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The expression and function of chemokines involved in CNS inflammation

Eroboghene E. Ubogu[1,2], Michael B. Cossoy[1] and Richard M. Ransohoff[1,3]

[1]Neuroinflammation Research Center, Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA
[2]Department of Neurology, Case Western Reserve University School of Medicine and the Neurology Service (127W), Louis Stokes Cleveland Veterans Affairs Medical Center, 10701 East Boulevard, Cleveland, OH 44106-1703, USA
[3]Mellen Center for MS Treatment and Research, Cleveland Clinic Foundation, Cleveland, OH 44195, USA

Chemokines and their receptors have principal roles in leukocyte trafficking under normal physiological and pathological conditions. The differential expression of the chemokine system in different parts of the CNS provides insights into the processes that are required for normal immune surveillance and pathological immune-mediated effector processes. Insights derived from studying multiple sclerosis, an inflammatory disorder of the CNS in humans, and experimental autoimmune encephalomyelitis, an animal model of this disorder, aid in further understanding the complexities of chemokine-mediated inflammation. Knowledge of the molecular biology of chemokines and their receptors, and the roles of specific chemokine ligands and receptors in the CNS in health and in disease have made these proteins targets for therapeutic intervention in neuroinflammation. We also discuss currently proposed and potentially useful chemokine receptor antagonists.

Chemokines in health and disease

Chemokines and their receptors mediate leukocyte trafficking into the CNS in health and in disease, particularly during neuroinflammation. The chemokine system provides an avenue for therapeutically modulating the deleterious effects of leukocyte entry in neuroinflammation. In this review, we discuss chemokines and their receptors, routes of leukocyte entry into the CNS, the differential expression and function of chemokines in the normal and pathological CNS and how to modify the system therapeutically.

Chemokine structure and nomenclature

Chemokines ('chemotactic cytokines') are small (8–14 kD), structurally similar proteins that elicit leukocyte migration in a concentration-dependent fashion. The tertiary structure of chemokines is highly conserved, despite relatively low sequence homology [1]. In general, chemokines contain at least four cysteine residues that form two disulfide bonds. Chemokines are subdivided into four subfamilies, based on the organization of two positionally conserved cysteine residues near the N-terminus. These include the CC, CXC, CX_3C and C subfamilies. Table 1 demonstrates the basic structural and chemotactic functional differences between subfamilies.

Using standard nomenclature, a chemokine ligand or receptor is indicated by its subfamily, then either 'L' or 'R' to indicate whether it is a ligand or a receptor, respectively, and, finally, by its specific number, for example, CCL2 and CCR2, CXCL3 and CXCR1, and CX_3CL1 and CX_3CR1.

Basic chemokine function

The biological effects of chemokines are mediated via specific G-protein-coupled receptors with seven transmembrane regions [1]. Each chemokine receptor has relative affinity for different chemokines, which results in significant diversity of interaction. Most chemokine receptors are stimulated by more than one chemokine (e.g. CCR5 is stimulated by CCL3, CCL4, CCL5 and CCL8), and one ligand might stimulate more than one receptor (e.g. CXCL6 binds to both CXCR1 and CXCR2). In general, these relationships are restricted to within chemokine subfamilies. This promiscuity or redundancy might act as a safety factor in the chemokine system, to ensure adequate host defenses. Alternatively, it might indicate intricate complex interactions between closely related chemokines and their receptors, or both [1].

Currently, ~50 human and mouse chemokines (http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/Chemokine.html) and 18 chemokine receptors (ten CCR, six CXCR, one XCR and one CX_3CR) (http://www.gpcr.org/7tm/) have been identified. These act as mediators of cellular migration in embryogenesis (including CNS development) and leukocyte migration in immune surveillance, and mediate effector responses in lymphoid and non-lymphoid tissue. Although, classically, chemokines are considered as regulators of leukocyte migration (which is important for neuroinflammation), phylogenetic evidence indicates that this role might be relatively new [2–5].

Corresponding author: Ransohoff, R.M. (ransohr@ccf.org).
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Table 1. Classification of chemokines

Chemokine subfamily	Alternative name	Number of residues between cysteine residues near N-terminus	Responding leukocyte population	Examples
CC	β	0	Mononuclear cells, eosinophils, basophils, natural killer cells	CCL2, CCL3, CCL4, CCL5, CCL11, CCL20
CXC	α	1	ELR[a] domain: neutrophils	CXCL5, CXCL8
			No ELR domain: multiple cell types	CXCL9, CXCL10, CXCL12, CXCL13
CX_3C	δ	3	Monocytes, natural killer cells	CX_3CL1
C	γ	Has one cysteine residue	T cells, natural killer cells	XCL1, XCL2

[a]Abbreviations: ELR, glutamic acid-leucine-arginine.

Leukocyte migration into the CNS: three (or more) compartments

The potential sites for leukocyte migration into the CNS are important because these might determine the most effective routes of drug administration for neuroinflammation. Previously, the CNS was characterized as an immunologically privileged site because of the limited inflammatory capacity and lack of lymphatic drainage. However, more recent studies in rodents and ruminants have shown that the CNS is an immunologically specialized site [6,7].

There are at least three distinct routes of leukocyte entry into the CNS: (i) migration from blood to cerebrospinal fluid (CSF) via either the choroid plexus or meningeal vessels in the subarachnoid space; (ii) from blood to the parenchymal perivascular space of the brain; and (iii) from blood to the parenchymal perivascular space of the spinal cord [6,7]. Route (i) involves crossing the blood–CSF barrier, whereas the other routes involve crossing the blood–brain barrier (BBB) and blood–spinal cord barrier (Figure 1). Detailed reviews of the routes of leukocyte entry into the CNS have been published recently [6,7].

Migration from the blood to the CSF via the choroid plexus (which is the site of CSF production) is an important source of leukocytes in the CSF. The blood–CSF barrier

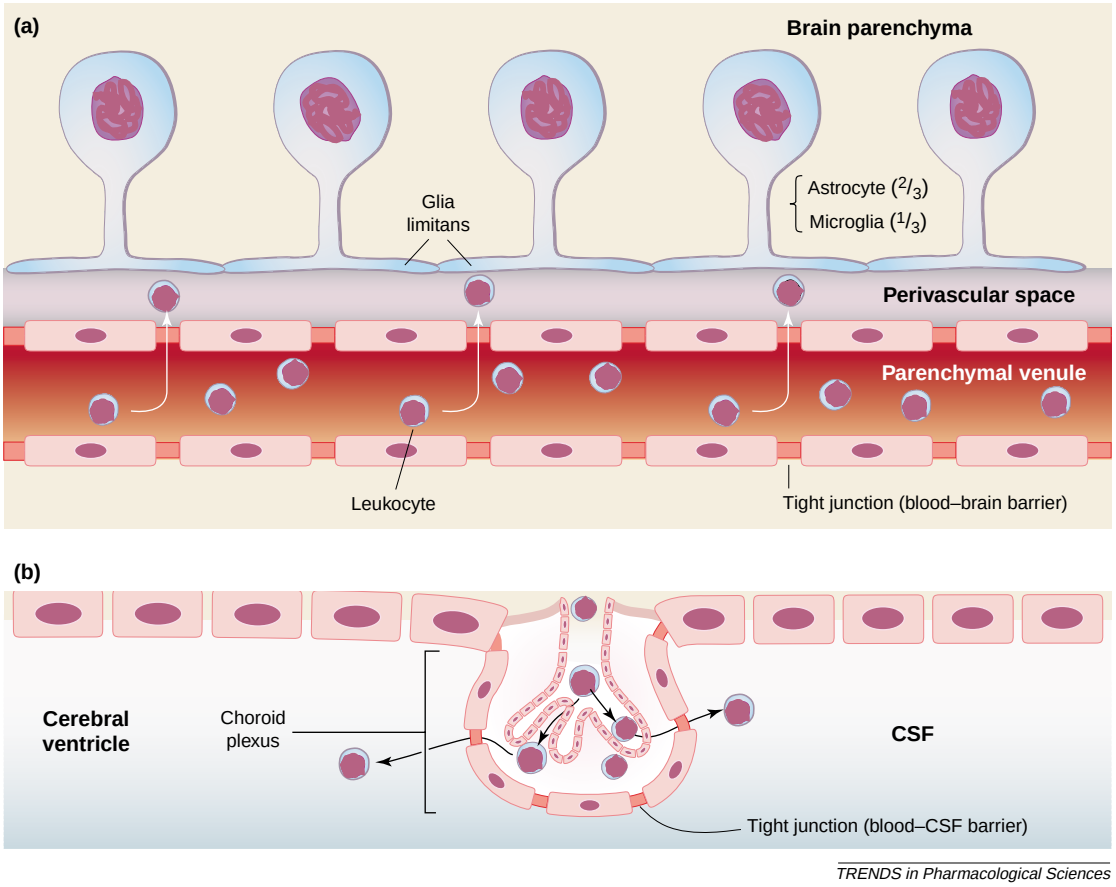

Figure 1. Routes of leukocyte entry into the brain. Two major routes of leukocyte entry into the brain are shown. **(a)** The blood–brain barrier (BBB) consists of parenchymal venules with tight inter-endothelial junctions. Arrows show the movement of leukocytes from the parenchymal venules to the perivascular space, from which leukocytes can enter the brain parenchyma. Two thirds of the cell processes that form the glia limitans are from astrocytes, whereas a third of these processes are from microglia. Leukocytes extravasate both across the endothelial cells of the BBB and at inter-endothelial junctions. **(b)** The blood–cerebral spinal fluid (CSF) barrier consists of epithelial cells of the choroid plexus, which possess tight junctions; blood vessels of the choroid plexus do not have tight junctions. The cerebral ventricles are lined by ependymal cells that do not participate in the blood–CSF barrier. Arrows show the movement of leukocytes from the choroid plexus blood vessels to the choroid plexus epithelium and the CSF. The blood–CSF barrier has a major role in the entry of leukocytes during routine immune surveillance whereas the BBB has an important role in pathological states.

consists of tight intercellular junctions that join the epithelial cells of the choroid plexus. Normally, P-selectin, E-selectin and intercellular adhesion molecule 1 (ICAM-1), which are important determinants of leukocyte trafficking, are expressed in vessels of the choroid plexus. The $CD4^+$T cells in CSF (~80% of CSF leukocytes) are predominantly $CD45RO^+/CD27^+$ memory cells that express L-selectin, CCR7 and CXCR3 [6,7]. L-selectin and CCR7 are important for migration across high endothelial venules of secondary lymphoid organs, and the return of memory lymphocytes into lymph nodes following passage through non-lymphoid organs [6,7].

Another potential site for leukocyte entry into the CSF is via veins in the subarachnoid space [6,7]. The endothelial cells of these veins express P-selectin, E-selectin and ICAM-1 [6,7]. This might facilitate CNS immune surveillance by activated memory T cells from the systemic circulation in normal physiological conditions.

Other potential sites of leukocyte migration from the blood to parenchymal perivascular space (across the BBB) might be more important in CNS disease. ICAM-1 is expressed by a minority of parenchymal microvessels in the brain in non-inflammatory disease states and is upregulated during inflammatory disease in mice and humans [6,7]. Studies of the BBB *in vitro* and *in vivo* have shown that endothelial activation increases adhesion and migration of leukocytes, as discussed in the following section.

Leukocyte migration across BBB following endothelial activation

Leukocyte migration across the BBB is vital for host immune processes in pathological conditions. Understanding the mechanisms by which these processes take place is important to elucidate potential targets for pharmacological intervention.

Previous studies indicate that the extravasation of T-cell blasts into the CNS parenchyma depends on activation with either mitogens or encephalitogenic neuroantigens, but the mechanisms involved are not elucidated fully [8]. Recent intravital-microscopy studies in mice show that highly activated, neuroantigen-specific encephalitogenic T cells only interact with superficial cerebral microvessels after the endothelium has been activated by intravenous injection of either tumor necrosis factor α (TNF-α) or lipopolysaccharide (LPS) [9]. These observations indicate that peripheral activation of T cells in lymphoid tissue and systemically induced activation of the microvascular endothelium have roles in CNS leukocyte trafficking.

Studies *in vitro* show that the adherence of resting or activated T cells to endothelial cells from the brain microvasculature increases following endothelial activation [which upregulates ICAM-1, vascular cell-adhesion molecule (VCAM-1) and E-selectin] with TNF-α, interferon γ (IFN-γ), interleukin 1β (IL-1β), CD40L and LPS [9]. CCL2, CCL3, CCL4 and CCL5 activate and induce chemotaxis of T cells and monocytes [10,11]. *In vitro* studies with CCL4 and CCL5 demonstrate increased adhesion of recently activated antigen-specific T cells and memory $CD4^+$T cells to resting and activated endothelial-cell monolayers [12].

Preliminary studies *in vitro* indicate that the binding of CCL2 to CCR2, and of CX_3CL1 to CX_3CR1 induces efficient transmigration of monocytes and macrophages across the microvascular endothelial layers of the brain [13,14]. The mechanisms by which activation of cells within the CNS parenchyma following antigen presentation in the perivascular space might enable leukocytes to transmigrate through the BBB are illustrated in Figure 2. Leukocyte-transmigration assays should help to decipher the mediators of cellular trafficking into the CNS, and to optimize the design of drugs that are specific for different stages of neuroinflammation.

Differential expression and physiological functions of the chemokine system in the CNS

Knowledge of the intrinsic properties and the distribution of the chemokine system in the CNS are important for developing specific pharmacological therapies for neuroinflammation. Intrinsic cellular components of the CNS that are involved in inflammation include astrocytes and microglia.

Astrocytes are the source of several cytokines (e.g. IL-6) and chemokines (e.g. CCL2 and CXCL10) in the CNS [15,16]. In addition, they have fundamental roles in supporting neuronal function via the provision of metabolic and trophic factors, aiding repair processes, and regulating brain homeostasis and development [17]. Microglia are the resident mononuclear phagocytes in the CNS, and are responsible for initial host-immune defenses. Some chemokines are chemotactic to microglia [17].

Participation of the chemokine system in normal CNS homeostasis and in pathological states such as MS is not fully elucidated, so information on the differential expression of chemokines consists of both 'highly validated' and 'preliminary' data. In this regard, comparisons of *in vitro* studies of explanted CNS cells with *in vivo* data (e.g. *in-situ* hybridization) show that tissue disruption and cell culture dysregulates the chemokine system. Therefore, it is perilous to extrapolate the situation *in vivo* from results *in vitro*.

CC subfamily: roles in pathophysiology and host defense

Resident cells of the CNS express chemokine ligands and receptors in this subfamily. *In vivo* models of brain axonal injury in CCR2-deficient mice verify the importance of these cytokines, especially CCL2–CCR2 interactions, as initial, crucial mediators of leukocyte migration to sites of injury [18]. CCL2 exacerbates acute *N*-methyl *D*-aspartate (NMDA)-mediated brain injury in neonatal rats [19], and stimulates the migration of astrocytes from neonatal and adult mice *in vitro* [17]. CCL2 and CCR2 are expressed in normal and inflamed rat brain, and in human fetal astrocytes *in vitro* [17]. There is evidence for an alternative receptor for CCL2 on astrocytes [20], which indicates a specialized role for CCL2 in chemokine function in the CNS.

In addition, CCR5 knockout mice are more susceptible to *Cryptococcus neoformans*-induced encephalitis [21] but respond adequately to pulmonary *Cryptococcus*

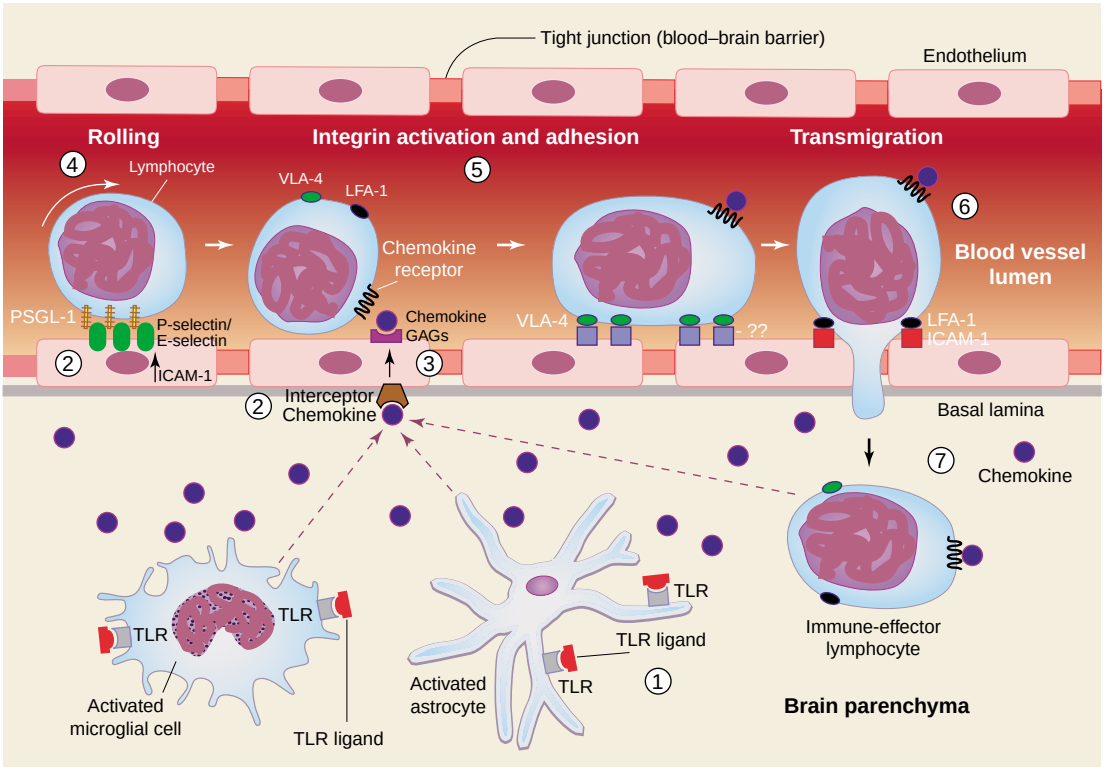

Figure 2. Potential mechanisms for the transmigration of lymphocytes across the blood–brain barrier (BBB) during infection to illustrate neuroinflammatory processes: (1) engagement of Toll-like receptors (TLRs) by their ligands, and secretion of pro-inflammatory cytokines [e.g. tumor necrosis factor α (TNF-α)]; (2) pro-inflammatory cytokine-mediated stimulation of both chemokine secretion (blue circles) and endothelial activation; (3) chemokine transport across activated endothelium; (4) lymphocyte rolling on activated endothelium; (5) activation of lymphocyte integrins and lymphocyte arrest and adhesion to activated endothelium; (6) lymphocyte diapedesis (either trans-endothelial or inter-endothelial) across activated endothelium; and (7) lymphocyte migration in the parenchyma to the site of chemokine secretion (chemotaxis), and elimination of pathogen (source of the TLR ligands). Abbreviations: GAGs, glycosaminoglycans; ICAM-1, intercellular adhesion molecule 1; LFA-1, leukocyte function-associated antigen 1 ($\alpha_L\beta_2$ integrin); PSGL-1, P-selectin glycoprotein ligand 1; VLA-4, very-late antigen 4 ($\alpha_4\beta_1$ integrin).

neoformans infection, which indicates an organ-specific role for CCR5 in host-defense in the former. The roles of the CC subfamily of chemokines in normal and pathological function of the CNS are preliminary and await further *in vivo* confirmation. However, these chemokines and their receptors might be potential initial targets for modulation in neuroinflammation.

CXC subfamily: chemokines that came from the CNS

It is likely that CXC chemokines and receptors arose early in vertebrate biology (coincident with jawless fish), and were involved initially in nervous system patterning and developmental positioning [22]. This concept is well validated for CXCR4 and CXCL12 (in neurons) [23], and for CXCR2 and CXCL1 (in glial cells), based on mouse knockout models [24]. In inflammation, astrocytes produce CXCL12 and CXCR4. CXCL12, by autocrine signaling, can stimulate astrocyte proliferation, chemotaxis and the production of cytokines and chemokines via a TNF-α-mediated process (with downregulation of glutamate transporter on glia) [5,17]. In line with their chemotactic properties, CXCR2–ligand interactions are also important for recruiting neutrophils during host-protective immune responses against *Staphylococcus aureus*-mediated brain abscesses in mice [25].

CXCR3 (the receptor for CXCL10) has an important role in recruiting microglia and the subsequent 'pruning' of denervated dendrites of interneurons in a mouse entorhinal cortex lesion model [26]. CXCR1 and CXCR2 (which are receptors for CXCL1 and CXCL8) are present on the surface of microglia from adult humans *in vitro* but their functions are unknown [17]. Several infectious and cytokine stimuli cause human adult microglia to produce either CXCL8 or CXCL10, which indicates a role for these chemokines in innate, host-immune responses [17].

In general, the roles of the CXC subfamily of chemokines in CNS physiology are the best validated because of the reproducible effects observed in animal knockout models. Blocking CNS CXC chemokine receptors might have unpredictable effects in addition to inhibiting inflammation because these chemokines have important roles in normal CNS development and physiology.

CX_3C and C subfamilies

CX_3CL1 is one of only two chemokines whose primary translation product is a transmembrane glycoprotein (the other is CXCL16). Subsequently, these are released by proteolysis by members of the ADAM (a disintegrin and a metalloprotease) family of proteases, the activity of which is either constitutive or induced [27]. This unique chemokine is produced in the CNS by neurons and is released in response to CNS pathology. CX_3CL1 signals to CX_3CR1 on microglia, which indicates a neuron–microglial signaling system [27]. CX_3CL1 induces microglial

chemotaxis *in vitro* [8], and inhibits cytokine production and apoptosis [28]; however, knockout models indicate that activation of microglia in response to either facial motor axotomy or laser lesioning of CNS microvessels [29] is not contingent on CX_3CR1 signaling.

Astrocytes, microglia and monocytes produce XCL1 in the CNS *in vivo* [30]. A recent report using HIV-1 Tat protein transgenic mice demonstrates that the expression of XCL1 (together with several CC and CXC chemokines) is upregulated in direct response to Tat protein in the brain, with a consequent increase in the infiltration of activated T cells [30]. These data suggest that XCL1 might have a pathogenic role in the development of HIV/AIDS-associated neurological disorders [30]. However, the roles of these subfamilies of chemokines require further validation.

Role of chemokines and their receptors in neuroinflammation

Multiple sclerosis (MS) and the animal model of experimental autoimmune encephalomyelitis (EAE) are classic examples of CNS inflammation. Therefore, it is pertinent to discuss the pathogenic role(s) of the chemokine system in these disorders. MS is an inflammatory, demyelinating disorder of the CNS that, commonly, results in axonal degeneration and progressive disability. MS lacks a direct counterpart in non-human species but it provides a platform for understanding the roles of some chemokines in neuroinflammation. Genetic studies indicate that CC chemokines are involved in the susceptibility to MS [31], and the spatial and temporal expression of the chemokine system in MS and EAE are important to understanding these roles.

Spatial expression of the chemokine system in neuroinflammation

The concentration of CXCL10 is increased and CXCR3 is present on lymphocytes from the CSF of MS patients, which indicates that these chemokines and their receptors might be involved in recruiting leukocytes from either the blood–CSF barrier or the BBB [1]. CXCR3 is a surface marker for T cells that can migrate across microvascular endothelial layers in human brain, and does not have an active role in transmigration *in vitro* [32]. In MS lesions, there is increased expression of CCL2 in astrocytes and of CCL5 in leukocytes [1,33], which indicates that these cytokines might be important for early transmigration of leukocytes across the BBB.

In MS, inflammatory cells accumulate in the perivascular space before they infiltrate the parenchyma. There is increased expression of CCL3, CCL4, CCL5 and CCL7 (and receptors CCR1, CCR2 and CCR5) in perivascular leukocytes and nearby microglia [1,11,17]. CCL2 and CXCL10 are produced by astrocytes around the lesions [1] and might signal to CCR2 or CXCR3 on leukocytes. These chemokines mediate chemotaxis of T cells and monocytes/macrophages. The production of CNS chemokines by astrocytes and microglia in response to pro-inflammatory cytokines such as IFN-γ and TNF-α might result in further migration of inflammatory leukocytes from the perivascular space into the parenchyma [1,16].

Studies of EAE further support the importance of chemokines in neuroinflammation. Leukocytes in the perivascular space and in focal parenchymal lesions express CCL2 and CCL5 in addition to CCL3, CCL4 and CXCL10 [33,34]. CCL2 and CXCL10 are expressed mainly by astrocytes in the CNS of mice with EAE [17,34,35]. However, these observations are mainly descriptive because, in general, chemokine genes are 'early-response' genes that are induced readily and nonspecifically by cytokines and other inflammatory stimuli.

Knockout mice that lack either chemokines or chemokine receptors provide a more direct test of the importance of individual chemokines in neuroinflammation. The severity of EAE is reduced in either CCR1 knockout or CCR2 knockout mice [36]. However, the development of EAE is not inhibited in CCR5 knockout mice [37], which might illustrate redundancy in the chemokine system to facilitate host immune effector mechanisms. More pertinent to MS, homozygotes for the Δ32 gene polymorphism ('human knockouts' who produce no functional CCR5) are not protected against developing MS [38]. There is modest evidence that Δ32 heterozygotes with MS experience a milder course of disease [39].

The role of CXCR3 and CXCL10 in EAE is less certain. Several reports using either CXCL10 knockout mice [40] or neutralizing monoclonal antibodies to CXCL10 [41] demonstrate increased severity of disease, whereas others show that inhibiting ligand–receptor interactions using antisense oligonucleotides, anti-CXCL10 antibodies [42] and vaccination with plasmid-encoding DNA [43] causes clinical and histological improvement in EAE. There is a need to correlate the observations from knockout animals with receptor-blockade assays to confirm the roles of these chemokines in neuroinflammation.

Temporal expression of the chemokine system in neuroinflammation

At its onset, MS can be divided into relapsing–remitting MS (RRMS; 85% of patients) and primary–progressive MS (PPMS; 5–10% of patients). Secondary–progressive MS (SPMS) is characterized by a continual decline after a period of RRMS. Current treatments are weakly effective and only benefit RRMS patients. It is possible that the nature of the inflammatory process, including the expression of chemokine ligands and receptors, differs between the forms and the phases of MS. Targeting differential, temporal expression in the chemokine system might improve therapeutic interventions.

There are few studies on the differential expression of the chemokine system in the subtypes of MS. There is increased expression of CXCL10 in peripheral blood mononuclear cells, serum and CSF in RRMS/SPMS compared with PPMS [11,44], but another study reports that the increase in CXCL10 is unrelated to MS subtype [11]. However, chemokines produced by circulating leukocytes are unlikely to reflect production by the CNS parenchyma. Increased expression of CXCR3 on T cells has been reported in RRMS, and increases in CCR5 and CXCR3 in progressive MS [45].

Studying the differential expression of chemokines during the relapse and remission phases of RRMS might

also provide some insights into innate recovery from CNS inflammation that might be useful therapeutically. CSF from patients with active disease provides an opportunity to evaluate chemokine levels in the extracellular space of CNS white matter. Fortunately, the production of chemokines by leukocytes in the CSF, which might confound these analyses, appears to be the same in patients with MS and in controls without CNS inflammation [1].

During attacks of RRMS, there is a consistent increase in the concentration of CXCL10 in the CSF, and a decrease in CCL2 [1]. The concentration of CCL2 rises gradually, whereas the concentration of CXCL10 declines with increased time after an attack. However, for unknown reasons, the CCL2 concentration remains lower than that in normal controls (and in patients with other forms of inflammatory demyelination such as Devic's disease and Japanese opticospinal MS). Although the expression of CXCL10 increases during an attack, preliminary studies do not correlate CXCL10 in the CSF with MRI gadolinium enhancement of CNS MS lesions as a marker of active disease [46].

The temporal relationships between chemokine expression and neuroinflammation are studied more conveniently in EAE. Models of acute EAE correlate with an MS attack in RRMS, whereas chronic EAE correlates to long-term RRMS. The full induction of EAE depends on CCR1, CCL20, CCR2 and CCL2 [11]. Upregulation of CXCL1, CXCL10 and CCR2 occurs in acute EAE, whereas relapses are associated with CCL2–CCR2 and CCL20–CCR6 interactions [11,47,48]. These observations provide insights into the selective modulation of the chemokine-mediated inflammatory response in EAE and, possibly, MS.

Therapeutic modulation of the chemokine system in neuroinflammation

Chemokines and their receptors, by virtue of their potential roles in mediating CNS inflammation in MS, provide potential sites for pharmacological intervention. Chemokine ligand–receptor interactions can be modulated in several ways (Figure 3). These include: (i) reducing signal-transduction mechanisms that are required for chemokine synthesis and to mediate the effects of receptor–ligand interactions [49]; (ii) reducing translation of chemokine mRNA [50]; (iii) producing neutralizing antibodies against either chemokines or their receptors [51]; (iv) modifying the structure of chemokines to reduce binding to, or activation of, receptors [52]; (v) generating chemokine mutants with reduced binding to glycosaminoglycans [53]; and (vi) administering small-molecule antagonists of chemokine receptors [54]. Here, we discuss briefly small-molecule antagonists of chemokine receptors as potential therapeutic modulators of CNS inflammation.

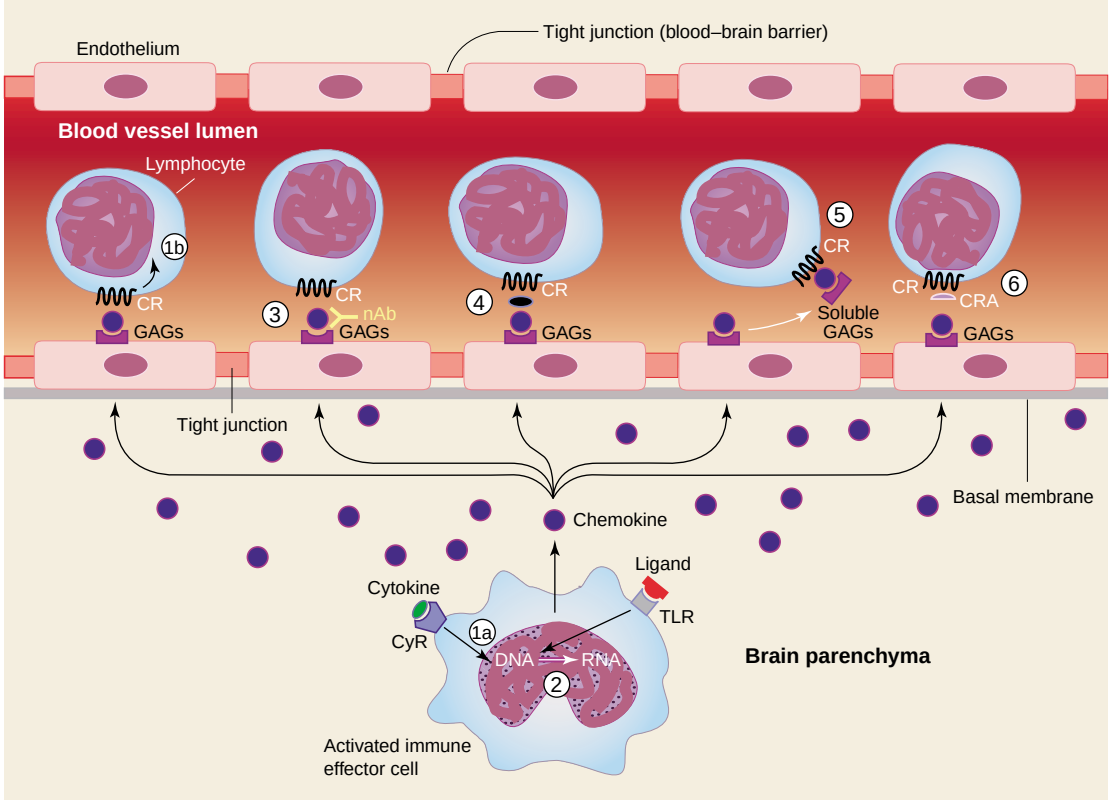

Figure 3. Potential sites for therapeutic modulation of the chemokine system in neuroinflammation: (1a) inhibition of second-messenger systems that produce chemokines in immune-effector cells; (1b) inhibition of second-messenger systems in activated lymphocytes following chemokine ligand–receptor interactions; (2) inhibition of chemokine translation (e.g. small-interfering RNA); (3) neutralizing antibodies (nAbs) against either chemokine ligands or chemokine receptors (CRs); (4) structurally modified chemokines; (5) reducing chemokine ligand–receptor binding with soluble glycosaminoglycans (GAGs); and (6) chemokine receptor antagonists (CRAs). Abbreviations: CyR, cytokine receptor; TLR, Toll-like receptor.

Small-molecule receptor antagonists

Chemokine receptors are G-protein-coupled transmembrane receptors and, thus, potential targets for small-molecule antagonists. Several chemokine receptor antagonists have been designed and are being studied currently *in vitro*, in animal models and in early clinical trials. These include antagonists of CCR1, CCR2, CCR3, CCR5, CXCR1, CXCR2, CXCR3 and CXCR4 (listed with the United States Patent and Trademark Office at http://www.uspto.gov/patft/index.html). It is difficult to perform proof-of-principle experiments in mice and rats with many of these agents because they are selective for the human receptors.

Antagonists to CCR1, CCR2, CCR5 and CXCR3 might be useful in MS. Data published from small clinical studies has shown efficacy of an oral CCR1 antagonist in treating rheumatoid arthritis [55] but failure to meet therapeutic endpoints in larger clinical trials. A novel CCR1 antagonist, BX471 (also known as ZK811752), is a potent, selective, orally available agent that was safe in Phase I clinical trials in MS [56], but unsuccessful in larger, Phase II trials.

TAK779, a quaternary ammonium salt, antagonizes the binding of chemokine ligands to CCR5 and CXCR3. Thus, it is potentially useful for treating MS, but is limited by poor oral absorption and rapid elimination [11,57]. Therapeutic application of these and other chemokine receptor antagonists has yet to be put to the test in clinical trials.

Concluding remarks

There has been significant progress in elucidating the roles of chemokines and their receptors in normal immune surveillance and in CNS inflammation, particularly in MS and its animal model EAE. However, much work is needed to further understand the interactions between the CNS and primary and secondary lymphoid organs in health and in disease. We also need more information about the chemokine mediators of pathogenesis in RRMS and PPMS, the roles of B-cell infiltration and immunoglobulin synthesis in MS, and specific chemokine ligands and receptor targets for therapeutic intervention.

We also need to consider regional differences between CNS sites. The roles of chemokines in leukocyte trafficking to the spinal cord, for example, have not been elucidated. These studies are required to define the pathogenic mechanisms of CNS inflammation in MS and EAE. Further challenges arise from the clinical heterogeneity of MS compared with animal models. Characterization of lesions, coupled with clinical and radiological findings, might help to classify CNS inflammation more specifically, and provide a framework for elucidating the differential expression of the chemokine system in clinically heterogeneous neuroinflammatory diseases. Such work is a prerequisite for the development of novel, efficacious and safe drugs to treat CNS inflammation.

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