

The Chemokine System in Neuroinflammation: An Update

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Chemokines and their receptors govern physiologic and pathologic leukocyte trafficking. The function of the chemokine system may be of particular interest for hematogenous leukocyte infiltration of the central nervous system (CNS) because of the distinct character of CNS inflammation and the exquisite specificity with which the chemokine system regulates cellular migration events. This review summarizes recent information about the expression and function of elements of the chemokine system in CNS inflammatory processes. Animal models of CNS demyelinating disease and the corresponding human disorder, multiple sclerosis are both considered.

Background

Migration of hematogenous cells into the central nervous system (CNS). Characteristics of CNS inflammation are distinct from those in other organs because of the presence of the blood-brain barrier (BBB), which affords partial isolation from the circulating cellular and molecular elements of the immune system [1]. It has been proposed that activated or memory T cells can penetrate an intact BBB and enter the CNS perivascular or subarachnoid space, regardless of specificity for CNS antigens [2–4]. This transit of T cells is believed to support protective immunologic surveillance of the CNS [5]. T cells that encounter cognate antigen, presented by perivascular or meningeal antigen-presenting cells (APC) [1], persist in the CNS and initiate a local inflammatory process [2].

Circulating leukocytes cross an endothelium in discrete steps [6]. Interactions between selectins and their carbohydrate counter receptors, in the presence of shear forces of the bloodstream, mediate leukocyte “rolling” on endothelial surfaces. Rolling of T cells on CNS microvessels during immune-mediated inflammation requires interactions between endothelial P-selectin and P-selectin glycoprotein ligand-1 on the T cell. [7].

Activated leukocyte integrins mediate arrest on the endothelium through firm adhesion to cell adhesion molecules (CAMs) or extracellular matrix components. Recent findings implicate intercellular CAM-1 and lymphocyte function-associated antigen-1 in this process [7]. However, in the model of experimental autoimmune encephalomyelitis (EAE), antibodies to the leukointegrin very late activation antigen (VLA)-4 blocked full expression of disease [8] and were effective in sup-

pressing EAE even after T cell entry into the CNS, indicating an effect on secondary recruitment of inflammatory cells. Of interest, anti-VLA-4 diminished the pathologic impact of infection with either of two neurotrophic viruses, Borna or Semliki Forest, without evident enhancement of viral replication within the CNS [9, 10].

The activation of integrins (a lightning-like process required for firm leukocyte-endothelial adhesion) requires signaling through G α i-linked receptors, of which the best characterized are members of a superfamily of receptors for chemokines, formyl peptides, and leukotrienes [11–13]. The role of chemokines in leukocyte entry into lymphoid organs is well established [14], and there is now support for extending this paradigm to CNS endothelial beds [7]. After firm adhesion, migrating inflammatory cells penetrate the endothelial layer and migrate into the CNS perivascular space, a process dependent on platelet endothelial adhesion molecule-1 [15].

Migration of inflammatory cells within the CNS parenchyma is poorly understood but may be directed by chemotactic gradients created by chemokines that diffuse from sites of production at foci of inflammation [16]. CNS glial cells can be stimulated for chemokine production by cytokines produced by T cells that recognize their cognate antigen, presented by resident APC [17]. In vivo studies indicate that resident glia, including astrocytes, express chemokines in great abundance [18] in both autoimmune and antiviral inflammatory responses [19].

Chemokines. More than 40 human chemokines have been identified to date [20] (available at <http://cytokine.medic.kumamoto-u.ac.jp/CFC/CK/Chemokine.html>). One major question confronting “chemokineologists” is why so many chemokines exist, given a limited number of target cell populations. A collateral issue concerns overlapping chemokine-chemokine receptor interactions, since one chemokine receptor may respond to several chemokine ligands, and individual chemokines may engage more than one receptor [21]. There are two broad proposals to account for these phenomena. The first is that redundancy provides a sufficient safety factor in the system to ensure adequate host defense [22]. An alternative concept specifies intricate interplay among chemokines and their receptors,

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parceling out individual functions among closely related molecules [23]. It is evident that the two notions are not mutually exclusive.

On the basis of chemokine structure there are four chemokine subfamilies [20] and two main chemokine subfamilies: CC and CXC. CC chemokines are typified by the presence of two adjacent cysteines near the N-terminus; in CXC chemokines the corresponding cysteine residues are separated by 1 amino acid. Two additional chemokine subfamilies, C and CXXXC, have at present two members each. CX3CL1 and CXCL16 are anchored to cellular plasma membranes by a transmembrane domain and a cytoplasmic tail, with the chemokine domain perched at the terminus of an extracellular stalk-like motif.

Structural differences in chemokine families carry functional implications. CC chemokines attract receptor-bearing mononuclear leukocytes (monocytes, lymphocytes), basophils, eosinophils, and dendritic and NK cells. One large subgroup of CXC chemokines containing an ELR (glutamic acid-leucine-arginine) motif located near the N-terminus is uniformly neutrophil-specific. CXC chemokines lacking the ELR motif are inert toward neutrophils but are potent lymphocyte chemoattractants. C and CXXXC chemokines predominantly stimulate migration of mononuclear inflammatory cells.

Chemokine receptors. Chemokines act on target cells through specific receptors belonging to the enormous superfamily of heptahelical G protein-coupled receptors (available at <http://www.gpcr.org/7tm/>). There are receptor subfamilies for each chemokine subfamily. Predictably, these are designated CCR, CXCR, XCR, and CXXXCR. To date, 6 CXC chemokine receptors (CXCR1-6) and 10 CC chemokine receptors (CCR1-10) have been assigned formal chemokine receptor designation based on demonstrated signaling in response to defined ligands. C and CXXXC chemokine subfamilies (lymphotactin and fractalkine) have 1 identified receptor (XCR1 and CXXXCR1, respectively) each [20, 24].

Chemokine receptors can be stimulated by ≥ 1 chemokine ligand [25]. Chemokine receptors usually do not cross subfamily barriers. Instructive exceptions include the Duffy antigen receptor for chemokines (DARC) and D6, each of which binds multiple chemokines from both CXC and CC subfamilies, but neither of which generates cellular responses to receptor engagement. DARC is localized on erythrocytes, postcapillary venules, and cerebellar Purkinje cells [26]. Because of its abundance on blood erythrocytes, DARC is suspected to be a “sink” for chemokines, binding chemokine peptides whose presence in high concentration in the circulation would cancel gradients into tissue.

Viruses, particularly those of the herpesvirus and poxvirus families, encode chemokine and chemokine receptor homologues and chemokine-binding proteins. It has been proposed that such interference with the endogenous chemokine system might promote viral propagation or allow viruses to elude host defenses [27, 28]. Chemokine receptors also support pathogen

entry, most prominently for human immunodeficiency virus (HIV) and *Plasmodium falciparum*. There is an intricate relationship between the chemokine system and HIV infection of the CNS [29]. Considerable attention has been devoted to the potential role of HIV envelope/chemokine receptor interactions in HIV-associated encephalopathy [30, 31].

State of the Art

Chemokines and receptors during inflammatory demyelination. About one decade ago, studies showed high-level expression of many chemokines during the active phase of EAE [32–36]. This expression was mostly detected in astrocytes localized around inflammatory foci [18]. The kinetics of chemokine expression suggested that they were probably responsible for amplification of the ongoing inflammatory reaction but not its initiation [37, 38]. In the chronic relapsing variant of EAE, chemokines were produced during spontaneous disease relapse, some (monocyte chemoattractant protein CCL2 [trivial nomenclature: MCP-1], CXCL10 [IP-10], and CXCL1 [KC] by astrocytes, and others, CCL3 (MIP)-1 α and CCL5 (RANTES) by migrating inflammatory cells [39].

These observational studies led to functional analysis of the chemokine system by using either blockade with neutralizing antibodies or examination of disease in chemokine or receptor knockout mice. The results indicated complex regulation of EAE by chemokines and their receptors [40] (e.g., anti-CCL3 inhibited disease), while CCL3 knockout mice experienced EAE equally as severe as their littermates [41, 42]. Satisfying concordance was found, however, for CCL2 as neutralizing antibodies suppressed relapses of disease in chronic EAE, and knockout mice exhibited a compatible phenotype in the same disease paradigm [43, 44]. Other agreeably interpretable results came from studying mice deficient for either CCL2 or CCR2, its major leukocyte receptor, both of which were resistant to induction of monophasic EAE [44–46]. Results with other knockout or antibody-blockade experiments have yet to be satisfactorily explained. For example, mice lacking CCR1 experience significantly milder disease than controls, but assignment of the relevant ligand has not been achieved [47].

In viral models of neuroinflammation, especially those that produce demyelination, CNS chemokine expression resembles that observed in EAE [48–51]. For example, CXCL10 and CCL5 are robustly produced in the virus-infected CNS both acutely and chronically [50, 51]. CXCL10 has been assigned a critical role in mouse hepatitis virus (MHV)-induced disease. It was implicated in early responses that were required for efficient clearance of virus. However, at later time points, elevated CNS CXCL10 levels in MHV-infected mice were associated with virus-induced inflammatory demyelination [52, 53]. Compatible contributions of CXCL10 to demyelination were delineated in the EAE model by use of anti-CXCL10 antibodies

[54]. Further EAE experiments in CXCL10-deficient mice will provide an important complement to these studies.

Examining the chemokine system in multiple sclerosis (MS) patients. Results obtained in MS animal models accelerated research on the human disease. Shortly after initial data from EAE were published, MS results were reported and were gratifyingly compatible with data from animal studies [55–60]. Similarities between MS and EAE concerned both chemokines that were present at high levels and the cellular sources of these chemokines. By *in situ* hybridization, CCL5 was expressed by inflammatory cells in perivascular foci in MS brains as previously shown in EAE [61]. CCL2 and CXCL10 were detected predominantly in astrocytes in active MS lesions, similar to EAE [62, 63]. Further, infiltrating inflammatory cells expressed the cognate receptors for these chemokines: CXCR3 (CXCL10 receptor) was localized on lymphocytes in MS lesions, with CCR5 (CCL5 receptor) on lymphocytes, macrophages, and microglial cells in active MS lesions [58, 64].

The cerebrospinal fluid (CSF) is in equilibrium with the extracellular space of the CNS white matter and is separated from the systemic circulation by the blood-CSF barrier [65]. Unlike most animal models of demyelination, human CSF cells and supernatants are accessible for study. A colleague and I recently reviewed opportunities and challenges for examining chemokines and their receptors in such material [55].

In another study, my colleagues and I observed that about 90% of T cells from the CSF of MS patients expressed CXCR3 [64], but levels in corresponding blood samples were significantly lower (<40%). These findings were reproducibly observed in both MS patients and controls. Enrichment of CXCR3⁺ cells in CSF was not specific for MS; similar proportions of CXCR3⁺ T cells in CSF and peripheral blood were found in patients with aseptic meningitis [64].

We made similar comparisons for other chemokine receptors (CCR1–6, CXCR3). Because CSF T cells are enriched for the CD4⁺/CD45RO⁺ subset, corrections were introduced to ensure that comparable cell populations were analyzed in blood and CSF. We then found that CXCR3, but not other receptors, was present at higher levels on CSF cells than on the corresponding population in blood [66]. We also showed enrichment for CCR1⁺/CCR5⁺ monocytes in the CSF as compared with circulation [67]. There were no differences between MS patients and controls without inflammatory CNS disease in regard to expression of chemokine receptors on CSF cells. We interpret this result as indicating that a population of tissue-infiltrating cells is competent to enter the CSF. These cells reenter the circulation, unless specific stimuli provoke their retention. During CNS inflammation, these T cells and monocytes receive signals via chemokine receptors and are retained in the subarachnoid space [55].

CSF chemokine levels differ in MS patients and controls. The differences extend both to inflammatory and noninflammatory controls; further, patients with active MS exhibit dif-

ferent CSF chemokine levels than those with quiescent disease. In particular, increased concentrations of CXCL10 and decreased amounts of CCL2, as compared with levels found in noninflammatory controls, typify the CSF of patients during attacks of MS but not in periods of disease inactivity [64, 68, 69]. Furthermore, reduced CSF CCL2 correlates with likelihood of detecting active (gadolinium-enhancing) lesions on magnetic resonance imaging of the brain [70]. Patients with aseptic meningitis have elevated CXCL10 with raised CSF CCL2 concentrations [70, 71]. Patients with HIV-associated dementia exhibit massive increases in CSF concentration of CCL2 [72]. It has been tempting to speculate that decreased CSF CCL2 in active MS might reflect a polarization of the immune response toward a type 1 cytokine profile as CCL2 is associated with type 2 responses in some experimental systems [73].

Summary

Analysis of the chemokine system promises to provide insight into mechanisms of CNS inflammatory reaction; however, the complexity of neuroinflammation is matched by the diversity and large number of chemokines and chemokine receptors. Nevertheless, significant advantages to study of chemokines are evident: Genetic murine models abound and reagents for analyzing chemokines and their receptors have proliferated in recent years. At this point, it appears feasible to obtain useful information about neurologic disease mechanisms by examining the chemokine system. Of most importance, the availability of small molecule inhibitors or agonists for chemokine receptors provides a near-term practical motivation for continuing these studies.

References

- Lowenstein PR. Immunology of viral-vector-mediated gene transfer into the brain: an evolutionary and developmental perspective. *Trends Immunol* **2002**; 23:23–30.
- Wekerle H, Sun D, Oropeza-Wekerle RL, Meyermann R. Immune reactivity in the nervous system: modulation of T-lymphocyte activation by glial cells. *J Exp Biol* **1987**; 132:43–57.
- Hickey WF, Hsu BL, Kimura H. T-lymphocyte entry into the central nervous system. *J Neurosci Res* **1991**; 28:254–60.
- Hickey WF. Migration of hematogenous cells through the blood-brain barrier and the initiation of CNS inflammation. *Brain Pathol* **1991**; 1:97–105.
- Hickey WF. Basic principles of immunological surveillance of the normal central nervous system. *Glia* **2001**; 36:118–24.
- Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* **1994**; 76:301–14.
- Piccio L, Rossi B, Scarpini E, et al. Molecular mechanisms involved in lymphocyte recruitment in inflamed brain microvessels: critical roles for P-selectin glycoprotein ligand-1 and heterotrimeric G(1)-linked receptors. *J Immunol* **2002**; 168:1940–9.
- Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. *Nature* **1992**; 356:63–6.
- Rubin SA, Yednock TA, Carbone KM. *In vivo* treatment with anti- α 4 in-

- tegrin suppresses clinical and pathological evidence of Borna disease virus infection. *J Neuroimmunol* **1998**;84:158–63.
10. Smith JP, Morris-Downes M, Brennan FR, Wallace GJ, Amor S. A role for $\alpha 4$ -integrin in the pathology following Semliki Forest virus infection. *J Neuroimmunol* **2000**;106:60–8.
 11. Baggiolini M. Chemokines and leukocyte traffic. *Nature* **1998**;392:565–8.
 12. Ransohoff RM, Bacon KB. Chemokine receptor antagonism as a new therapy for multiple sclerosis. *Expert Opin Investig Drugs* **2000**;9:1079–97.
 13. Bacon KB, Harrison JK. Chemokines and their receptors in neurobiology: perspectives in physiology and homeostasis. *J Neuroimmunol* **2000**;104:92–7.
 14. Cyster JG. Chemokines and cell migration in secondary lymphoid organs. *Science* **1999**;286:2098–102.
 15. Qing Z, Sandor M, Radvany Z, et al. Inhibition of antigen-specific T cell trafficking into the central nervous system via blocking PECAM1/CD31 molecule. *J Neuropathol Exp Neurol* **2001**;60:798–807.
 16. Huang D, Han Y, Rani MR, et al. Chemokines and chemokine receptors in inflammation of the nervous system: manifold roles and exquisite regulation. *Immunol Rev* **2000**;177:52–67.
 17. Hickey WF, Kimura H. Perivascular microglial cells of the CNS are bone marrow-derived and present antigen in vivo. *Science* **1988**;239:290–2.
 18. Glabinski AR, Tani M, Strieter RM, Tuohy VK, Ransohoff RM. Synchronous synthesis of alpha- and beta-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. *Am J Pathol* **1997**;150:617–30.
 19. Asensio VC, Campbell IL. Chemokines in the CNS: plurifunctional mediators in diverse states. *Trends Neurosci* **1999**;22:504–12.
 20. Murphy PM, Baggiolini M, Charo IF, et al. International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* **2000**;52:145–76.
 21. Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* **2000**;18:217–42.
 22. Mantovani A. The chemokine system: redundancy for robust outputs. *Immunol Today* **1999**;20:254–7.
 23. Rollins BJ. Chemokines and atherosclerosis: what Adam Smith has to say about vascular disease. *J Clin Invest* **2001**;108:1269–71.
 24. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* **2000**;12:121–7.
 25. Horuk R. Chemokine receptors. *Cytokine Growth Factor Rev* **2001**;12:313–35.
 26. Hesselgesser J, Horuk R. Chemokine and chemokine receptor expression in the central nervous system. *J Neurovirol* **1999**;5:13–26.
 27. Murphy PM. Viral exploitation and subversion of the immune system through chemokine mimicry. *Nat Immunol* **2001**;2:116–22.
 28. McFadden G, Murphy PM. Host-related immunomodulators encoded by poxviruses and herpesviruses. *Curr Opin Microbiol* **2000**;3:371–8.
 29. He J, Chen Y, Farzan M, et al. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **1997**;385:645–9.
 30. Loetscher P, Moser B, Baggiolini M. Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol* **2000**;74:127–80.
 31. Wu DT, Woodman SE, Weiss JM, et al. Mechanisms of leukocyte trafficking into the CNS. *J Neurovirol* **2000**;6(suppl 1):S82–5.
 32. Eng LF, Ghirnikar RS, Lee YL. Inflammation in EAE: role of chemokine/cytokine expression by resident and infiltrating cells. *Neurochem Res* **1996**;21:511–25.
 33. Hulkower K, Brosnan CF, Aquino DA, et al. Expression of CSF-1, c-fms, and MCP-1 in the central nervous system of rats with experimental allergic encephalomyelitis. *J Immunol* **1993**;150:2525–33.
 34. Tani M, Ransohoff RM. Do chemokines mediate inflammatory cell invasion of the central nervous system parenchyma? *Brain Pathol* **1994**;4:135–43.
 35. Ransohoff RM, Hamilton TA, Tani M, et al. Astrocyte expression of mRNA encoding cytokines IP-10 and JE/MCP-1 in experimental autoimmune encephalomyelitis. *FASEB J* **1993**;7:592–600.
 36. Godiska R, Chantry D, Dietsch GN, Gray PW. Chemokine expression in murine experimental allergic encephalomyelitis. *J Neuroimmunol* **1995**;58:167–76.
 37. Glabinski AR, Tani M, Tuohy VK, Tuthill RJ, Ransohoff RM. Central nervous system chemokine mRNA accumulation follows initial leukocyte entry at the onset of acute murine experimental autoimmune encephalomyelitis. *Brain Behav Immun* **1995**;9:315–30.
 38. Gerard C, Rollins BJ. Chemokines and disease. *Nat Immunol* **2001**;2:108–15.
 39. Glabinski AR, Tani M, Strieter RM, Tuohy VK, Ransohoff RM. Synchronous synthesis of alpha- and beta-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. *Am J Pathol* **1997**;150:617–30.
 40. Karpus WJ, Ransohoff RM. Chemokine regulation of experimental autoimmune encephalomyelitis: temporal and spatial expression patterns govern disease pathogenesis. *J Immunol* **1998**;161:2667–71.
 41. Karpus WJ, Lukacs NW, McRae BL, Strieter RM, Kunkel SL, Miller SD. An important role for the chemokine macrophage inflammatory protein-1 alpha in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. *J Immunol* **1995**;155:5003–10.
 42. Tran EH, Kuziel WA, Owens T. Induction of experimental autoimmune encephalomyelitis in C57BL/6 mice deficient in either the chemokine macrophage inflammatory protein-1 α or its CCR5 receptor. *Eur J Immunol* **2000**;30:1410–5.
 43. Karpus WJ, Kennedy KJ. MIP-1 α and MCP-1 differentially regulate acute and relapsing autoimmune encephalomyelitis as well as Th1/Th2 lymphocyte differentiation. *J Leukoc Biol* **1997**;62:681–7.
 44. Huang DR, Wang J, Kivisakk P, Rollins BJ, Ransohoff RM. Absence of monocyte chemoattractant protein 1 in mice leads to decreased local macrophage recruitment and antigen-specific T helper cell type 1 immune response in experimental autoimmune encephalomyelitis. *J Exp Med* **2001**;193:713–26.
 45. Fife BT, Huffnagle GB, Kuziel WA, Karpus WJ. CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis. *J Exp Med* **2000**;192:899–906.
 46. Izikson L, Klein RS, Charo IF, Weiner HL, Luster AD. Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR) 2. *J Exp Med* **2000**;192:1075–80.
 47. Rottman JB, Slavin AJ, Silva R, Weiner HL, Gerard CG, Hancock WW. Leukocyte recruitment during onset of experimental allergic encephalomyelitis is CCR1 dependent. *Eur J Immunol* **2000**;30:2372–7.
 48. Lane TE, Asensio VC, Yu N, Paoletti AD, Campbell IL, Buchmeier MJ. Dynamic regulation of alpha- and beta-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. *J Immunol* **1998**;160:970–8.
 49. Manchester M, Eto DS, Oldstone MB. Characterization of the inflammatory response during acute measles encephalitis in NSE-CD46 transgenic mice. *J Neuroimmunol* **1999**;96:207–17.
 50. Murray PD, Krivacic K, Chernosky A, Wei T, Ransohoff RM, Rodriguez M. Biphasic and regionally-restricted chemokine expression in the central nervous system in the Theiler's virus model of multiple sclerosis. *J Neurovirol* **2000**;6:S44–52.
 51. Ransohoff RM, Wei T, Pavelko KD, Lee JC, Murray PD, Rodriguez M. Chemokine expression in the central nervous system of mice with a viral disease resembling multiple sclerosis: Roles of CD4(+) and CD8(+) T cells and viral persistence. *J Virol* **2002**;76:2217–24.
 52. Liu MT, Keirstead HS, Lane TE. Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. *J Immunol* **2001**;167:4091–7.
 53. Liu MT, Chen BP, Oertel P, et al. The T cell chemoattractant IFN-inducible protein 10 is essential in host defense against viral-induced neurologic disease. *J Immunol* **2000**;165:2327–30.
 54. Fife BT, Kennedy KJ, Paniagua MC, et al. CXCL10 (IFN-gamma-inducible protein-10) control of encephalitogenic CD4⁺ T cell accumulation in the

- central nervous system during experimental autoimmune encephalomyelitis. *J Immunol* **2001**;166:7617–24.
55. Trebst C, Ransohoff RM. Investigating chemokines and chemokine receptors in patients with multiple sclerosis: opportunities and challenges. *Arch Neurol* **2001**;58:1975–80.
 56. Kivisäkk P, Trebst C, Eckstein DJ, Kerza-Kwiatecki AP, Ransohoff RM. Chemokine-based therapies for MS: how do we get there from here? *Trends Immunol* **2001**;22:591–3.
 57. Lucchinetti C, Mandler R, McGavern D, et al. A role for humoral mechanisms in the pathogenesis of Devic's neuromyelitis optica. *Brain* **2002**;125:1450–61.
 58. Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1 α and IP-10 are expressed in demyelinating brain lesions. *Proc Natl Acad Sci USA* **1999**;96:6873–8.
 59. Simpson J, Rezaie P, Newcombe J, Cuzner ML, Male D, Woodroffe MN. Expression of the beta-chemokine receptors CCR2, CCR3 and CCR5 in multiple sclerosis central nervous system tissue. *J Neuroimmunol* **2000**;108:192–200.
 60. Woodroffe N, Cross AK, Harkness K, Simpson JE. The role of chemokines in the pathogenesis of multiple sclerosis. *Adv Exp Med Biol* **1999**;468:135–50.
 61. Hvas J, McLean C, Justesen J, et al. Perivascular T cells express the pro-inflammatory chemokine RANTES mRNA in multiple sclerosis lesions. *Scand J Immunol* **1997**;46:195–203.
 62. McManus C, Berman JW, Brett FM, Staunton H, Farrell M, Brosnan CF. MCP-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: an immunohistochemical and in situ hybridization study. *J Neuroimmunol* **1998**;86:20–9.
 63. Van Der Voorn P, Tekstra J, Beelen RH, Tensen CP, Van Der Valk P, de Groot CJ. Expression of MCP-1 by reactive astrocytes in demyelinating multiple sclerosis lesions. *Am J Pathol* **1999**;154:45–51.
 64. Sørensen TL, Tani M, Jensen J, et al. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J Clin Invest* **1999**;103:807–15.
 65. Weller RO. Pathology of cerebrospinal fluid and interstitial fluid of the CNS: significance for Alzheimer disease, prion disorders and multiple sclerosis. *J Neuropathol Exp Neurol* **1998**;57:885–94.
 66. Kivisäkk P, Trebst C, Liu Z, et al. T-cells in the cerebrospinal fluid express a similar repertoire of inflammatory chemokine receptors in the absence or presence of CNS inflammation: implications for CNS trafficking. *Clin Exp Immunol* **2002**;129:510–8.
 67. Trebst C, Sørensen TL, Kivisäkk P, et al. CCR1⁺/CCR5⁺ mononuclear phagocytes accumulate in the central nervous system of patients with multiple sclerosis. *Am J Pathol* **2001**;159:1701–10.
 68. Sørensen TL, Sellebjerg F, Jensen CV, Strieter RM, Ransohoff RM. Chemokines CXCL10 and CCL2: differential involvement in intrathecal inflammation in multiple sclerosis. *Eur J Neurol* **2001**;8:665–72.
 69. Franciotta D, Martino G, Zardini E, et al. Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. *J Neuroimmunol* **2001**;115:192–8.
 70. Sindern E, Niederkinkhaus Y, Henschel M, Ossege LM, Patzold T, Malin JP. Differential release of beta-chemokines in serum and CSF of patients with relapsing-remitting multiple sclerosis. *Acta Neurol Scand* **2001**;104:88–91.
 71. Lahrtz F, Piali L, Spanaus KS, Seebach J, Fontana A. Chemokines and chemotaxis of leukocytes in infectious meningitis. *J Neuroimmunol* **1998**;85:33–43.
 72. Conant K, Garzino-Demo A, Nath A, et al. Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. *Proc Natl Acad Sci USA* **1998**;95:3117–21.
 73. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ. Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. *Nature* **2000**;404:407–11.