



HAL
open science

The blood-brain barrier, chemokines and multiple sclerosis

David W. Holman, Robyn S. Klein, Richard M. Ransohoff

► **To cite this version:**

David W. Holman, Robyn S. Klein, Richard M. Ransohoff. The blood-brain barrier, chemokines and multiple sclerosis. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2010, 1812 (2), pp.220. 10.1016/j.bbadis.2010.07.019 . hal-00652656

HAL Id: hal-00652656

<https://hal.science/hal-00652656>

Submitted on 16 Dec 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

The blood-brain barrier, chemokines and multiple sclerosis

David W. Holman, Robyn S. Klein, Richard M. Ransohoff

PII: S0925-4439(10)00156-0
DOI: doi: [10.1016/j.bbadis.2010.07.019](https://doi.org/10.1016/j.bbadis.2010.07.019)
Reference: BBADIS 63144

To appear in: *BBA - Molecular Basis of Disease*

Received date: 15 December 2009
Revised date: 9 July 2010
Accepted date: 26 July 2010



Please cite this article as: David W. Holman, Robyn S. Klein, Richard M. Ransohoff, The blood-brain barrier, chemokines and multiple sclerosis, *BBA - Molecular Basis of Disease* (2010), doi: [10.1016/j.bbadis.2010.07.019](https://doi.org/10.1016/j.bbadis.2010.07.019)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The Blood-Brain Barrier, Chemokines and Multiple Sclerosis

David W. Holman¹, Robyn S. Klein^{1,2,3}, Richard M. Ransohoff⁴

Departments of ¹Internal Medicine, ²Pathology and Immunology, ³Anatomy and Neurobiology
Washington University School of Medicine, St. Louis, MO 63110

⁴Neuroinflammation Research Center, Department of Neurosciences, Lerner Research
Institute, Cleveland Clinic, Cleveland, Ohio 44195, USA

Correspondence:

Robyn S. Klein, M.D., Ph.D.

Department of Internal Medicine, Division of Infectious Diseases

Washington University School of Medicine

660 South Euclid Ave

St. Louis, MO 63110

Tel. (314) 286-2140

Fax (314) 362-9230

Address: rklein@id.wustl.edu

Introduction

The infiltration of leukocytes into the central nervous system (CNS) is an essential step in the neuropathogenesis of Multiple Sclerosis (MS). Leukocyte extravasation from the bloodstream is a multistep process that depends on several factors including fluid dynamics within the vasculature and molecular interactions between circulating leukocytes and the vascular endothelium. An important step in this cascade is the presence of chemokines on the vascular endothelial cell surface. Chemokines displayed along the endothelial lumen bind chemokine receptors on circulating leukocytes, initiating intracellular signaling that culminates in integrin activation, leukocyte arrest, and extravasation. The presence of chemokines at the endothelial lumen can help guide the movement of leukocytes through peripheral tissues during normal immune surveillance, host defense or inflammation. The expression and display of homeostatic or inflammatory chemokines therefore critically determines which leukocyte subsets extravasate and enter peripheral tissues. Within the CNS, however, infiltrating leukocytes that cross the endothelium face additional boundaries to parenchymal entry, including the abluminal presence of localizing cues that prevent egress from perivascular spaces. This review focuses on the differential display of chemokines along endothelial surfaces and how they impact leukocyte extravasation into parenchymal tissues, especially within the CNS. In particular, the display of chemokines by endothelial cells of the blood brain barrier may be altered during CNS autoimmune disease, promoting leukocyte entry into this immunologically distinct site. Recent advances in microscopic techniques, including two-photon and intravital imaging have provided new insights into the mechanisms of chemokine-mediated capture of leukocytes within the CNS.

I. CNS Vascular Compartments

Vascular Anatomy

Homeostasis of the CNS is maintained within strict limits by several specializations, both anatomical and immunological, that prevent unrestricted access of cells, solutes and macromolecules to the brain parenchymal tissue. These anatomical specializations consist of a series of barriers, including the blood-cerebrospinal fluid (CSF) barrier that prevents free exchange of solutes between the blood and cerebrospinal fluid [1-3]. The blood-CSF barrier is due not to the presence of a specialized vascular endothelium, but rather to the presence of the choroid plexus epithelium in which the cells are linked by tight junctions and are responsible for secreting CSF into the brain ventricles. The blood brain barrier (BBB), which consists of the endothelial cells of the cerebral microvasculature and surrounding pericytes and astrocytic endfeet, has been well characterized in terms of its morphological and biochemical specializations [2].

While the BBB is typically described as a monolithic entity, there is accumulating evidence of heterogeneity within the BBB [4] that varies depending on the vascular bed as well as the anatomical location of the blood vessels. This heterogeneity is evident in the peripheral vasculature as well and has been shown in gene expression microarray analysis comparing blood vessels of different organs, different caliber vessels, arteries and veins [5], blood vessels and lymphatic vessels [6], and tumor angiogenesis [7]. Relatively less is known about the differences within the cerebral vascular bed; however there appear to be differences in the expression of transporters between different caliber vessels. The efflux transporter P-glycoprotein has been found in cerebral capillaries but not in parenchymal or superficial venules

and arterioles [8], while differences in the expression of the Glut-1 transporter and the Transferrin receptor between different size cerebral vessels has also been noted [4].

The heterogeneity of the vascular endothelium extends to the structural make-up of interendothelial junctions. In early studies, the organization of interendothelial protein strands was found to vary between arterioles, capillaries and venules in the periphery [9, 10]. Freeze fracture investigations revealed that arterioles and capillaries displayed complex, continuous junctional strands while junctional strands were fewer and more loosely organized in venules. Consistent with these observations, Nagy et al. observed that tight junctions of post-capillary brain venules were discontinuous and less complex than those of brain capillaries [11].

Confirmation of these anatomical observations was provided by electrophysiological studies showing that *in situ* transendothelial electrical resistance (TEER) across rat pial arterioles were significantly higher than resistances measured across pial venules (means values of 2050 and 800 Ωcm^2 respectively) [12, 13], suggesting that arteriole tight junctions are more complex, as TEER values correlate positively with tight junction strand number and complexity [14, 15]. In addition, these studies highlight the fact that results of studying electrophysiological and ultrastructural features of pial microvessels may differ from those obtained by examining deeper cerebral cortical microvessels. Allt and Lawrenson reviewed the morphological and molecular differences between pial microvessels and cerebral cortical microvessels [16] and conclude that there are many similarities between these two types of vessels. The authors do note however that tight junctions between cerebral microvascular endothelial cells were marked by consistent fusion of adjacent endothelial cell membranes, while the majority of endothelial cell junctions in pial microvessels were typified by membrane separation at the inter-endothelial cell cleft. Notably, parenchymal but not pial vessels were invested with astrocyte endfeet of the glial

limitans. It is not clear at this time what effect, these differences might have on the barrier properties of microvessels in these CNS compartments.

While there appear to be parallels between pial and parenchymal microvessels, there is a differential display of adhesion molecules involved in leukocyte extravasation between more superficial pial or meningeal vessels and parenchymal vessels of the brain and spinal cord. Specifically, P- and E-selectin have been detected in microvessels of the subarachnoid space, primarily in venules, but not in the deeper parenchymal vessels [17]. This differential pattern of expression is consistent with reports of P-selectin mediated rolling of T-cells along meningeal vessels [18, 19] but not along vessels within the spinal cord white matter where leukocytes do not roll but rather undergo immediate arrest [20]. Some studies have also suggested that regional differences in BBB chemokine expression are important determinants of leukocyte extravasation. In particular, polarized expression of the chemokine CXCL12 at the abluminal endothelial membrane has been identified in both parenchymal arterioles and venules, but CXCL12 relocation in MS and EAE appears to occur exclusively at the level of the post capillary venule and correlates strongly with the perivascular infiltration of T-cells [21, 22]. The reasons for BBB heterogeneity remain unclear, but may relate to functional requirements for different regions of the vascular bed including immune effector responses and the initiation of parenchymal inflammation.

CNS Immune Privilege

The concept of immune privilege developed as a result of studies finding that antigenic material, including foreign tumors and tissue grafts, failed to elicit a systemic, T-cell mediated immunological response when transplanted into certain tissues including the brain parenchyma

and corneal tissue (reviewed in [23, 24]). Subsequent studies confirmed that in addition to xenografts, the immune privilege of the CNS extended to include bacteria, viruses, and viral vectors [25-28]. These observations correlate teleologically with the limited regenerative capacity of the CNS, where tissue injury from an inflammatory response is potentially more damaging than in the periphery. Further, the brain parenchyma is encased within an inexpandable skull and therefore has no ability to accommodate changes in volume brought about by cellular infiltrates and/or swelling.

Presently it is recognized that the CNS immune privilege is not absolute and that foreign material implanted within the CNS can elicit an immune response, albeit one that is delayed and more carefully regulated than in the periphery [1]. In this sense, the CNS is more accurately described as an immunologically specialized site rather than immunologically privileged.

Interestingly, the location of the foreign material within the CNS has a significant effect on the severity of the immune response. The intensity of immune responses increases with proximity to the ventricles of the brain [29-32], and materials implanted within the subarachnoid space and meninges are capable of eliciting a robust immune response. The propensity of these sites to elicit a typical peripheral immune response suggests that the ventricular and subarachnoid CSF may function as sites of physiological immune surveillance. Consistent with this hypothesis, cellular infiltrates that accumulate within the meningeal membranes have been observed to arrange structures resembling secondary lymphoid structures [33], while infiltrates within the parenchyma do not exhibit the features of lymphoid neogenesis. More recently, Kivisakk et al. have shown that CD4⁺ T-cells are restimulated within the subarachnoid space by encounters with MHC class II⁺ antigen presenting cells prior to the onset of inflammation in a murine model of CNS autoimmune disease, experimental autoimmune encephalomyelitis (EAE),

providing further support to the concept of the subarachnoid space and meninges as a site of routine immunological surveillance [34].

II. Chemokines displayed on the surfaces of post-capillary venules lead to the arrest, activation and adhesion of rolling leukocytes

Contact between circulating leukocytes and vascular endothelium is initially promoted by fluid dynamics within blood vessels during a process called margination [35, 36]. Leukocyte margination describes the phenomenon whereby leukocytes flowing in a blood vessel tend to be positioned close to the vascular wall rather than within the rapidly flowing center of the vessel. This phenomenon occurs predominantly at low-caliber post-capillary venules, where blood flows relatively slowly. Further, red blood cells aggregate in the center of the vessel, displacing leukocytes to the periphery where the flow velocity is much lower [35, 37, 38]. Leukocytes at the blood-endothelium interface exhibit enhanced intercellular interactions, facilitating contact and tethering of leukocytes to the endothelial wall.

Rolling along the vascular wall slows the circulating lymphocytes considerably and permits the sampling of the luminal surface of the endothelial cells, leading to activation of integrins on leukocytes resulting in adhesion strengthening and firm arrest [36, 39, 40]. Signals to activate integrins are transduced from the vascular wall to the rolling leukocyte by chemokines displayed on the endothelial lumen. Chemokines bind their receptors on the leukocyte surface and initiate G-protein coupled signaling that results in increased integrin binding affinity as well as clustering at the cell surface [40-43]. Integrin activation leads to enhanced avidity of the adhesion molecule for its endothelial ligands which include members of the Ig-superfamily, specifically VCAM-1 and ICAM-1, as well as fibronectin CS1 epitope (FNCS1).

Chemokines are secreted molecules and are often described in terms of their ability to induce cellular migration along a chemotactic gradient. This idea implies that chemokines present within blood or interstitial fluid form soluble gradients that drive cell movement. In tissues however, chemokines are more commonly associated with extracellular matrix components or bound to cell surface proteoglycans [44, 45]. In this context, the differential display of chemokines along the endothelial surface in association with proteoglycans can provide arrest and migration cues that drive leukocyte migration.

Transendothelial migration can occur via two pathways

Integrin mediated adhesion of circulating lymphocytes to the vascular endothelium is followed by crawling along and then extravasation across the vascular wall. This transmigration step leads to the accumulation of lymphocytes in the perivascular space of the CNS and can occur via paracellular pathways between adjacent endothelial cells or directly through the endothelial cell itself via transendothelial migration. The mechanisms of endothelial transmigration in the peripheral vasculature have been studied intensively and much is now known concerning the interactions between adhesion molecules on extravasating leukocytes and the molecules that make up the interendothelial junctions (for review see [46, 47]).

Platelet endothelial cell adhesion molecule-1 (PECAM-1 or CD31) is a transmembrane protein expressed at interendothelial junctions as well as on neutrophils, monocytes and some subsets of T-cells. PECAM-1 appears to play a role in guiding leukocytes to interendothelial junctions. *In vitro* and *in vivo* studies utilizing PECAM-1 blocking antibodies have demonstrated that blocking homophilic interactions between PECAM-1 on circulating leukocytes and endothelial cells causes leukocytes to accumulate at the apical side of the

endothelium or on the abluminal side between the endothelium and the basement membrane [48-51]. The location of immune cell accumulation in these studies appears to depend upon homophilic interactions between different Ig domains of the PECAM-1 protein located on the luminal and abluminal side of the endothelium.

More recently the protein CD99, ubiquitously expressed on by leukocytes, has been identified at the apical surface of endothelial cells and also at interendothelial junctions. *In vitro* studies with HUVEC monolayers demonstrated that anti-CD99 blocking antibodies cause monocytes to accumulate between endothelial adjacent endothelial cells, with a portion of the monocyte visible on the apical side, while a portion of the monocyte was also detectable on the abluminal side between the endothelium and basement membrane [52, 53]. These results suggest that the expression of CD99 and PECAM-1 along the luminal endothelial surface and at inter-endothelial junctions may be responsible for guiding leukocytes to and through the paracellular pathway.

While many of the relevant molecules in the transmigration cascade have been identified, at present it is not clear to what extent these molecules mediate passage through the transcellular versus the paracellular pathways [54]. Many of the adhesion molecules relevant for transendothelial migration are expressed at the luminal endothelial surface as well as at the interendothelial cell junctions (PECAM-1, CD99, ICAMs), making their relative contributions to these two pathways obscure. Other molecules, such as the junctional adhesion molecules (JAMs), ESAM, and VE-cadherin are expressed exclusively at endothelial cell junctions and may contribute solely to paracellular transmigration. The relative contribution of these pathways, remains to be determined, but likely depends upon numerous variables including the type of

leukocyte extravasating, location within the vascular bed, the target tissue, and the initiating stimulus for transmigration.

Within the CNS, leukocyte entry across the endothelium can take place via the transcellular pathway, leaving interendothelial cell junctions intact [55, 56]. This may be due to the specializations of the endothelial tight junctions at the BBB, where the transmembrane protein strands comprising the junctional domain are more numerous and complex than in the peripheral tissues [11, 57]. T-cells within the CNS interact with the vascular wall either by rolling (superficial, meningeal microvessels) or immediate capture (spinal cord white matter microvessels). However, T-cell transmigration appears to occur exclusively at the levels of the post-capillary venule. The reason for this specificity is not known but may relate to heterogeneity of the BBB with regards to anatomical organization, complexity of the tight junctions and expression of chemokines by the endothelium [4, 58].

The activation state of the vasculature in different brain regions may also be an important determinant of the location of T-cell transmigration. Kivisakk et al. have shown that P- and E-selectin are expressed on meningeal vessels but not parenchymal vessels in humans both with and without CNS inflammation [17]. Further, CD4⁺ T-cells are restimulated by interactions with antigen presenting cells within the subarachnoid space during the early phases of EAE, followed by their proliferation and accumulation within the subarachnoid space and appearance within the spinal cord parenchyma at the onset of EAE signs [34]. Together these studies support the idea that the differential expression of adhesion molecules might direct leukocyte transmigration across superficial subarachnoid blood vessels into the subarachnoid space as an early event during immune effector responses within the CNS [59, 60].

III. Chemokine expression at endothelial cell barriers outside the CNS: lessons from lymphoid and visceral tissues

The expression patterns of endothelial cell chemokines depend on their cellular sources and whether they are translocated from abluminal to luminal surfaces of venules where they can exert actions towards circulating leukocytes. A critical aspect of chemokine function, therefore, is their localization along endothelial cell surfaces and extracellular matrix. In this section, we will discuss the evidence that immobilized chemokines play a role in leukocyte selection for entry and response within lymphoid and visceral tissues during immune surveillance and in response to inflammatory cues.

Chemokine display in lymphoid tissues

The discovery of chemokines as agents of leukocyte mobility and directed migration led to numerous *in vitro* analyses examining the movement of cells towards increasing concentrations of soluble chemokines using Boyden chambers [61]. Although these studies were critical for elucidating many aspects of chemokine biology, they fostered the notion that leukocytes respond to soluble chemokine gradients *in vivo*. It is now apparent that chemokines immobilized on endothelium and extracellular matrix direct rolling leukocytes to firmly adhere to the endothelium through integrin activation [62]. Interactions with surface matrix molecules are believed to provide a mechanism to concentrate and localize chemokines, preventing their rapid diffusion into blood and tissue fluids with subsequent loss of immunological effects [63]. Also, because sheer stress, as demonstrated by experiments performed under physiological flow conditions [64-66], is critical for chemokine-mediated integrin activation, arrest and crawling of

leukocytes, chemokine immobilization is likely to be crucial for the recruitment of leukocytes across endothelial cell barriers

Although the extracellular matrix molecules responsible for chemokine binding are unknown, chemokines are highly basic proteins, which typically interact well with glycosaminoglycans (GAGs), acidic, sulfated linear polysaccharides that are major components of extracellular matrix. Although there are at least six major classes of GAGs, studies examining chemokine interactions with this group of molecules have focused primarily on binding to heparin [67]. Many chemokines contain well-established heparin-binding domains and exhibit conformational changes upon interaction with heparan sulfate proteoglycans [68-70]. In *in vitro* systems, immobilized heparin mediates chemokine oligomerization while solubilized heparin displaces membrane-associated chemokines from cultured endothelial cells and decreases the transendothelial migration of T cells [71, 72]. Mutations that inhibit binding to heparin do not affect chemokine binding to cognate receptors yet diminishes their ability to induce cell migration *in vivo* [73-75]. Indeed, systemic heparinization of patients leads to increased plasma levels of the proinflammatory chemokines CXCL9, CXCL10 and CXCL11, which decrease upon subsequent administration of the heparin antagonist protamine [72]. Chemokine classes exhibit rank order of affinity for GAGs and single chemokines may exhibit different forms of GAG-binding epitopes, suggesting additional regulatory mechanisms for chemokine display along the extracellular matrix [67, 76]. These data confirm the importance of chemokine oligomerization and GAG binding and support the notion that chemokines are not likely to exert their *in vivo* effects via soluble concentration gradients.

Leukocyte recruitment into peripheral tissues

The migration of leukocytes into peripheral tissues relies on the combined expression of chemokine receptors, adhesion molecules and lipid chemoattractant receptors. These receptors allow leukocytes to interact with endothelial cell barriers and gain access to parenchymal tissues as part of immunosurveillance or control of pathogens. Several of these adhesion molecules have been shown to convey tissue-specific homing properties to memory T cells, such as CCR7 and CD62L into lymphoid tissues, cutaneous lymphocyte-associated antigen (CLA), CCR4 and CCR10 into skin and $\alpha 4\beta 7$ integrin and CCR9 into gut [77]. During inflammation, a stereotypical pattern of adhesion and chemokine receptor expression occurs on leukocytes that generally match the inflammatory-mediated induction of proinflammatory chemokine ligands and adhesion receptors at endothelial cell barriers. Thus, migration of leukocytes occurs across inflamed endothelium, which display one or more chemokines immobilized along the luminal surface via binding to GAGs. In support of this, inactivation of genes involved in heparin sulfation resulted in decreased leukocyte extravasation in inflammatory models with a reduction in L-selectin-mediated adhesion and in the transcytosis and binding of chemokines to endothelial cells [78]. Circulating leukocytes are recruited into inflamed tissues mainly in small vessels such as capillaries and venules. Consistent with this, chemokine expression is higher in microvascular endothelium than in aortic endothelial cells. Low shear stress was found to activate endothelial chemokine production via cell surface heparan sulfates, beta3-integrins, focal adhesion kinase, MAP kinase p38beta, mitogen- and stress-associated protein kinase-1 [79].

During inflammation vascular endothelium in most tissues upregulate E- and P-selectin and chemokine ligands of chemokine receptors expressed by cells of both innate and adaptive immune responses. While some of these chemokines are up-regulated at the mRNA level in

response to pro-inflammatory cytokines, the endothelium of certain tissues also stores chemokines within Weibel-Palade bodies (WPBs). WPBs are organelles that stores molecules, such as P-selectin, that can be rapidly released in response to inflammatory cues, such as TNF- α , to mediate tethering and rolling of leukocytes. CXCL8, for example, is found in WPBs of intestinal and nasal mucosal endothelium, and is rapidly released in response to histamine or thrombin [80-82]. Although microvascular endothelium within the CNS does not contain stored P-selectin, despite that fact that they contain WPBs [83, 84], IL-1 β and TNF- α have been shown to lead to P-selectin upregulation, providing a means for tethering of leukocytes at the endothelium specifically during inflammatory states. Chemokines also induce leukocyte movement to inter-endothelial junctions where they extend their processes to seek abluminal chemokines, which then localize transendothelially migrating leukocytes to perivascular sites where GAG-immobilized chemokines establish haptotactic gradients [66, 85].

The endothelial display of several proinflammatory chemokines has been studied in detail. CXCL10 is selectively upregulated by injured renal microvascular endothelium within tubulointerstitial area while CCL2 is upregulated at the glomerulus and tubulointerstitium, the former correlating with T cell infiltrates and the latter with monocyte entry at these distinct sites. Anti-CXCL10 antibodies decreased T cell infiltration and improved renal function [86, 87].

DARC is expressed on endothelial cells of capillaries and postcapillary venules and on epithelial cells within the inflamed synovium, kidney and lung. During inflammation, endothelial DARC is involved in the transport of chemokines from the inflamed tissue to the luminal side of the endothelium, facilitating leukocyte recruitment and aggravating inflammation. In the lung, chemokines are presented on the pulmonary endothelium, causing neutrophils to adhere and transmigrate into the lung parenchyma [88]. DARC was recently

shown to limit PMN migration into the alveolar space via RBC-mediated chemokine sequestration. Use of bone-marrow chimeric mice using wild-type vs. DARC-deficient mice, however, did not reveal a role for endothelial cell DARC in leukocyte trafficking [89]. An alternative method of chemokine display along endothelial surfaces is exhibited by CX₃CL1. CX₃CL1 is released from the cell surface by constitutive and inducible protease cleaving [90]. Once cleaved, CX₃CL1 is not captured by the extracellular matrix, suggesting it exerts its effects as a mucin stalk or in solution [91]. CX₃CL1 is expressed by TNF- α - and IL-1-activated endothelium within the kidney and heart where it induces integrin-independent leukocyte capture, acting as an adhesion molecule [92-94]. In the CNS, CX₃CL1 is expressed by neurons and recent data suggest it is important for the recruitment of NK cells at this site [95, 96], as discussed below.

IV. Expression and function of chemokines and chemokine receptors at the BBB

Compared to peripheral tissues, less is known regarding the expression and regulation of lymphoid and inflammatory chemokines within the brain microvasculature. Several homeostatic or lymphoid chemokines are known to be expressed by the blood vessels of the CNS, including CCL19, CCL21, and CXCL12 as discussed in detail below. In contrast to the lymphoid chemokines, the expression of inflammatory chemokines has not been as well characterized at the human or murine BBB *in vivo*, however several inflammatory chemokines have been identified in *in vitro* preparations of brain microvascular endothelial cells including CCL2 [97-99], CCL4 and CCL5 [100]. These molecules bind chemokine receptors expressed by activated mononuclear cells including CCR1, CCR2, and CCR5 [101]. Use of mice with targeted deletions of these chemokines and their receptors revealed a role for CCL2 and CCR2 in the

induction of EAE via effects on infiltrating monocytes [102-110]. Studies examining both mice and individuals without functional CCR5 have excluded a role for this receptor in susceptibility to EAE (in mice) or MS (in humans) [111, 112]. Further research utilizing *in vitro* as well as *in vivo* approaches is necessary to elucidate the role of inflammatory chemokines in neuroinflammation and CNS autoimmunity. The remainder of this section focuses on the homeostatic chemokines that have been identified within the CNS microvasculature and their potential role in CNS inflammation.

CCR7 and CCL19/CCL21

The chemokines CCL19 and CCL21 are known as homeostatic, or lymphoid, chemokines due to their role in guiding B-cells, naïve T-cells, and mature dendritic cells (DCs) into lymphoid tissue under physiological conditions [113, 114]. Within the CNS the expression of lymphoid chemokines has been examined under normal as well as neuroinflammatory conditions such as MS and its mouse model EAE. Alt et al., using SJL mice with actively induced EAE, examined the expression of an array of chemokines in brain and spinal cord tissue sections[115]. While several chemokines were expressed in parenchymal tissue and inflammatory cells during EAE, only CCL19 mRNA transcripts were constitutively expressed on the endothelial cells of post-capillary venules in normal as well as EAE brains and spinal cords. In addition, CCL21 transcripts were induced at post-capillary venules during EAE. Further, the authors of this study found that encephalitogenic T-cells stained positively for CCR7 and CXCR3 and that adhesion of these T-cells to frozen sections from EAE brains was inhibited by preincubation with CCL19 and CCL21. The expression of these lymphoid chemokines at the BBB and their ability to attract CCR7 expressing encephalitogenic T-cells led the authors of this study to speculate that

these typically homeostatic chemokines may contribute to maintaining chronic CNS inflammation in EAE.

The role of CCL19 and CCL21 in EAE was further characterized in relapsing-remitting and chronic relapsing models in SJL and Biozzi AB/H mice respectively [116]. CCL19 mRNA levels in CNS tissue homogenates were found to be low under normal condition but increased with disease progression in both models, while CCL21 levels were low to undetectable under all conditions. Further, CCL19 protein was expressed by some astrocytes and microglia within the parenchyma and immune cells accumulating in the meninges, but not the endothelial cells of post-capillary venules. CCL21 protein expression was not detected, except in meningeal vessels of the spinal cord in some atypical cases in which mice developed chronic stable EAE. The expression of these lymphoid chemokines in the CNS during EAE led these authors to suggest that CCL19, and to a lesser extent CCL21, may function to signal circulating lymphocytes that would normally home to the lymphoid tissues. The expression of these chemokines during neuroinflammation would then help to retain CCR7 positive T-cells within the CNS and favor interaction with mature DCs. CCL19 and CCR7 interaction in the CNS in EAE may thus be responsible for the local retention of memory T-cells and contribute to maintaining cycles of neuroinflammation.

Expression of the chemokines CCL19 or CCL21 in transgenic mice led to CNS inflammation when CCL21, but not CCL19, was ectopically expressed by oligodendrocytes [117]. In mice expressing the CCL21 construct, approximately 70% displayed motor deficits and tremors and did not survive past 4 weeks, while CCL19 expressing mice showed no such clinical signs and developed normally. CCL21 expressing mice were found to have leukocytic infiltrates that were predominantly neutrophils and eosinophils, reactive gliosis of microglia and

astrocytes, and defects in myelin sheaths that resulted in hypomyelination of the parenchyma and spinal cord. Interestingly, expression of CCL21 did not lead to lymphocyte infiltration, indicating that the expression of CCL21 alone is not sufficient to promote lymphocyte trafficking to the CNS. The ability of CCL21, but not CCL19, to induce CNS inflammation led the authors to speculate that CCL21 may be acting through a distinct chemokine receptor from the shared CCR7 receptor, possibly through the receptor CXCR3, which can be expressed by microglia [118-121].

Kivisakk et al. examined the expression of CCR7, CCL19 and CCL21 in brain autopsy material and CSF samples from MS patients [122]. These authors found large numbers of CCR7+ cells in the inflammatory cuffs of MS lesions, a subpopulation of which also expressed MHC type II and CD86, indicating antigen presenting competence and suggesting that these cells may be maturing DC's. However, in contrast to previously reported EAE models, CD3 positive T-cells in acute and chronic MS lesions did not express CCR7. Further, within the CSF of MS patients the majority (>90%) of T-cells, expressing a central memory phenotype, also expressed CCR7. In pooled CSF samples of patients with inflammatory neurological diseases, including MS, a subpopulation of CSF DCs (~33%) expressed CCR7. Overall, however, DCs comprised about 0.5% of CSF cells. This study also reported no CCL19 or CCL21 protein expression in endothelial or parenchymal cells of non-lesioned white matter or in active or chronic MS lesions, but did find strong CCL21 immunoreactivity within the choroid plexus epithelium. The authors of this paper suggest a model in which activated microglia and parenchymal macrophages express signals, including CCR7, associated with maturing DCs. These CCR7 positive cells are available to restimulate central memory T cells locally. Subpial macrophages might also transport myelin associated antigens from the CNS via the CSF to deep cervical lymph nodes.

According to this scenario, after priming in the periphery, central memory T cells enter the CSF, in part via signaling between CCR7 and CCL21 or CCR6 and CCL20 expressed by the choroid plexus epithelium [123, 124] and are stimulated to differentiate into effector type upon restimulation by APCs within the meninges.

In humans, CCL19 and CCL21 expression levels have also been determined in tissue samples of patients with neuroinflammation including MS as well as other inflammatory neurological disorders [125]. Under normal conditions, CCL19 transcripts were detected in brain tissue homogenates, while expression levels were elevated in homogenates from active and inactive MS lesions. CCL21 expression levels were very low under all conditions. CCL19 protein levels in lysates of brain tissue as well as CSF samples were found to be elevated in MS. Despite the presence of low levels of CCL21 mRNA transcripts in CNS tissue, no CCL21 protein was detected in brain tissue lysates or CSF samples from normal or MS patients. The authors did however report positive CCL21 staining of brain endothelial cells. The authors of this study attribute the differences in CCL21 expression levels between human and animal models of disease to the presence of one CCL21 gene in humans, compared to the presence of 2-3 CCL21 genes in mice. Based on the constitutive expression of CCL19 within the CNS tissues, the authors speculate that this lymphoid chemokine may function in physiological immune surveillance of the CNS by recruiting and retaining CCR7 expressing T cells. Further the authors of this study propose that the expression of CCL19 also functions to recruit other types of immune cells to the CNS including B-cells which also express the chemokine receptor CCR7.

CXCR4/CXCR7 and CXCL12

The chemokine CXCL12, or stromal cell-derived factor 1 (SDF-1), is a potent chemoattractant for monocytes and lymphocytes [126]. CXCL12 is expressed as three alternatively spliced isoforms within the CNS, with CXCL12 α expressed by neurons and CXCL12 β and CXCL12 γ expressed by endothelial cells [127]. In the periphery CXCL12 and its receptor CXCR4 play important roles in the patterning of the immune system, as embryonic expression of CXCL12 plays a role in chemoattraction and proliferation of CXCR4 expressing B- and T-cell precursors. Similarly, CXCL12 and CXCR4 are involved in patterning and plasticity of the central nervous system both during development as well as in the modulation of synapse formation [128]. These parallel roles for CXCL12 and CXCR4 in immune and nervous system function suggest an avenue for crosstalk and communication between these two systems (reviewed by [129]).

Within the central nervous system, the expression patterns for CXCL12 and CXCR4 are widespread and include the cortex, olfactory bulb, hippocampus, cerebellum, meninges, and the endothelium of the BBB. Further, CXCR4 expression has been detected in numerous types of cells including astrocytes, microglia, oligodendrocytes, neurons, and endothelial cells of the BBB [130-133]. Interestingly, these studies have identified the endothelium of the BBB as a source of the chemokine ligand CXCL12 as well as its receptor CXCR4, suggesting a role for CXCL12 in recruiting CXCR4+ circulating lymphocytes but also a potential feedback mechanism for CXCR4 expressed at the BBB.

Krumbholz et al. identified CXCL12 protein expression along the blood vessels of the BBB as well as in astrocytes and noted that CXCL12 expression was increased in both inactive and active MS lesions [133]. These initial observations of CXCL12 expression at the BBB were expanded by McCandless et al. who studied the role of CXCL12 at the BBB of mice in an active

immunization model of EAE [22]. CXCL12 message was expressed in unimmunized mice and levels were significantly increased in mice at peak of clinical disease. CXCL12 expression was localized to the vasculature of the spinal cord where CXCL12 protein was detected along the abluminal surface of the endothelial cells of the BBB in unimmunized mice and mice 10 days post-immunization. In contrast at 14 days post-immunization, during peak of disease, CXCL12 polarity is lost and its localization shifts toward a more luminal expression pattern, which was associated with dense perivascular infiltrates of CXCR4 positive mononuclear cells. Treatment of mice with the CXCR4 antagonist AMD3100 during the induction of EAE led to significant worsening of disease associated with more widespread parenchymal infiltration, increased demyelination, and larger lesion areas. Similar studies in a viral model of encephalitis produced identical results with regard to CXCR4+ leukocyte trafficking, but in this case associated with improved viral clearance (McCandless, Zhang et al. 2008). Taken together these results suggest that abluminal expression of CXCL12 at the CNS vasculature is important in restricting the parenchymal access of CXCR4-expressing immune cells, which are instead localized to perivascular spaces.

A subsequent study extended these findings to human neuroinflammation, comparing CXCL12 and CXCR4 expression in tissue specimens from MS and non-MS patients [21]. Similar to mice, in non-MS patients and uninflamed regions of the MS brain, CXCL12 expression was polarized primarily along the parenchymal facing surface of the endothelial BBB in both arterioles and venules of the CNS. Consistent with EAE in mice, CXCL12 polarity was lost in CNS venules of MS patients, with CXCL12 expression shifting from the abluminal to luminal endothelial surface, while CXCL12 polarity was maintained in CNS arterioles. Morphometric analysis indicated that CXCL12 relocation was associated with the presence of

perivascular infiltrates within active MS lesions, which exhibited activated CXCR4, as detected with an antibody that recognizes the phosphorylated form of the receptor. The display of CXCL12 was also associated with the detection of activated CXCR4 on leukocytes within the blood (McCandless, Piccio et al. 2008), suggesting that relocation of CXCL12 not only promotes the egress of leukocytes from perivascular spaces but also increases their capture at the BBB.

Mechanistic details of CXCL12 relocation at the BBB remain unclear, but recent evidence has pointed to a role for the pro-inflammatory cytokine IL-1 β [134]. Injection of mice with IL-1 β induces pathologic relocation of CXCL12 at the BBB while injection of TNF- α does not. Further, IL-1 β receptor knockout mice are protected from EAE and do not exhibit CXCL12 relocation, while TNF- α receptor knockout mice showed loss of CXCL12 polarity.

Until recently it was believed that CXCL12 mediated its effects exclusively via interactions with CXCR4, however the receptor CXCR7, formerly the orphan receptor RDC1, has now been shown to bind CXCL12 as well as CXCL11 [135, 136]. While CXCR7/RDC1 possesses homology with conserved domains of G-protein coupled receptors and is structurally similar to other CXC receptors, ligand binding does not initiate typical intracellular signaling pathways. In particular, CXCR7 binds CXCL12 with high affinity but ligand binding does not induce intracellular calcium mobilization or cell migration. However CXCR7 expression does confer growth and survival advantages and increased adhesive strength in vitro [136, 137]. Other studies investigating the role of CXCR7 as a signaling receptor have given conflicting results [135, 138-142].

Another potential role of CXCR7 in regulating CXCL12 mediated signaling may involve the ability of CXCR7 to bind to and sequester CXCL12, regulating local CXCL12 levels in the extracellular space. Boldajipour et al. demonstrated that CXCR7 expression was critical in

regulating primordial germ cell migration in the developing zebrafish by its ability to bind and internalize CXCL12 [143, 144]. CXCR7 expression within the somatic environment functioned to sequester and regulate the extracellular level of CXCL12 and thereby control the migration of CXCR4 expressing primordial germ cells. Studies demonstrating such effects in mammals have not been reported.

CXCR7 expression within the CNS of rats was detected via *in situ* hybridization, with CXCR7 mRNA transcripts identified in the ventricular ependyma, the choroid plexus, neuronal and astroglial cells as well as cells of the vasculature [145, 146]. After cerebral ischemia, CXCR7 mRNA levels increased along some blood vessels suggesting that CXCR7 may be involved in angiogenesis after ischemia, a role that would be consistent with previous reports of CXCR7 expression during cardiac development [138] and tumor angiogenesis [147]. While these studies have identified the CNS vasculature as a source of CXCR7 within the CNS, the function of CXCR7 in regulating lymphocyte trafficking into the central nervous system in normal as well as pathological conditions remains undefined. Importantly, CXCR7 expression at the mRNA level is not uniformly followed by protein production, indicating translational control.

Some clues however may be provided by a recent study by Zabel et al. that described a role for CXCR7 in regulating CXCL12/CXCR4 mediated transendothelial migration (TEM) [148]. Using human umbilical vein endothelial cells (HUVECs) grown on transwell membrane inserts, the authors demonstrated that CXCR7 was expressed on the endothelial surface via flow cytometry, although it was not noted whether receptor expression was restricted to the luminal or abluminal side of the cells. TEM assays clearly demonstrated an essential role for CXCR7 in guiding CXCR4/CXCL12 mediated migration of human tumor cells expressing both CXCR4 and CXCR7 receptors. Further, the endogenous ligand CXCL11 or a novel small molecule

CXCR7 receptor antagonist CCX771 were capable of binding endothelial CXCR7 and inhibiting TEM. Interestingly, although the migration was driven by CXCR4 –CXCL12 interactions, the CXCR7 antagonist CCX771 was more than 100 times more potent at inhibiting TEM than the CXCR4 receptor antagonist AMD3100. Taken together these results suggest an important role for CXCR7 in mediating the TEM of circulating tumor cells. Whether this receptor plays a similar role in mediating the TEM of circulating immune cells remains to be determined.

Innate immune cell entry into the CNS

Several chemokines play roles in the recruitment of innate immune cells including natural killer (NK) cells, neutrophils and monocytes. NK cells are cytotoxic lymphocytes that destroy cells via granzyme- and perforin-mediated apoptosis (Yokoyama WM, Altfeld M, Hsu KC. Biol Blood Marrow Transplant. 2009). NK cells express several chemokine receptors including CCR4, CCR7, CXCR4, and CX₃CR1 and play important roles in immune responses directed against tumor and virally infected cells [149]. Studies utilizing the EAE model indicate that CX₃CR1 is required for the trafficking of regulatory NK cells into the CNS, which refrain disease severity [96]. Consistent with this, CX₃CR1-deficiency was recently associated with increased disease severity in experimental autoimmune uveitis [150]. In contrast, neutrophil recruitment to the CNS in the setting of EAE is associated with worsening disease severity [151], promoting leukocyte infiltration and extensive demyelination. Neutrophils, granulocytes traditionally associated with clearance of bacterial pathogens, express CXCR1 and CXCR2, which bind CXCL1 and CXCL2, respectively. Recent studies identifying molecular cues involved in neutrophil recruitment indicate that Th17 expressed by infiltrating CD4⁺ T cells mediates the CNS expression of CXCL1 and CXCL2, which are required for CNS neutrophil

recruitment and induction of EAE [152]. These authors also demonstrated that neutrophil depletion prevented induction of EAE and that transfer of wild-type neutrophils into CXCR2^{-/-} mice, which are resistant to EAE, restored susceptibility.

Chronic over-expression of the inflammatory cytokine IL-1 β within the central nervous system using a transgenic mouse model led to BBB disruption, hippocampal CCL2 expression, and infiltration of leukocytes including T-cells, macrophages, DCs, and neutrophils [153]. Interestingly, leukocyte entry into the CNS did not lead to overt neuronal degeneration despite neutrophil infiltration that persisted as long as one year after induction of IL-1 β expression. Neutrophil infiltration in this study also coincided with hippocampal up-regulation of the neutrophil chemoattractants CXCL1 and CXCL2. Induction of IL-1 β in mice lacking CXCR2, the neutrophil receptor for CXCL1 and CXCL2, led to a 96% reduction of neutrophil infiltration, but did not significantly reduce BBB disruption. Taken together these results suggest that IL-1 β is a potent stimulus for leukocyte infiltration to the CNS including the recruitment of neutrophils, however IL-1 β expression alone is not sufficient to induce neuron inflammation or degeneration.

Within the CNS, microglia act as the main form of immune defense and share many phenotypical and functional characteristics of peripheral macrophages [154]. In many CNS pathologies, including neuroinflammation, microglia alter their morphology and phenotype and proliferate rapidly, a phenomena termed microgliosis [155, 156]. While the response of microglia to CNS inflammation has been well-characterized, the origin of these cells remains controversial. Many studies have relied upon bone marrow chimeras produced by irradiating mice to kill the host's bone marrow cells and injecting labeled bone marrow cells from a non-irradiated donor. A drawback to these studies is that injecting bone marrow cells into the peripheral circulation may lead to the non-physiological presence of hematopoietic precursors

persisting within the circulation. Additionally, irradiation has been shown to lead to the induction of inflammatory cytokines and BBB disruption [157, 158] which may permit the entry of hematopoietic cells that would otherwise be excluded from the CNS.

To circumvent these issues, Ajami et al. used parabiosis to join the circulatory system of two mice, one of which expressed GFP in all non-erythroid hematopoietic cells [159]. In parabiotic recipients, 30-40% of the circulating hematopoietic cells became GFP positive. Despite the presence of GFP positive hematopoietic cells within the circulation, the authors were unable to demonstrate the presence of GFP positive microglial cells within the CNS parenchyma under normal conditions. Additionally, there were no GFP positive cells within the CNS parenchyma during both acute and chronic models of CNS microgliosis, indicating that CNS microglia are capable of rapid and sustained self-renewal.

Further these authors demonstrated that irradiation of the parabiotic recipient, but not the donor, did not induce the infiltration of peripheral hematopoietic cells into the CNS. The results of this study suggest that the presence of donor-derived microglial cells in the recipient CNS parenchyma reported in previous bone marrow chimera studies [160-164] may result from the non-physiological introduction of bone marrow progenitor cells into the peripheral circulation, along with cranial irradiation. Mildner et al. investigated the role of radiation treatment in bone chimera studies using a targeted radiation approach whereby mice received selective-body radiation that spared the head [165]. Microglial populations in these protected mice excluded peripheral blood derived macrophages under normal conditions as well as during acute microgliosis and chronic neuroinflammation (using cuprizone induced corpus callosum demyelination). Together the studies by Ajami et al. and Mildner et al. demonstrate that CNS irradiation is necessary but not sufficient to permit blood myeloid cells in the periphery to cross

the BBB into the CNS and reside as parenchymal microglia. Finally, in irradiated bone marrow chimeras and adoptive transfer experiments Mildner et al. identified peripheral monocytes expressing Ly-6C and CCR2 as being preferentially recruited to the CNS during microgliosis and inflammation. The authors identify this monocyte subpopulation as the direct precursor to parenchymal macrophages with microglial morphology in the adult brain, suggesting that these cells may be used as vehicles to deliver genes or other therapeutics across the BBB to the CNS.

Concluding Remarks

In this review we have outlined the role of chemokines in providing cues to extravasating leukocytes, directing their movement into peripheral tissues as well as those of the CNS. The immobilization of chemokines along the endothelial surface via binding to cell surface proteoglycans and extracellular matrix proteins provides important localizing cues for circulating immune cells. Further, the presentation and translocation of these chemokines between luminal and abluminal endothelial surfaces are critical in promoting leukocyte adhesion and transendothelial migration into secondary lymphoid organs or peripheral tissues. Within the CNS, less is known regarding the mechanisms and movement of chemokines along the cerebral vasculature in normal and pathological conditions. Studies on the role of chemokines in neuroinflammation suggest a role for homeostatic chemokines including CCL19, CCL21 and CXCL12 in autoimmune disease models, such as EAE, and in the important neurologic condition MS. CXCL12 expression in the normal CNS is restricted to the abluminal or parenchymal facing vascular surface, while in MS CXCL12 translocates toward the vessel lumen leading to parenchymal infiltration and subsequent demyelination. These studies suggest that abluminal CXCL12 expression may be important in restricting parenchymal tissue inflammation within the

CNS. The role of inflammatory chemokines at the CNS microvasculature remains undefined and is primarily limited to *in vitro* studies. Regardless, it is clear that the localization of chemokines within the CNS microvasculature is important in controlling leukocyte access to parenchyma and the molecular mechanisms regulating the movements of these chemokines in neuroinflammation will be crucial in developing novel therapeutics specifically directed towards diseases of disrupted immune privilege, such as MS.

Figure Legend**Figure 1: CXCL12 receptors regulate leukocyte access to CNS parenchyma.**

Within the CNS, CXCL12 expression is localized to the microvasculature along the parenchymal facing surface of endothelial cells under physiological conditions. This abluminal expression pattern of CXCL12 functions to retain CXCR4 and CXCR7 expressing leukocytes within the perivascular space, preventing their access to the brain parenchyma proper. In neuroinflammatory conditions such as the animal model EAE and in human MS, CXCL12 polarization is lost and CXCL12 translocates towards the vessel lumen. CXCR4 and CXCR7 expressing leukocytes lose their localizing cues and can infiltrate further into the parenchymal tissues subsequently inducing demyelination. Further, display of CXCL12 along the luminal endothelial surface may provide further signaling to circulating CXCR4/CXCR7⁺ T-cells leading to increased T-cell recruitment and transendothelial migration.

References

- [1] R.M. Ransohoff, P. Kivisakk and G. Kidd, Three or more routes for leukocyte migration into the central nervous system, *Nat Rev Immunol* 3 (2003) 569-81.
- [2] N.J. Abbott, L. Ronnback and E. Hansson, Astrocyte-endothelial interactions at the blood-brain barrier, *Nat Rev Neurosci* 7 (2006) 41-53.
- [3] C.E. Johanson, J.A. Duncan, 3rd, P.M. Klinge, T. Brinker, E.G. Stopa and G.D. Silverberg, Multiplicity of cerebrospinal fluid functions: New challenges in health and disease, *Cerebrospinal Fluid Res* 5 (2008) 10.
- [4] S. Ge, L. Song and J.S. Pachter, Where is the blood-brain barrier ... really?, *J Neurosci Res* 79 (2005) 421-7.
- [5] J.T. Chi, H.Y. Chang, G. Haraldsen, F.L. Jahnsen, O.G. Troyanskaya, D.S. Chang, Z. Wang, S.G. Rockson, M. van de Rijn, D. Botstein and P.O. Brown, Endothelial cell diversity revealed by global expression profiling, *Proc Natl Acad Sci U S A* 100 (2003) 10623-8.
- [6] S. Podgrabinska, P. Braun, P. Velasco, B. Kloos, M.S. Pepper and M. Skobe, Molecular characterization of lymphatic endothelial cells, *Proc Natl Acad Sci U S A* 99 (2002) 16069-74.
- [7] B. St Croix, C. Rago, V. Velculescu, G. Traverso, K.E. Romans, E. Montgomery, A. Lal, G.J. Riggins, C. Lengauer, B. Vogelstein and K.W. Kinzler, Genes expressed in human tumor endothelium, *Science* 289 (2000) 1197-202.
- [8] D. Virgintino, D. Robertson, M. Errede, V. Benagiano, F. Girolamo, E. Maiorano, L. Roncali and M. Bertossi, Expression of P-glycoprotein in human cerebral cortex microvessels, *J Histochem Cytochem* 50 (2002) 1671-6.
- [9] M. Simionescu, N. Simionescu and G.E. Palade, Segmental differentiations of cell junctions in the vascular endothelium. Arteries and veins, *J Cell Biol* 68 (1976) 705-23.
- [10] M. Simionescu, N. Simionescu and G.E. Palade, Segmental differentiations of cell junctions in the vascular endothelium. The microvasculature, *J Cell Biol* 67 (1975) 863-85.
- [11] Z. Nagy, H. Peters and I. Huttner, Fracture faces of cell junctions in cerebral endothelium during normal and hyperosmotic conditions, *Lab Invest* 50 (1984) 313-22.
- [12] A.M. Butt and H.C. Jones, Effect of histamine and antagonists on electrical resistance across the blood-brain barrier in rat brain-surface microvessels, *Brain Res* 569 (1992) 100-5.
- [13] A.M. Butt, H.C. Jones and N.J. Abbott, Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study, *J Physiol* 429 (1990) 47-62.
- [14] P. Claude, Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens, *J Membr Biol* 39 (1978) 219-32.
- [15] P. Claude and D.A. Goodenough, Fracture faces of zonulae occludentes from "tight" and "leaky" epithelia, *J Cell Biol* 58 (1973) 390-400.
- [16] G. Allt and J.G. Lawrenson, Is the pial microvessel a good model for blood-brain barrier studies?, *Brain Res Brain Res Rev* 24 (1997) 67-76.
- [17] P. Kivisakk, D.J. Mahad, M.K. Callahan, C. Trebst, B. Tucky, T. Wei, L. Wu, E.S. Baekkevold, H. Lassmann, S.M. Staugaitis, J.J. Campbell and R.M. Ransohoff, Human cerebrospinal fluid central memory CD4+ T cells: evidence for trafficking through

- choroid plexus and meninges via P-selectin, *Proc Natl Acad Sci U S A* 100 (2003) 8389-94.
- [18] S.M. Kerfoot and P. Kubes, Overlapping roles of P-selectin and alpha 4 integrin to recruit leukocytes to the central nervous system in experimental autoimmune encephalomyelitis, *J Immunol* 169 (2002) 1000-6.
- [19] L. Battistini, L. Piccio, B. Rossi, S. Bach, S. Galgani, C. Gasperini, L. Ottoboni, D. Ciabini, M.D. Caramia, G. Bernardi, C. Laudanna, E. Scarpini, R.P. McEver, E.C. Butcher, G. Borsellino and G. Constantin, CD8⁺ T cells from patients with acute multiple sclerosis display selective increase of adhesiveness in brain venules: a critical role for P-selectin glycoprotein ligand-1, *Blood* 101 (2003) 4775-82.
- [20] P. Vajkoczy, M. Laschinger and B. Engelhardt, Alpha4-integrin-VCAM-1 binding mediates G protein-independent capture of encephalitogenic T cell blasts to CNS white matter microvessels, *J Clin Invest* 108 (2001) 557-65.
- [21] E.E. McCandless, L. Piccio, B.M. Woerner, R.E. Schmidt, J.B. Rubin, A.H. Cross and R.S. Klein, Pathological expression of CXCL12 at the blood-brain barrier correlates with severity of multiple sclerosis, *Am J Pathol* 172 (2008) 799-808.
- [22] E.E. McCandless, Q. Wang, B.M. Woerner, J.M. Harper and R.S. Klein, CXCL12 limits inflammation by localizing mononuclear infiltrates to the perivascular space during experimental autoimmune encephalomyelitis, *J Immunol* 177 (2006) 8053-64.
- [23] I. Galea, I. Bechmann and V.H. Perry, What is immune privilege (not)?, *Trends Immunol* 28 (2007) 12-8.
- [24] M.J. Carson, J.M. Doose, B. Melchior, C.D. Schmid and C.C. Ploix, CNS immune privilege: hiding in plain sight, *Immunol Rev* 213 (2006) 48-65.
- [25] M.K. Matyszak and V.H. Perry, Demyelination in the central nervous system following a delayed-type hypersensitivity response to bacillus Calmette-Guerin, *Neuroscience* 64 (1995) 967-77.
- [26] P.G. Stevenson, S. Hawke, D.J. Sloan and C.R. Bangham, The immunogenicity of intracerebral virus infection depends on anatomical site, *J Virol* 71 (1997) 145-51.
- [27] A.P. Byrnes, R.E. MacLaren and H.M. Charlton, Immunological instability of persistent adenovirus vectors in the brain: peripheral exposure to vector leads to renewed inflammation, reduced gene expression, and demyelination, *J Neurosci* 16 (1996) 3045-55.
- [28] P.R. Lowenstein, Immunology of viral-vector-mediated gene transfer into the brain: an evolutionary and developmental perspective, *Trends Immunol* 23 (2002) 23-30.
- [29] M.K. Matyszak and V.H. Perry, A comparison of leucocyte responses to heat-killed bacillus Calmette-Guerin in different CNS compartments, *Neuropathol Appl Neurobiol* 22 (1996) 44-53.
- [30] V.H. Perry, A revised view of the central nervous system microenvironment and major histocompatibility complex class II antigen presentation, *J Neuroimmunol* 90 (1998) 113-21.
- [31] D.W. Mason, H.M. Charlton, A.J. Jones, C.B. Lavy, M. Puklavec and S.J. Simmonds, The fate of allogeneic and xenogeneic neuronal tissue transplanted into the third ventricle of rodents, *Neuroscience* 19 (1986) 685-94.
- [32] R. Magliozzi, O. Howell, A. Vora, B. Serafini, R. Nicholas, M. Puopolo, R. Reynolds and F. Aloisi, Meningeal B-cell follicles in secondary progressive multiple sclerosis

- associate with early onset of disease and severe cortical pathology, *Brain* 130 (2007) 1089-104.
- [33] B. Serafini, B. Rosicarelli, R. Magliozzi, E. Stigliano and F. Aloisi, Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis, *Brain Pathol* 14 (2004) 164-74.
- [34] P. Kivisakk, J. Imitola, S. Rasmussen, W. Elyaman, B. Zhu, R.M. Ransohoff and S.J. Khoury, Localizing central nervous system immune surveillance: meningeal antigen-presenting cells activate T cells during experimental autoimmune encephalomyelitis, *Ann Neurol* 65 (2009) 457-69.
- [35] H.L. Goldsmith and S. Spain, Margination of leukocytes in blood flow through small tubes, *Microvasc Res* 27 (1984) 204-22.
- [36] K. Ley, Molecular mechanisms of leukocyte recruitment in the inflammatory process, *Cardiovasc Res* 32 (1996) 733-42.
- [37] G.W. Schmid-Schonbein, S. Usami, R. Skalak and S. Chien, The interaction of leukocytes and erythrocytes in capillary and postcapillary vessels, *Microvasc Res* 19 (1980) 45-70.
- [38] U. Nobis, A.R. Pries, G.R. Cokelet and P. Gaehtgens, Radial distribution of white cells during blood flow in small tubes, *Microvasc Res* 29 (1985) 295-304.
- [39] K. Ley, C. Laudanna, M.I. Cybulsky and S. Nourshargh, Getting to the site of inflammation: the leukocyte adhesion cascade updated, *Nat Rev Immunol* 7 (2007) 678-89.
- [40] B. Johnston and E.C. Butcher, Chemokines in rapid leukocyte adhesion triggering and migration, *Semin Immunol* 14 (2002) 83-92.
- [41] R. Alon and S. Feigelson, From rolling to arrest on blood vessels: leukocyte tap dancing on endothelial integrin ligands and chemokines at sub-second contacts, *Semin Immunol* 14 (2002) 93-104.
- [42] G. Cinamon, V. Grabovsky, E. Winter, S. Franitza, S. Feigelson, R. Shamri, O. Dwir and R. Alon, Novel chemokine functions in lymphocyte migration through vascular endothelium under shear flow, *J Leukoc Biol* 69 (2001) 860-6.
- [43] P. Adamson, B. Wilbourn, S. Etienne-Manneville, V. Calder, E. Beraud, G. Milligan, P.O. Couraud and J. Greenwood, Lymphocyte trafficking through the blood-brain barrier is dependent on endothelial cell heterotrimeric G-protein signaling, *Faseb J* 16 (2002) 1185-94.
- [44] A.E. Proudfoot, The biological relevance of chemokine-proteoglycan interactions, *Biochem Soc Trans* 34 (2006) 422-6.
- [45] C.R. Parish, The role of heparan sulphate in inflammation, *Nat Rev Immunol* 6 (2006) 633-43.
- [46] C.V. Carman and T.A. Springer, Trans-cellular migration: cell-cell contacts get intimate, *Curr Opin Cell Biol* 20 (2008) 533-40.
- [47] D. Vestweber, Adhesion and signaling molecules controlling the transmigration of leukocytes through endothelium, *Immunol Rev* 218 (2007) 178-96.
- [48] F. Liao, J. Ali, T. Greene and W.A. Muller, Soluble domain 1 of platelet-endothelial cell adhesion molecule (PECAM) is sufficient to block transendothelial migration in vitro and in vivo, *J Exp Med* 185 (1997) 1349-57.

- [49] F. Liao, H.K. Huynh, A. Eiroa, T. Greene, E. Polizzi and W.A. Muller, Migration of monocytes across endothelium and passage through extracellular matrix involve separate molecular domains of PECAM-1, *J Exp Med* 182 (1995) 1337-43.
- [50] G.S. Duncan, D.P. Andrew, H. Takimoto, S.A. Kaufman, H. Yoshida, J. Spellberg, J. Luis de la Pompa, A. Elia, A. Wakeham, B. Karan-Tamir, W.A. Muller, G. Senaldi, M.M. Zukowski and T.W. Mak, Genetic evidence for functional redundancy of Platelet/Endothelial cell adhesion molecule-1 (PECAM-1): CD31-deficient mice reveal PECAM-1-dependent and PECAM-1-independent functions, *J Immunol* 162 (1999) 3022-30.
- [51] M.W. Wakelin, M.J. Sanz, A. Dewar, S.M. Albelda, S.W. Larkin, N. Boughton-Smith, T.J. Williams and S. Nourshargh, An anti-platelet-endothelial cell adhesion molecule-1 antibody inhibits leukocyte extravasation from mesenteric microvessels in vivo by blocking the passage through the basement membrane, *J Exp Med* 184 (1996) 229-39.
- [52] A.R. Schenkel, Z. Mamdouh, X. Chen, R.M. Liebman and W.A. Muller, CD99 plays a major role in the migration of monocytes through endothelial junctions, *Nat Immunol* 3 (2002) 143-50.
- [53] G. Bixel, S. Kloep, S. Butz, B. Petri, B. Engelhardt and D. Vestweber, Mouse CD99 participates in T-cell recruitment into inflamed skin, *Blood* 104 (2004) 3205-13.
- [54] B. Engelhardt and H. Wolburg, Mini-review: Transendothelial migration of leukocytes: through the front door or around the side of the house?, *Eur J Immunol* 34 (2004) 2955-63.
- [55] C.S. Raine, B. Cannella, A.M. Duijvestijn and A.H. Cross, Homing to central nervous system vasculature by antigen-specific lymphocytes. II. Lymphocyte/endothelial cell adhesion during the initial stages of autoimmune demyelination, *Lab Invest* 63 (1990) 476-89.
- [56] H. Wolburg, K. Wolburg-Buchholz and B. Engelhardt, Diapedesis of mononuclear cells across cerebral venules during experimental autoimmune encephalomyelitis leaves tight junctions intact, *Acta Neuropathol* 109 (2005) 181-90.
- [57] U. Kniesel, W. Risau and H. Wolburg, Development of blood-brain barrier tight junctions in the rat cortex, *Brain Res Dev Brain Res* 96 (1996) 229-40.
- [58] T. Owens, I. Bechmann and B. Engelhardt, Perivascular spaces and the two steps to neuroinflammation, *J Neuropathol Exp Neurol* 67 (2008) 1113-21.
- [59] I. Bartholomaeus, N. Kawakami, F. Odoardi, C. Schlager, D. Miljkovic, J.W. Ellwart, W.E. Klinkert, C. Flugel-Koch, T.B. Issekutz, H. Wekerle and A. Flugel, Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions, *Nature* 462 (2009) 94-8.
- [60] R.M. Ransohoff, Immunology: In the beginning, *Nature* 462 (2009) 41-2.
- [61] C.O. Zachariae, Chemotactic cytokines and inflammation. Biological properties of the lymphocyte and monocyte chemotactic factors ELCF, MCAF and IL-8, *Acta Derm Venereol Suppl (Stockh)* 181 (1993) 1-37.
- [62] A. Rot, Neutrophil attractant/activation protein-1 (interleukin-8) induces in vitro neutrophil migration by haptotactic mechanism, *Eur J Immunol* 23 (1993) 303-6.
- [63] Z. Johnson, M.H. Kosco-Vilbois, S. Herren, R. Cirillo, V. Muzio, P. Zaratin, M. Carbonatto, M. Mack, A. Smailbegovic, M. Rose, R. Lever, C. Page, T.N. Wells and A.E. Proudfoot, Interference with heparin binding and oligomerization creates a novel

- anti-inflammatory strategy targeting the chemokine system, *J Immunol* 173 (2004) 5776-85.
- [64] A. Peled, V. Grabovsky, L. Habler, J. Sandbank, F. Arenzana-Seisdedos, I. Petit, H. Ben-Hur, T. Lapidot and R. Alon, The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow, *J Clin Invest* 104 (1999) 1199-211.
- [65] G. Cinamon, V. Shinder and R. Alon, Shear forces promote lymphocyte migration across vascular endothelium bearing apical chemokines, *Nat Immunol* 2 (2001) 515-22.
- [66] T.H. Schreiber, V. Shinder, D.W. Cain, R. Alon and R. Sackstein, Shear flow-dependent integration of apical and subendothelial chemokines in T-cell transmigration: implications for locomotion and the multistep paradigm, *Blood* 109 (2007) 1381-6.
- [67] T.M. Handel, Z. Johnson, S.E. Crown, E.K. Lau and A.E. Proudfoot, Regulation of protein function by glycosaminoglycans--as exemplified by chemokines, *Annu Rev Biochem* 74 (2005) 385-410.
- [68] D. Spillmann, D. Witt and U. Lindahl, Defining the interleukin-8-binding domain of heparan sulfate, *J Biol Chem* 273 (1998) 15487-93.
- [69] A. Amara, O. Lorthioir, A. Valenzuela, A. Magerus, M. Thelen, M. Montes, J.L. Virelizier, M. Delepiepierre, F. Baleux, H. Lortat-Jacob and F. Arenzana-Seisdedos, Stromal cell-derived factor-1alpha associates with heparan sulfates through the first beta-strand of the chemokine, *J Biol Chem* 274 (1999) 23916-25.
- [70] L. Martin, C. Blanpain, P. Garnier, V. Wittamer, M. Parmentier and C. Vita, Structural and functional analysis of the RANTES-glycosaminoglycans interactions, *Biochemistry* 40 (2001) 6303-18.
- [71] A.J. Hoogewerf, G.S. Kuschert, A.E. Proudfoot, F. Borlat, I. Clark-Lewis, C.A. Power and T.N. Wells, Glycosaminoglycans mediate cell surface oligomerization of chemokines, *Biochemistry* 36 (1997) 13570-8.
- [72] H. Ranjbaran, Y. Wang, T.D. Manes, A.O. Yakimov, S. Akhtar, M.S. Kluger, J.S. Pober and G. Tellides, Heparin displaces interferon-gamma-inducible chemokines (IP-10, I-TAC, and Mig) sequestered in the vasculature and inhibits the transendothelial migration and arterial recruitment of T cells, *Circulation* 114 (2006) 1293-300.
- [73] A.E. Proudfoot, T.M. Handel, Z. Johnson, E.K. Lau, P. LiWang, I. Clark-Lewis, F. Borlat, T.N. Wells and M.H. Kosco-Vilbois, Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines, *Proc Natl Acad Sci U S A* 100 (2003) 1885-90.
- [74] S. Ali, H. Robertson, J.H. Wain, J.D. Isaacs, G. Malik and J.A. Kirby, A non-glycosaminoglycan-binding variant of CC chemokine ligand 7 (monocyte chemoattractant protein-3) antagonizes chemokine-mediated inflammation, *J Immunol* 175 (2005) 1257-66.
- [75] F.C. Peterson, E.S. Elgin, T.J. Nelson, F. Zhang, T.J. Hoeger, R.J. Linhardt and B.F. Volkman, Identification and characterization of a glycosaminoglycan recognition element of the C chemokine lymphotactin, *J Biol Chem* 279 (2004) 12598-604.
- [76] Z. Johnson, A.E. Proudfoot and T.M. Handel, Interaction of chemokines and glycosaminoglycans: a new twist in the regulation of chemokine function with opportunities for therapeutic intervention, *Cytokine Growth Factor Rev* 16 (2005) 625-36.

- [77] W.W. Agace, Tissue-tropic effector T cells: generation and targeting opportunities, *Nat Rev Immunol* 6 (2006) 682-92.
- [78] L. Wang, M. Fuster, P. Sriramarao and J.D. Esko, Endothelial heparan sulfate deficiency impairs L-selectin- and chemokine-mediated neutrophil trafficking during inflammatory responses, *Nat Immunol* 6 (2005) 902-10.
- [79] S.S. Shaik, T.D. Soltau, G. Chaturvedi, B. Totapally, J.S. Hagood, W.W. Andrews, M. Athar, N.N. Voitenok, C.R. Killingsworth, R.P. Patel, M.B. Fallon and A. Maheshwari, Low intensity shear stress increases endothelial ELR+ CXC chemokine production via a focal adhesion kinase-p38 β MAPK-NF- κ B pathway, *J Biol Chem* 284 (2009) 5945-55.
- [80] J. Hol, A.M. Kuchler, F.E. Johansen, B. Dalhus, G. Haraldsen and I. Oynebraten, Molecular requirements for sorting of the chemokine IL-8/CXCL8 to endothelial Weibel-Palade bodies, *J Biol Chem* (2009).
- [81] J.O. Utgaard, F.L. Jahnsen, A. Bakka, P. Brandtzaeg and G. Haraldsen, Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells, *J Exp Med* 188 (1998) 1751-6.
- [82] B. Wolff, A.R. Burns, J. Middleton and A. Rot, Endothelial cell "memory" of inflammatory stimulation: human venular endothelial cells store interleukin 8 in Weibel-Palade bodies, *J Exp Med* 188 (1998) 1757-62.
- [83] F.J. Barkalow, M.J. Goodman, M.E. Gerritsen and T.N. Mayadas, Brain endothelium lack one of two pathways of P-selectin-mediated neutrophil adhesion, *Blood* 88 (1996) 4585-93.
- [84] F.J. Barkalow, M.J. Goodman and T.N. Mayadas, Cultured murine cerebral microvascular endothelial cells contain von Willebrand factor-positive Weibel-Palade bodies and support rapid cytokine-induced neutrophil adhesion, *Microcirculation* 3 (1996) 19-28.
- [85] A.E. Cardona, M. Li, L. Liu, C. Savarin and R.M. Ransohoff, Chemokines in and out of the central nervous system: much more than chemotaxis and inflammation, *J Leukoc Biol* 84 (2008) 587-94.
- [86] U. Panzer, O.M. Steinmetz, R.R. Reinking, T.N. Meyer, S. Fehr, A. Schneider, G. Zahner, G. Wolf, U. Helmchen, P. Schaerli, R.A. Stahl and F. Thaiss, Compartment-specific expression and function of the chemokine IP-10/CXCL10 in a model of renal endothelial microvascular injury, *J Am Soc Nephrol* 17 (2006) 454-64.
- [87] B.C. Kieseier, M. Tani, D. Mahad, N. Oka, T. Ho, N. Woodroffe, J.W. Griffin, K.V. Toyka, R.M. Ransohoff and H.P. Hartung, Chemokines and chemokine receptors in inflammatory demyelinating neuropathies: a central role for IP-10, *Brain* 125 (2002) 823-34.
- [88] J. Reutershan, M.A. Morris, T.L. Burcin, D.F. Smith, D. Chang, M.S. Saprito and K. Ley, Critical role of endothelial CXCR2 in LPS-induced neutrophil migration into the lung, *J Clin Invest* 116 (2006) 695-702.
- [89] J. Reutershan, B. Harry, D. Chang, G.J. Bagby and K. Ley, DARC on RBC limits lung injury by balancing compartmental distribution of CXC chemokines, *Eur J Immunol* 39 (2009) 1597-607.
- [90] C.L. Tsou, C.A. Haskell and I.F. Charo, Tumor necrosis factor- α -converting enzyme mediates the inducible cleavage of fractalkine, *J Biol Chem* 276 (2001) 44622-6.

- [91] D.D. Patel, W. Koopmann, T. Imai, L.P. Whichard, O. Yoshie and M.S. Krangel, Chemokines have diverse abilities to form solid phase gradients, *Clin Immunol* 99 (2001) 43-52.
- [92] A.M. Fong, L.A. Robinson, D.A. Steeber, T.F. Tedder, O. Yoshie, T. Imai and D.D. Patel, Fractalkine and CX3CR1 mediate a novel mechanism of leukocyte capture, firm adhesion, and activation under physiologic flow, *J Exp Med* 188 (1998) 1413-9.
- [93] H. Umehara and T. Imai, Role of fractalkine in leukocyte adhesion and migration and in vascular injury, *Drug News Perspect* 14 (2001) 460-4.
- [94] J.K. Harrison, Y. Jiang, S. Chen, Y. Xia, D. Maciejewski, R.K. McNamara, W.J. Streit, M.N. Salafra, S. Adhikari, D.A. Thompson, P. Botti, K.B. Bacon and L. Feng, Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia, *Proc Natl Acad Sci U S A* 95 (1998) 10896-901.
- [95] D. Sunnemark, S. Eltayeb, M. Nilsson, E. Wallstrom, H. Lassmann, T. Olsson, A.L. Berg and A. Ericsson-Dahlstrand, CX3CL1 (fractalkine) and CX3CR1 expression in myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis: kinetics and cellular origin, *J Neuroinflammation* 2 (2005) 17.
- [96] D. Huang, F.D. Shi, S. Jung, G.C. Pien, J. Wang, T.P. Salazar-Mather, T.T. He, J.T. Weaver, H.G. Ljunggren, C.A. Biron, D.R. Littman and R.M. Ransohoff, The neuronal chemokine CX3CL1/fractalkine selectively recruits NK cells that modify experimental autoimmune encephalomyelitis within the central nervous system, *Faseb J* 20 (2006) 896-905.
- [97] A.V. Andjelkovic, D.D. Spencer and J.S. Pachter, Visualization of chemokine binding sites on human brain microvessels, *J Cell Biol* 145 (1999) 403-12.
- [98] O.B. Dimitrijevic, S.M. Stamatovic, R.F. Keep and A.V. Andjelkovic, Effects of the chemokine CCL2 on blood-brain barrier permeability during ischemia-reperfusion injury, *J Cereb Blood Flow Metab* 26 (2006) 797-810.
- [99] S.M. Stamatovic, R.F. Keep, S.L. Kunkel and A.V. Andjelkovic, Potential role of MCP-1 in endothelial cell tight junction 'opening': signaling via Rho and Rho kinase, *J Cell Sci* 116 (2003) 4615-28.
- [100] J. Quandt and K. Dorovini-Zis, The beta chemokines CCL4 and CCL5 enhance adhesion of specific CD4+ T cell subsets to human brain endothelial cells, *J Neuropathol Exp Neurol* 63 (2004) 350-62.
- [101] A. Szczucinski and J. Losy, Chemokines and chemokine receptors in multiple sclerosis. Potential targets for new therapies, *Acta Neurol Scand* 115 (2007) 137-46.
- [102] L. Izikson, R.S. Klein, I.F. Charo, H.L. Weiner and A.D. Luster, Resistance to experimental autoimmune encephalomyelitis in mice lacking the CC chemokine receptor (CCR)2, *J Exp Med* 192 (2000) 1075-80.
- [103] L. Izikson, R.S. Klein, A.D. Luster and H.L. Weiner, Targeting monocyte recruitment in CNS autoimmune disease, *Clin Immunol* 103 (2002) 125-31.
- [104] B.T. Fife, G.B. Huffnagle, W.A. Kuziel and W.J. Karpus, CC chemokine receptor 2 is critical for induction of experimental autoimmune encephalomyelitis, *J Exp Med* 192 (2000) 899-905.
- [105] B.T. Fife, M.C. Paniagua, N.W. Lukacs, S.L. Kunkel and W.J. Karpus, Selective CC chemokine receptor expression by central nervous system-infiltrating encephalitogenic T cells during experimental autoimmune encephalomyelitis, *J Neurosci Res* 66 (2001) 705-14.

- [106] D. Mahad, M.K. Callahan, K.A. Williams, E.E. Ubogu, P. Kivisakk, B. Tucky, G. Kidd, G.A. Kingsbury, A. Chang, R.J. Fox, M. Mack, M.B. Sniderman, R. Ravid, S.M. Staugaitis, M.F. Stins and R.M. Ransohoff, Modulating CCR2 and CCL2 at the blood-brain barrier: relevance for multiple sclerosis pathogenesis, *Brain* 129 (2006) 212-23.
- [107] D.J. Mahad and R.M. Ransohoff, The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE), *Semin Immunol* 15 (2003) 23-32.
- [108] T.L. Sorensen, R.M. Ransohoff, R.M. Strieter and F. Sellebjerg, Chemokine CCL2 and chemokine receptor CCR2 in early active multiple sclerosis, *Eur J Neurol* 11 (2004) 445-9.
- [109] S. Gaupp, D. Pitt, W.A. Kuziel, B. Cannella and C.S. Raine, Experimental autoimmune encephalomyelitis (EAE) in CCR2(-/-) mice: susceptibility in multiple strains, *Am J Pathol* 162 (2003) 139-50.
- [110] D.R. Huang, J. Wang, P. Kivisakk, B.J. Rollins and R.M. Ransohoff, 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* 193 (2001) 713-26.
- [111] B.H. Bennetts, S.M. Teutsch, M.M. Buhler, R.N. Heard and G.J. Stewart, The CCR5 deletion mutation fails to protect against multiple sclerosis, *Hum Immunol* 58 (1997) 52-9.
- [112] E.H. Tran, W.A. Kuziel and T. Owens, Induction of experimental autoimmune encephalomyelitis in C57BL/6 mice deficient in either the chemokine macrophage inflammatory protein-1alpha or its CCR5 receptor, *Eur J Immunol* 30 (2000) 1410-5.
- [113] M.F. Bachmann, M. Kopf and B.J. Marsland, Chemokines: more than just road signs, *Nat Rev Immunol* 6 (2006) 159-64.
- [114] S.K. Bromley, T.R. Mempel and A.D. Luster, Orchestrating the orchestrators: chemokines in control of T cell traffic, *Nat Immunol* 9 (2008) 970-80.
- [115] C. Alt, M. Laschinger and B. Engelhardt, Functional expression of the lymphoid chemokines CCL19 (ELC) and CCL 21 (SLC) at the blood-brain barrier suggests their involvement in G-protein-dependent lymphocyte recruitment into the central nervous system during experimental autoimmune encephalomyelitis, *Eur J Immunol* 32 (2002) 2133-44.
- [116] S. Columba-Cabezas, B. Serafini, E. Ambrosini and F. Aloisi, Lymphoid chemokines CCL19 and CCL21 are expressed in the central nervous system during experimental autoimmune encephalomyelitis: implications for the maintenance of chronic neuroinflammation, *Brain Pathol* 13 (2003) 38-51.
- [117] S.C. Chen, M.W. Leach, Y. Chen, X.Y. Cai, L. Sullivan, M. Wiekowski, B.J. Dovey-Hartman, A. Zlotnik and S.A. Lira, Central nervous system inflammation and neurological disease in transgenic mice expressing the CC chemokine CCL21 in oligodendrocytes, *J Immunol* 168 (2002) 1009-17.
- [118] K. Biber, A. Sauter, N. Brouwer, S.C. Copray and H.W. Boddeke, Ischemia-induced neuronal expression of the microglia attracting chemokine Secondary Lymphoid-tissue Chemokine (SLC), *Glia* 34 (2001) 121-33.
- [119] I.M. Dijkstra, S. Hulshof, P. van der Valk, H.W. Boddeke and K. Biber, Cutting edge: activity of human adult microglia in response to CC chemokine ligand 21, *J Immunol* 172 (2004) 2744-7.

- [120] A. Rappert, K. Biber, C. Nolte, M. Lipp, A. Schubel, B. Lu, N.P. Gerard, C. Gerard, H.W. Boddeke and H. Kettenmann, Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a Cl⁻ current and chemotaxis in murine microglia, *J Immunol* 168 (2002) 3221-6.
- [121] H.R. Weering, A.P. Jong, A.H. Haas, K.P. Biber and H.W. Boddeke, CCL21-induced calcium transients and proliferation in primary mouse astrocytes: CXCR3-dependent and independent responses, *Brain Behav Immun* (2009).
- [122] P. Kivisakk, D.J. Mahad, M.K. Callahan, K. Sikora, C. Trebst, B. Tucky, J. Wujek, R. Ravid, S.M. Staugaitis, H. Lassmann and R.M. Ransohoff, Expression of CCR7 in multiple sclerosis: implications for CNS immunity, *Ann Neurol* 55 (2004) 627-38.
- [123] S. Buonamici, T. Trimarchi, M.G. Ruocco, L. Reavie, S. Cathelin, B.G. Mar, A. Klinakis, Y. Lukyanov, J.C. Tseng, F. Sen, E. Gehrie, M. Li, E. Newcomb, J. Zavadil, D. Meruelo, M. Lipp, S. Ibrahim, A. Efstratiadis, D. Zagzag, J.S. Bromberg, M.L. Dustin and I. Aifantis, CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia, *Nature* 459 (2009) 1000-4.
- [124] A. Reboldi, C. Coisne, D. Baumjohann, F. Benvenuto, D. Bottinelli, S. Lira, A. Uccelli, A. Lanzavecchia, B. Engelhardt and F. Sallusto, C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE, *Nat Immunol* 10 (2009) 514-23.
- [125] M. Krumbholz, D. Theil, F. Steinmeyer, S. Cepok, B. Hemmer, M. Hofbauer, C. Farina, T. Derfuss, A. Junker, T. Arzberger, I. Sinicina, C. Hartle, J. Newcombe, R. Hohlfeld and E. Meinl, CCL19 is constitutively expressed in the CNS, up-regulated in neuroinflammation, active and also inactive multiple sclerosis lesions, *J Neuroimmunol* 190 (2007) 72-9.
- [126] C.C. Bleul, R.C. Fuhlbrigge, J.M. Casasnovas, A. Aiuti and T.A. Springer, A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1), *J Exp Med* 184 (1996) 1101-9.
- [127] R.K. Stumm, J. Rummel, V. Junker, C. Culmsee, M. Pfeiffer, J. Kriegelstein, V. Holtt and S. Schulz, A dual role for the SDF-1/CXCR4 chemokine receptor system in adult brain: isoform-selective regulation of SDF-1 expression modulates CXCR4-dependent neuronal plasticity and cerebral leukocyte recruitment after focal ischemia, *J Neurosci* 22 (2002) 5865-78.
- [128] M. Li and R.M. Ransohoff, Multiple roles of chemokine CXCL12 in the central nervous system: a migration from immunology to neurobiology, *Prog Neurobiol* 84 (2008) 116-31.
- [129] R.S. Klein and J.B. Rubin, Immune and nervous system CXCL12 and CXCR4: parallel roles in patterning and plasticity, *Trends Immunol* 25 (2004) 306-14.
- [130] P. van der Meer, A.M. Ulrich, F. Gonzalez-Scarano and E. Lavi, Immunohistochemical analysis of CCR2, CCR3, CCR5, and CXCR4 in the human brain: potential mechanisms for HIV dementia, *Exp Mol Pathol* 69 (2000) 192-201.
- [131] E.E. Jazin, S. Soderstrom, T. Ebendal and D. Larhammar, Embryonic expression of the mRNA for the rat homologue of the fusin/CXCR-4 HIV-1 co-receptor, *J Neuroimmunol* 79 (1997) 148-54.
- [132] K.E. McGrath, A.D. Koniski, K.M. Maltby, J.K. McGann and J. Palis, Embryonic expression and function of the chemokine SDF-1 and its receptor, CXCR4, *Dev Biol* 213 (1999) 442-56.

- [133] M. Krumbholz, D. Theil, S. Cepok, B. Hemmer, P. Kivisakk, R.M. Ransohoff, M. Hofbauer, C. Farina, T. Derfuss, C. Hartle, J. Newcombe, R. Hohlfeld and E. Meinl, Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment, *Brain* 129 (2006) 200-11.
- [134] E.E. McCandless, M. Budde, J.R. Lees, D. Dorsey, E. Lyng and R.S. Klein, IL-1R signaling within the central nervous system regulates CXCL12 expression at the blood-brain barrier and disease severity during experimental autoimmune encephalomyelitis, *J Immunol* 183 (2009) 613-20.
- [135] K. Balabanian, B. Lagane, S. Infantino, K.Y. Chow, J. Harriague, B. Moepps, F. Arenzana-Seisdedos, M. Thelen and F. Bachelier, The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes, *J Biol Chem* 280 (2005) 35760-6.
- [136] J.M. Burns, B.C. Summers, Y. Wang, A. Melikian, R. Berahovich, Z. Miao, M.E. Penfold, M.J. Sunshine, D.R. Littman, C.J. Kuo, K. Wei, B.E. McMaster, K. Wright, M.C. Howard and T.J. Schall, A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development, *J Exp Med* 203 (2006) 2201-13.
- [137] B. Mazzinghi, E. Ronconi, E. Lazzeri, C. Sagrinati, L. Ballerini, M.L. Angelotti, E. Parente, R. Mancina, G.S. Netti, F. Becherucci, M. Gacci, M. Carini, L. Gesualdo, M. Rotondi, E. Maggi, L. Lasagni, M. Serio, S. Romagnani and P. Romagnani, Essential but differential role for CXCR4 and CXCR7 in the therapeutic homing of human renal progenitor cells, *J Exp Med* 205 (2008) 479-90.
- [138] F. Sierro, C. Biben, L. Martinez-Munoz, M. Mellado, R.M. Ransohoff, M. Li, B. Woehl, H. Leung, J. Groom, M. Batten, R.P. Harvey, A.C. Martinez, C.R. Mackay and F. Mackay, Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7, *Proc Natl Acad Sci U S A* 104 (2007) 14759-64.
- [139] J. Wang, Y. Shiozawa, J. Wang, Y. Wang, Y. Jung, K.J. Pienta, R. Mehra, R. Loberg and R.S. Taichman, The role of CXCR7/RDC1 as a chemokine receptor for CXCL12/SDF-1 in prostate cancer, *J Biol Chem* 283 (2008) 4283-94.
- [140] P. Proost, A. Mortier, T. Loos, J. Vandercappellen, M. Gouwy, I. Ronsse, E. Schutyser, W. Put, M. Parmentier, S. Struyf and J. Van Damme, Proteolytic processing of CXCL11 by CD13/aminopeptidase N impairs CXCR3 and CXCR7 binding and signaling and reduces lymphocyte and endothelial cell migration, *Blood* 110 (2007) 37-44.
- [141] D. Yoshida, R. Nomura and A. Teramoto, Signaling Pathway Mediated by CXCR7, an Alternative Chemokine Receptor for Stromal-Cell Derived Factor-1alpha, in AtT20 mouse ACTH-secreting pituitary adenoma cells, *J Neuroendocrinol* (2009).
- [142] T.N. Hartmann, V. Grabovsky, R. Pasvolsky, Z. Shulman, E.C. Buss, A. Spiegel, A. Nagler, T. Lapidot, M. Thelen and R. Alon, A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells, *J Leukoc Biol* 84 (2008) 1130-40.
- [143] B. Boldajipour, H. Mahabaleswar, E. Kardash, M. Reichman-Fried, H. Blaser, S. Minina, D. Wilson, Q. Xu and E. Raz, Control of chemokine-guided cell migration by ligand sequestration, *Cell* 132 (2008) 463-73.

- [144] H. Mahabaleshwar, B. Boldajipour and E. Raz, Killing the messenger: The role of CXCR7 in regulating primordial germ cell migration, *Cell Adh Migr* 2 (2008) 69-70.
- [145] B. Schonemeier, A. Kolodziej, S. Schulz, S. Jacobs, V. Hoell and R. Stumm, Regional and cellular localization of the CXCL12/SDF-1 chemokine receptor CXCR7 in the developing and adult rat brain, *J Comp Neurol* 510 (2008) 207-20.
- [146] B. Schonemeier, S. Schulz, V. Hoell and R. Stumm, Enhanced expression of the CXCL12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain, *J Neuroimmunol* 198 (2008) 39-45.
- [147] Z. Miao, K.E. Luker, B.C. Summers, R. Berahovich, M.S. Bhojani, A. Rehemtulla, C.G. Klee, J.J. Essner, A. Nasevicius, G.D. Luker, M.C. Howard and T.J. Schall, CXCR7 (RDC1) promotes breast and lung tumor growth in vivo and is expressed on tumor-associated vasculature, *Proc Natl Acad Sci U S A* 104 (2007) 15735-40.
- [148] B.A. Zabel, Y. Wang, S. Lewen, R.D. Berahovich, M.E. Penfold, P. Zhang, J. Powers, B.C. Summers, Z. Miao, B. Zhao, A. Jalili, A. Janowska-Wieczorek, J.C. Jaen and T.J. Schall, Elucidation of CXCR7-mediated signaling events and inhibition of CXCR4-mediated tumor cell transendothelial migration by CXCR7 ligands, *J Immunol* 183 (2009) 3204-11.
- [149] A.A. Maghazachi, G protein-coupled receptors in natural killer cells, *J Leukoc Biol* 74 (2003) 16-24.
- [150] A. Dagkalis, C. Wallace, B. Hing, J. Liversidge and I.J. Crane, CX3CR1-deficiency is associated with increased severity of disease in experimental autoimmune uveitis, *Immunology* 128 (2009) 25-33.
- [151] E.H. Tran, E.N. Prince and T. Owens, IFN-gamma shapes immune invasion of the central nervous system via regulation of chemokines, *J Immunol* 164 (2000) 2759-68.
- [152] T. Carlson, M. Kroenke, P. Rao, T.E. Lane and B. Segal, The Th17-ELR+ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease, *J Exp Med* 205 (2008) 811-23.
- [153] S.S. Shaftel, T.J. Carlson, J.A. Olschowka, S. Kyrkanides, S.B. Matousek and M.K. O'Banion, Chronic interleukin-1beta expression in mouse brain leads to leukocyte infiltration and neutrophil-independent blood brain barrier permeability without overt neurodegeneration, *J Neurosci* 27 (2007) 9301-9.
- [154] U.K. Hanisch, Microglia as a source and target of cytokines, *Glia* 40 (2002) 140-55.
- [155] G.W. Kreutzberg, Microglia: a sensor for pathological events in the CNS, *Trends Neurosci* 19 (1996) 312-8.
- [156] F. Gonzalez-Scarano and G. Baltuch, Microglia as mediators of inflammatory and degenerative diseases, *Annu Rev Neurosci* 22 (1999) 219-40.
- [157] Y.Q. Li, P. Chen, V. Jain, R.M. Reilly and C.S. Wong, Early radiation-induced endothelial cell loss and blood-spinal cord barrier breakdown in the rat spinal cord, *Radiat Res* 161 (2004) 143-52.
- [158] M. Diserbo, A. Agin, I. Lamproglou, J. Mauris, F. Staali, E. Multon and C. Amourette, Blood-brain barrier permeability after gamma whole-body irradiation: an in vivo microdialysis study, *Can J Physiol Pharmacol* 80 (2002) 670-8.
- [159] B. Ajami, J.L. Bennett, C. Krieger, W. Tetzlaff and F.M. Rossi, Local self-renewal can sustain CNS microglia maintenance and function throughout adult life, *Nat Neurosci* 10 (2007) 1538-43.

- [160] D.C. Hess, T. Abe, W.D. Hill, A.M. Studdard, J. Carothers, M. Masuya, P.A. Fleming, C.J. Drake and M. Ogawa, Hematopoietic origin of microglial and perivascular cells in brain, *Exp Neurol* 186 (2004) 134-44.
- [161] A.R. Simard and S. Rivest, Bone marrow stem cells have the ability to populate the entire central nervous system into fully differentiated parenchymal microglia, *Faseb J* 18 (2004) 998-1000.
- [162] J. Priller, A. Flugel, T. Wehner, M. Boentert, C.A. Haas, M. Prinz, F. Fernandez-Klett, K. Prass, I. Bechmann, B.A. de Boer, M. Frotscher, G.W. Kreutzberg, D.A. Persons and U. Dirnagl, Targeting gene-modified hematopoietic cells to the central nervous system: use of green fluorescent protein uncovers microglial engraftment, *Nat Med* 7 (2001) 1356-61.
- [163] D.W. Kennedy and J.L. Abkowitz, Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model, *Blood* 90 (1997) 986-93.
- [164] A. Flugel, M. Bradl, G.W. Kreutzberg and M.B. Graeber, Transformation of donor-derived bone marrow precursors into host microglia during autoimmune CNS inflammation and during the retrograde response to axotomy, *J Neurosci Res* 66 (2001) 74-82.
- [165] A. Mildner, H. Schmidt, M. Nitsche, D. Merkler, U.K. Hanisch, M. Mack, M. Heikenwalder, W. Bruck, J. Priller and M. Prinz, Microglia in the adult brain arise from Ly-6ChiCCR2+ monocytes only under defined host conditions, *Nat Neurosci* 10 (2007) 1544-53.

