cells in peripheral blood MNCs from patients with SP-MS, while the same authors reported that IFN-β1a suppressed the expression of CXCR3 on T cells in RR-MS.[68] On the other hand, Martínez-Cáceres *et al.*[66] found a significant increase in the percentage of CXCR3 in $CD14^+$ macrophages in peripheral blood by flow cytometry with no differences between RR-, SP- and PP-MS.

The above studies suggest that the role of IP-10 in RR-MS might be different from those in the progressive forms of MS, although conflicting results make it difficult to draw strong conclusions at this time. The characteristic clinical course of TMEV induced demyelination is a gradual, nearly continuously worsening baseline without distinct relapses. Thus, TMEV infection is an animal

model for the progressive forms of MS. This is different from the other animal models for MS; most of their clinical courses are either acute monophasic or RR with a few exceptions.[63] Therefore, the distinct outcome seen in neutralization of IP-10 in TMEV infection might be due to a common unique immunological pathomechanism in progressive forms of demyelinating diseases. Thus, the clinical course as well as the genetic background of each patient with MS should be considered for the clinical application of neutralization of IP-10 in humans.

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