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Inflammatory cell trafficking across the blood-brain barrier (BBB): Chemokine regulation and *in vitro* models

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Summary

The blood-brain barrier (BBB) is the brain-specific capillary barrier that is critical for preventing toxic substances from entering the central nervous system (CNS). In contrast to vessels of peripheral organs, the BBB limits the exchange of inflammatory cells and mediators under physiological and pathological conditions. Clarifying these limitations and the role of chemokines in regulating the BBB would provide new insights into neuroprotective strategies in neuroinflammatory diseases. Because there is a paucity of *in vitro* BBB models, however, some mechanistic aspects of transmigration across the BBB still remain largely unknown. In this review, we summarize current knowledge of BBB cellular components, the multi-step process of inflammatory cells crossing the BBB, functions of CNS-derived chemokines and *in vitro* BBB models for transmigration, with a particular focus on new and recent findings.

Keywords

in vitro BBB model; BBB components; CNS chemokine; endothelial cell line; shear stress

Cellular components of the blood-brain barrier

The blood-brain barrier (BBB) is primarily formed by microvascular endothelial cells, which are surrounded by basement membranes, pericytes, and astrocytes (Fig. 1). The endothelial basement membrane delimits the vascular aspect of the perivascular space. Astrocytic endfoot processes form the glia limitans, which, along with its own basement membrane, provide the parenchymal aspect of the perivascular space (1) (Fig. 2). This endothelial layer and glia limitans represent physical barriers to cellular entry to the central nervous system (CNS) parenchyma. Neuronal and microglial processes also contribute to the glia limitans. Interactions between endothelial cells and these surrounding cells and processes enhance BBB function and consequently result in the maintenance of proper brain homeostasis (2). More detail about each cell type is provided below.

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Endothelial cells

Brain microvascular endothelial cells (BMVECs) directly mediate BBB function. Resting on a basement membrane, which consists mainly of collagen IV, fibronectin, laminin, and proteoglycans, BMVECs act as mediators between the blood and brain (3). They have specialized transport systems, uniform thickness with no transendothelial fenestrations, low pinocytic activity, continuous intercellular tight junctions, and high mitochondrial volume (4, 5). In addition, they have a negative luminal surface charge that repulses negatively charged compounds (5). Owing to no fenestrations and the diminished pinocytic activity, paracellular flux is limited. Uptake of essential molecules occurs through specific carrier and transport systems (6, 7). In addition, because of the presence of continuous tight junctions and adherens junctions, the paracellular space between adjacent lateral endothelial membranes is almost completely sealed (7–11). As they have a greater number and volume of mitochondria compared with endothelia in other organs, brain endothelial cells can provide energy and regulate the selective transport and metabolism of substances from blood to brain as well as from the parenchyma back to the systemic circulation (12).

Astrocytes

Astrocytes are important components of the BBB. Astrocytic endfeet ensheathe 99% of the surface of brain microvessels from which their endfoot processes are separated only by a thin basal membrane (13). Astrocytes are a source of important regulatory factors such as transforming growth factor-β (TGF-β) (14), glial-derived neurotropic factor (GDNF) (15), and the fibroblast growth factor (FGF) (16), and they can provide these secreted factors to endothelial cells (17). The current predominant view is that astrocytes regulate various aspects of BBB physiology with secreted factors and influence particular BBB features such as permeability, leading to tight junction formation and expression in endothelial cells (18).

Pericytes

Pericytes are important cellular constituents of capillaries and post-capillary venules. They share the same basement membrane with the endothelial cell (19) and cover 22–32% of the capillaries in CNS (20). The extent of pericyte coverage of BBB vessels is highest among the varied types of vessels (21). Pericytes regulate many neurovascular functions such as angiogenesis, BBB formation in embryogenesis, maintenance, vascular stability, regulation of capillary blood flow, and clearance of toxic cellular products (22). Pericytes can control the expression of tight junction molecules in endothelial cells by secreting factors such as TGF-β, (14) and angiopoietin (23). TGF-β enhances BBB function by inhibiting the migration of leukocytes and the proliferation of endothelial cells. The release of angiopoietin can induce remodeling and stabilization of capillaries. Platelet-derived growth factor-β (PDGFβ), secreted by endothelial cells, is an essential factor for recruitment and maintenance of pericytes on vessels and vascular maturation (24, 25).

Other cell types

The interaction between endothelial cells and neurons plays an essential role in the neurovascular network. Neurons can regulate BBB function by expressing BBB-related enzymes (26). Microglial endfoot processes, found in the perivascular glia limitans, are hypothesized to influence BBB properties. However, their contribution to BBB function remains unknown.

Multi-step process of leukocytes crossing into the CNS

Intrusion of leukocytes into CNS

The CNS exhibits strictly controlled inflammatory reactions, in part because the BBB and other vascular-tissue barriers limit the exchange of inflammatory cells and mediators. There are several routes that leukocytes can use to enter into CNS: migration from the microvessels into parenchymal perivascular space, migration via the choroid plexus into the cerebrospinal fluid, and migration through post-capillary venules at the pial surface into subarachnoid and Virchow-Robin perivascular spaces (27–29). A fourth route has also been suggested that involves migration from subependymal vessels via the ependyma into the ventricles (30). These routes involve crossing the BBB, the blood–cerebrospinal fluid (CSF) barrier and the blood–spinal cord (BSC) barrier (27, 31).

Multi-step process of crossing into the CNS

Transendothelial leukocyte migration through the BBB is a multi-step process characterized by a series of sequential and tightly controlled steps that follow the paradigm of leukocyte extravasation across all vascular beds (1, 32–34) (Fig. 2). The steps are: (i) rolling: weak adhesion of leukocytes to endothelial cells mainly through interactions between selectins and their carbohydrate counter-receptors; (ii) activation: leukocyte activation through chemokine stimulation of G-protein-linked receptors, resulting in functional activation of adhesion molecules along their surface; (iii) arrest: leukocyte attachment to endothelial cells through interactions between integrins associated with leukocytes and cell adhesion molecules (CAMs) on endothelial cells; (iv) crawling: leukocytes seeking preferred sites of transmigration across the endothelium; (v) transmigration: migration of leukocytes across CNS endothelia into the perivascular space and progression across the glia limitans into the brain parenchyma, a process driven in part by chemokine–chemokine receptor interactions. By interacting pairs of selectins and their ligands, integrins and CAMs, and chemokines and chemokine receptors, brain-specific processes are determined. Each of these steps and interacting pairs is described in more detail below.

Rolling

This multi-step process starts with a short and initial transient contact of the circulating leukocytes with the endothelial cell through E- and P-selectin and carbohydrate adducts on their leukocyte ligand P-selectin glycoprotein 1 (PSGL1) (35). Very late antigen-4 (VLA-4) can also support rolling. The interactions between selectins and their ligands are of low-affinity and leukocytes roll along the vascular wall with gradually reduced velocity. Recent studies have shown that despite the blockade or absence of P-selectin, immune-reactions induced in mouse models of experimental autoimmune encephalomyelitis (EAE) are indistinguishable from wildtype EAE (36, 37). This observation suggests that P-selectin is not required for leukocytes to migrate across the CNS parenchymal vessels. On the other hand, P-selectin is stored in the Weibel-Palade bodies of endothelial cells of meningeal and the fenestrated choroid plexus capillaries (38, 39). P-selectin is believed to be important for leukocyte recruitment across meningeal and choroid plexus vessels (40).

Activation

Rolling along the vascular wall slows circulating lymphocytes and permits factors such as chemokines, immobilized on the endothelial cell surface, to activate integrins on leukocytes (37, 41, 42). Chemokine receptors such as CXCR4 on rolling leukocytes interact with chemokines such as CXCL12 on endothelial cells. Chemokine receptors enhance a G-protein intracellular signal, which induces conformational changes of leukocyte integrins. Chemokines activate several signaling pathways (PI3K, PLC, RAS- and RHO-family

GTPase, and MAPK), leading to opened integrin conformation (43–45). As a result, adhesion molecules such as LFA-1 and VLA-4 are activated on the leukocyte surface. Integrin activation leads to enhanced avidity and affinity of the leukocyte integrin for its endothelial ligands, specifically VCAM-1 or fibronectin CS1 epitope (46, 47) and intercellular adhesion molecule-1 (ICAM-1).

Arrest

During arrest, adhesion molecules in leukocytes (VLA-4, LFA-1, and Mac-1) and endothelial receptors such as ICAM-1 and VCAM-1 play important roles (32). ICAM-1 and VCAM-1 are the major ligands for leukocyte integrins to attach to the endothelial cell against shear flow. Under normal conditions, ICAM-1 is detected on a small number of CNS microvessels and strongly upregulated by inflammatory stimuli (48). In contrast, VCAM-1 upregulation on human CNS microvessels is still matter of debate (49–51). Binding of these integrins to their endothelial ligands, such as VLA-4/VCAM-1 and LFA-1, Mac-1/ICAM-1, generates cytoplasmic signaling cascades in both leukocytes and endothelial cells. As a result, leukocytes arrest on the endothelial cells.

Crawling

After leukocytes arrest, they crawl via tightly regulated integrin/CAM interactions (LFA-1/ICAM-1, VLA-4/VCAM-1) (52). These interactions initiate essential signaling within the endothelial cells and promote indentification of optimal sites for transmigration (53). Leukocytes crawl inside blood vessels in a MAC1- and ICAM1-dependent manner (52, 54). Recently an immobilized intravascular gradient of the chemokine CXCL-1 was shown to guide crawling neutrophils to transmigration sites (55). It is unknown if CNS-derived chemokines play a similar role by directing leukocytes crawling against the direction of blood flow.

Transmigration

The last stage in the multi-step process is transmigration. It is not clear whether the leukocytes cross the endothelial cell through tight junctions, via a large pore or vacuole in the endothelial cell, or through some other site (56). Until recently, leukocyte migration across the endothelial cell was thought to occur through the paracellular pathway only but leukocyte migration through the transcellular route occurs in the CNS, and in various inflammatory conditions (57, 58), and *in vitro* models (59–61). Transmigration of leukocytes appears to be regulated by CAMs (ICAM-1, VCAM-1) and chemokine signaling processes (32). If crawling is inhibited, transmigration is delayed and occurs preferentially through the transcellular pathway as opposed to the paracellular pathway (53). While in peripheral tissues migrated cells directly enter the tissue parenchyma, in the CNS, migrated cells can only access perivascular spaces. To access the CNS parenchyma, they need to reach beyond the glia limitans, which is unique to the architecture of the BBB. Currently, there are no suitable *in vitro* BBB models to analyze transmigration of cells, therefore it remains incompletely understood which molecules including chemokines and chemokine receptors are critical for this process.

Chemokines and chemokine receptors

Chemokines

Chemokines play critical roles in the initial inflammatory recruitment of leukocytes. In addition to leukocyte chemotaxis, chemokines are involved in neuronal positioning during development, modulating synaptic transmission, regulating cell adhesion, phagocytosis, cytokine secretion, matrix metalloproteinase release, T-cell differentiation and activation, apoptosis, and angiogenesis (62–65).

Chemokines are a group of small (8–14 kDa) structurally related molecules released by a variety of cell types. Approximately 50 human chemokine genes have been identified to date (Table 1). In spite of a variable amino acid sequence, all chemokines share a characteristic tertiary structure called the 'chemokine fold'(66). Chemokines are divided into four subfamilies according to the configuration of two positionally conserved cysteine residues near the N-terminus. These include the CC subfamily (CCL1-CCL28), CXC subfamily (CXCL1-CXCL16), C subfamily (XCL1-XCL2), and CX3C subfamily (CX3CL1) and their nomenclature has been reviewed (66, 67).

CC chemokines have a large spectrum of action and can attract monocytes, eosinophils, basophils, T lymphocytes, natural killer (NK) cells, and dendritic cells. Most CC chemokines are clustered on chromosome 17 in humans.

The CXC chemokines are distinguished by the presence or absence of a specific amino acid sequence, called the ELR-motif (glutamic acid-leucine-arginine) located near the N-terminus. The ELR^+ CXC chemokines bind the neutrophil receptors CXCR2 and some also bind CXCR1. On the other hand, the ELR^- CXC chemokines are inactive towards neutrophils but are potent chemoattractants for other leukocytes appropriate receptors (68).

The C chemokines, which comprise XCL1 and XCL2, are distinguished from the other chemokine subfamilies by the presence of only two of the four conserved cysteine residues (69). C chemokines chemoattract lymphocytes but not neutrophils or monocytes.

The CX3C chemokine is CX3CL1, which is characterized by the presence of three amino acids between the first two cysteine residues as well as transmembrane and mucin-like domains in C-terminal sequence. CX3CL1 can be soluble or membrane-bound (70) and acts as an adhesion molecule or a chemoattractant for T lymphocytes, NK cells, and mononuclear phagocytes (71).

Chemokine receptors

Chemokines exert their biological functions by binding to seven transmembrane-domain receptors on target cells. Chemokine receptors are classified according to the ligand family to which they respond (Table 2). The 19 known receptors often bind multiple chemokines in a subclass-restricted manner although some (such as CCR1) are highly promiscuous, while others (such as CCR8) respond only to a single unique ligand. Chemokine receptors are rhodopsin-like G protein-coupled receptors, with an acidic N-terminal extracellular domain and serine/threonine-rich intracellular C-terminal domain (72). Some chemokine receptors are widely expressed throughout the entire body, whereas others are expressed in certain specific cells or tissues or in specific activation or differentiation states of the receptor-bearing cell (73).

CNS chemokines and receptors

The expression of chemokines and their receptors in the CNS has been described by several authors through immunohistochemistry. It has been difficult to produce specific and sensitive antibodies for chemokines and receptors. Unfortunately, many preliminary reports could not be confirmed by critical studies using wildtype and gene-deficient mice (74). As a result, compared to peripheral tissues, chemokine functions in the CNS are less known. Chemokines and receptors that are constitutively expressed or developmentally regulated in the CNS include CXCL12-CXCR4/CXCR7, CXCL1-CXCR2, and CX3CL1-CX3CR1. CXCL12-CXCR4, which are selectively expressed in the developing and adult brain, control the migration and survival of neural precursors and stimulate astrocyte proliferation. The functions of CXCL12-CXCR7 still remain incompletely understood (75). CXCL1-CXCR2 are also implicated in the migration and proliferation of oligodendrocyte progenitors (76,

77). CX3CL1-CX3CR1, which are constitutively expressed in CNS modify inflammatory reactions of microglia and are required for recruitment of NK cells (78–82).

Chemokine control of cell migration

Chemokine signaling results in molecular and functional changes in leukocytes. Chemokines and their receptors are involved in multiple steps during leukocyte transendothelial migration (Fig. 2). Chemokines presented on luminal endothelial surfaces can trigger integrin activation. As representative arrest chemokines, CXCL12, CCL11, and CCL21 can trigger integrin-dependent adhesion of leukocytes, preceding crawling towards interendothelial junctions (1, 83–88).

There are other important molecules in this step, namely Duffy antigen receptor for chemokines (DARC) and D6. DARC can bind multiple chemokines from CXC and CC subfamilies, although their binding does not induce G protein-coupled cellular responses (89, 90). DARC is expressed on endothelium of capillaries and post-capillary venules, transferring chemokines across the endothelium to the lumen where the chemokine can be bound to glycosaminoglycans (GAGs) or ‘presented’ by Duffy (91,92). As a result, chemokines can be immobilized at high local concentrations on endothelial cells in the flowing blood. D6 another chemokine receptor like molecule that lacks G-protein coupling can bind to at least 12 CC chemokines (93) and is expressed on lymphatic endothelial cells, where it controls tissue concentration of CC chemokines by internalizing and degrading its ligands (94, 95).

In vitro dynamic model of BBB

After leukocyte arrest on the vascular lumen, signaling from chemokines on the abluminal aspect of the endothelium may initiate leukocyte transmigration. Using a modified Boyden chamber and human umbilical vein endothelial cells (HUVECs) under physiological flow conditions, it was shown that CXCL12 on the luminal side induces two steps in transendothelial migration for T-lymphocytes: arrest and crawling on activated HUVEC layers under shear forces. These actions of CXCL12 enhance final transmigration to abluminal CCL5, a weak subendothelial chemokine stimulus (96). This research set the stage for development of a new generation of *in vitro* BBB models.

Leukocyte migration across the BBB has been shown in many neurological disorders such as multiple sclerosis and stroke (97, 98), but the precise functions of chemokines in mediating leukocyte-endothelial interactions at the BBB remain incompletely understood. Models of the human BBB are being developed to address these issues. The existence of a large number of different *in vitro* models suggest that there is no one perfect model system and that certain models can be advantageous in specific situations. To closely mimic *in vivo* conditions, *in vitro* BBB models should have four important properties. Initially, cells for *in vitro* experiments should be isolated from human sources and their physiological and morphological properties should remain consistent and BBB-like. Secondly, endothelial cells should be co-cultured with other BBB components. Thirdly, the model should incorporate shear forces. Finally, the model should allow the transendothelial migration of inflammatory cells that can be recovered for further analysis. Further, the model should permit addition of chemokines.

Cell lines for BBB experiments

Primary cultures of brain microvascular endothelial cells (BMECs) represent the closest possible approximation to the *in vivo* BBB (99). The most widely used primary BMECs originate from rat, mouse, pig, and cow (100). The use of human BMECs is rare and limited (101, 102) due to the restricted availability of human brain tissue as well as the high cost and

special skills necessary for isolation and culture of primary human BMECs. Unfortunately most primary BMECs lose their specific characteristics in culture within limited passages and rapidly cease being useful as *in vitro* models of the human BBB (103).

To address these issues, immortalized human BMECs were generated by expressing simian virus 40 large T antigen (SV40-LT) (104,105), human papilloma E6E7 gene (106), E1A adenovirus gene (107), or Rous sarcoma virus (108), as well as by incorporating human telomerase (109). Well-characterized human BMEC lines include human cerebral microvascular endothelial cells (hCMEC/D3) and transfected human brain microvascular endothelial cells (THBMECs) (104,109). hCMEC/D3 were established by transducing primary human brain endothelial cellswith lentiviral vectors incorporating human telomerase and SV40-LT. They have high expression of junctional proteins and have been widely used for cell signaling and drug transport studies (110–119). THBMECs were isolated from human brain microvessels and immortalized by transfection with SV40-LT (104). They share characteristics of primary human BMECs including expression of tight junction-associated proteins, high transendothelial electrical resistance (TEER) (104,120), expression of factor VIII-related antigen and gamma-glutamyl transpeptidase, and uptake of 1, 1′-dioctadecyl-3, 3, 3, 3′-tetramethylindocarboxyamine perchlorate-labeled acetylated low-density lipoprotein (121). However these human BMEC lines lack contact inhibition and can lose the morphological and physiological properties of their *in vivo* siblings because of their immortalization particularly at high passage number or super-confluence. Under those conditions, they can present transudative intercellular junctions and lack paracellular barrier properties, which limit their effective use as an *in vitro* BBB model (122). Moreover, complex karyotype changes were recently reported in immortalized BMECs, rendering important the genetic testing of cell lines before their application to *in vitro* studies (123). As a general statement, there are few cell lines that are appropriate for *in vitro* BBB experiments.

Anew conditionally immortalized human BMEC cell line was established recently using a temperature-sensitive SV40-LT in order to improve BBB-like differentiated characteristics of these immortalized cell lines (124). At 33°C, SV40-T antigen binds and inhibits p53 and Rb, which are strong tumor suppressors, leading to continuous cell proliferation. At 37°C, SV40-LT is inactivated, and the cells exhibit growth arrest and differentiate into endothelial cells. These conditionally immortalized cells express occludin and claudin-5 at intercellular boundaries as well as influx and efflux transporters. At 37°C, conditional immortalized human BMEC cells retain the physiological and morphological properties of human BMECs and may represent a usefull cellular model for *in vitro* experiments.

In vitro BBB model for co-culture experiments

Co-culture systems, incorporating communication between endothelial cells and other BBB components, provide a closer reproduction of *in vivo* conditions. A significant step towards the understanding of co-culture models was the discovery that glial cells enhanced BBB properties (125). After hollow fibers with transmural microperforations were generated, many *in vitro* co-culture models incorporating brain endothelial cells and glial cells were developed.

Astrocyte endfeet are the cell components in closest proximity to brain capillary endothelial cells (126–130). Most co-culture BBB models focused on reconstructing the brain microenvironment by incorporating astrocyte co-cultures, or astrocyte-conditioned medium, to further induce BMECs (126, 131–135). It is now possible to evaluate endothelial cells in the presence of other types of cells, such as pericytes (136, 137), neurons (138), and microglia (139). Moreover, studies have been conducted with triple cultures of BMVECs, astrocytes, and pericytes (103,140), as well as with BMVECs, astrocytes and neurons (141),

all extracted from rodent brain. The establishment of human multi- culture systems using BMECs and other BBB components is challenging because of the limited availability of human cell lines and the complexity of these multi- culture systems (142–145).

Dynamic model of BBB for shear stress

There is increasing evidence that shear stress affects endothelial-leukocyte interactions in a complex and subtle fashion (146). This understanding led to the development of dynamic *in vitro* models. Among the first dynamic models were co-cultures of bovine aortic endothelial cells and glial cells (135, 147). Recently a dynamic model allowing pulsatile flow and using hCMEC/D3 cell line and astrocytes was developed (134, 148). The model showed much higher TEER than static models. Now that it is clear that shear stress allows i*n vitro* endothelial cells to incorporate many physiological, anatomical, and biochemical BBB characteristics, including leukocyte transmigration and drug-resistant properties (134, 135, 149, 150), flow-based models are beginning to be applied for *in vitro* BBB studies.

Dynamic model of BBB for transmigration in response to chemokines Pioneering flow-based models showed convincingly that shear forces in the presence of chemokines control the processes of leukocyte transmigration, including arrest and crawling (83, 96, 151, 152). Recently, due to the construction of more physiological shear stress systems and the development of hollow fiber technology, some attractive artificial BBB models for migration have been developed (149, 153). For example, Man *et al.* (149) constructed a model that allows the transendothelial migration of inflammatory cells with the addition of chemokines. This model demonstrated that monocytes selectively adhered to BBB endothelium in response to CXCL12 and facilitated lymphocyte migration across BBB. This model provides a three-dimensional, controllable and physiologically relevant environment where vascular endothelial cells can be exposed to physiological levels of flow and chemokines, and might be useful for future transmigration experiments.

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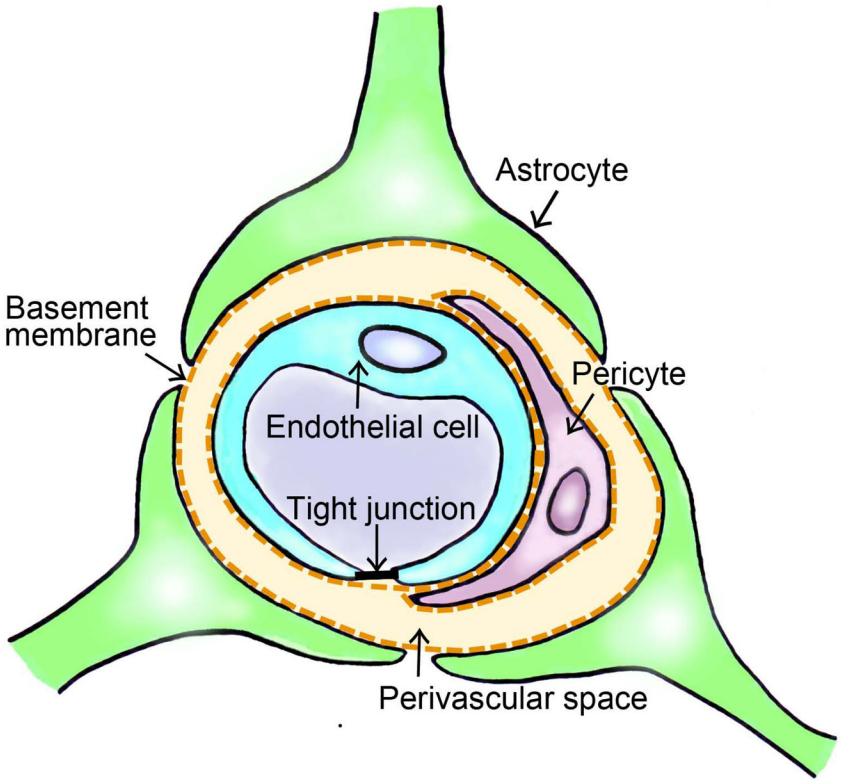

Fig. 1. Cellular structure of the BBB

Endothelial cells have luminal tight junctions and form the capillary and the barrier. There is a basement membrane that surrounds the pericyte and astrocyte outside endothelial cells. Astrocytic endfeet are in close proximity to all of these structures.

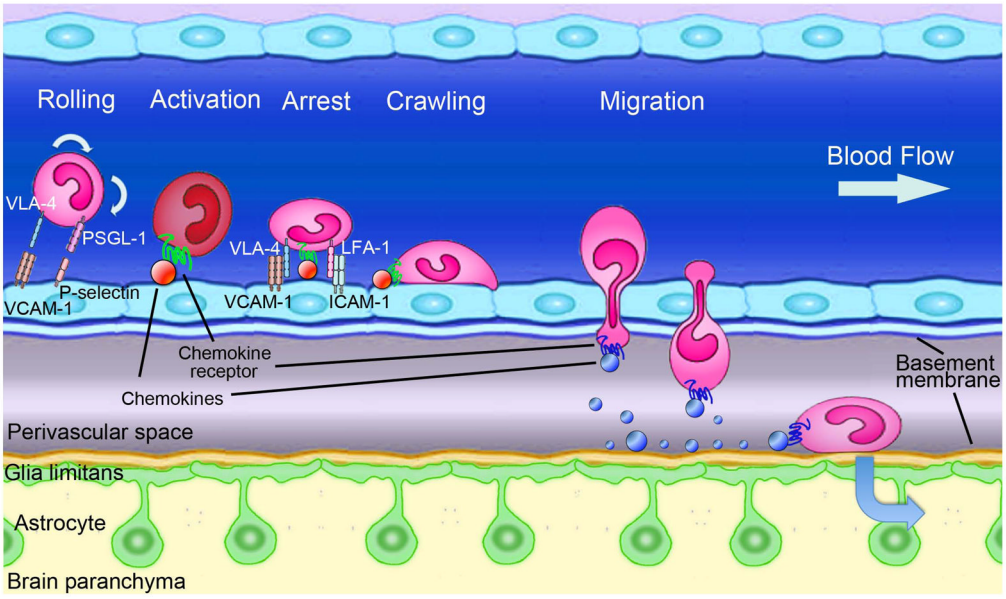

Fig. 2. Multi-step recruitment of leukocytes into the CNS

The five steps are shown. *Rolling:* the binding of P-selectin and PSGL-1 in leukocytes and VCAM1 and VLA-4 in leukocytes allows the leukocyte to slow on endothelial cells; *Activation:* chemokines on the endothelial cells activate the rolling leukocyte; *Arrest:* activated leukocyte upregulates the VLA-4 and LFA-1. Binding to VCAM-1 and ICAM-1 on the endothelial cell allows the activated leukocyte attach to endothelial cells; *Crawling:* arrested leukocyte crawls to preferred site for migration; *Migration:* crawling leukocytes migrate across the endothelial cell via the paracellar or transcellar pathway. Luminal chemokines allow the crawling leukocyte to cross the endothelial cell. By abluminal chemokines leukocytes migrate to the CNS across the glia limitans. Key molecules involved in each step: PSGL-1, P-selectin glycoprotein ligand 1; VLA-4, very late antigen 4; VCAM-1, vascular cell adhesion molecule; LFA, lymphocyte function-associated antigen 1; ICAM-1, intercellular adhesion molecule-1. Adapted from (1).

Table 1

Chemokines and their related receptors (67, 72,154–157).

Subfamily		
Subgroup		
Chemokine name	**Alternative name**	**Chemokine Receptor**
CXC family		
ELR motif(+)		
CXCL1	Gro-alpha, MGSA, N51/KC, MIP-2	CXCR2
CXCL2	Gro-beta, MIP-2 alpha	CXCR2
CXCL3	Gro-gamma, MIP-2 beta	CXCR2
CXCL5	ENA-78	CXCR2
CXCL6	GCP-2	CXCR1, CXCR2
CXCL7	beta-TG, CTAP-III, NAP-2	CXCR2
CXCL8	IL-8	CXCR1, CXCR2
CXCL15	Lungkine	Unknown
ELR motif(−)		
CXCL4	Platelet factor 4(PF4)	Unknown
CXCL9	MIG	CXCR3
CXCL10	IP10, CRG-2	CXCR3
CXCL11	I-TAC, beta-R1, IP9, H174	CXCR3, CXCR7
CXCL12	SDF-1 alpha, SDF1 beta, PBSF	CXCR4, CXCR7
CXCL13	BCA-1, BLC	CXCR5
CXCL14	BRAK, bolekine	Unknown
CXCL16	SR-PSOX	CXCR6
CC family		
CCL1	I-309	CCR8
CCL2	MCP-1	CCR2
CCL3	MIP-1, LD78	CCR1, CCR5
CCL4	MIP-1, Act-2	CCR5
CCL5	RANTES	CCR1, CCR3, CCR5
CCL6	mC10	CCR1
CCL7	MCP-3, FIC, MARC	CCR1, CCR2, CCR3, CCR5
CCL8	MCP-2	CCR1, CCR2, CCR3, CCR5
CCL9/10	MIP-1gammma	CCR1
CCL11	Eotaxin	CCR3, CCR5
CCL12	MCP-5	CCR2
CCL13	MCP-4, CK10	CCR1, CCR2, CCR3, CCR5
CCL14	HCC, CK1	CCR1
CCL15	HCC-2, MIP-5, MIP-1	CCR1, CCR3
CCL16	HCC-4, CK12	CCR1

Subfamily		
Subgroup		
Chemokine name	**Alternative name**	**Chemokine Receptor**
CCL17	TARC	CCR4
CCL18	DC-CK1, PARC, MIP-4, CK7	Unknown
CCL19	MIP-3	CCR7, CCR11
CCL20	MIP-3, LARC, Exodus-1, CK4	CCR6
CCL21	SLC, 6Ckine, Exodus-2, TCA4	CCR7, CCR11, CXCR3,
CCL22	MDC	CCR4
CCL23	MPIF-1, CK8, MIP-3	CCR1,CCR12
CCL24	MPIF-2, CK6, Eotaxin-2	CCR3
CCL25	TECK, CK15	CCR9, CCR11
CCL26	Eotaxin-3, MIP-4	CCR3, CCR10
CCL27	CTAK, Eskine	CCR10
CCL28	skinkine, MEC	CCR3, CCR10
C family		
XCL1	Lymphotactin alpha, SCM-1 alpha,ATAC alpha	XCR1
XCL2	Lymphotactin beta, SCM-1 beta, ATAC beta	XCR1
CX3C family		
CX3CL1	Fractalkine	CX3CR1

Table 2

Chemokines receptors and their related chemokines (68, 71,157–160).

Receptor	Chemokine Ligands
CXC family	
CXCR1	CXCL6, CXCL8
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8
CXCR3	CXCL9, CXCL10, CXCL11
CXCR4	CXCL12
CXCR5	CXCL13
CXCR6	CXCL16
CXCR7	CXCL11, CXCL12
CC family	
CCR1	CCL2, CCL3, CCL5, CCL6, CCL7, CCL8, CCL9/10, CCL13, CCL14, CCL15, CCL16, CCL23
CCR2	CCL2, CCL7, CCL8, CCL12, CCL13
CCR3	CCL5, CCL7, CCL8, CCL11, CCL13, CCL15, CCL24, CCL26, CCL28
CCR4	CCL17, CCL22
CCR5	CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL15
CCR6	CCL20
CCR7	CCL19, CCL21
CCR8	CCL1
CCR9	CCL25
CCR10	CCL27, CCL28
CCR11	CCL19, CCL21, CCL25
CCR12	CCL23
C family	
XCR1	XCL1, XCL2
CX3C family	
CX3CR1	CX3CL1