

# Vascular inflammation in central nervous system diseases: adhesion receptors controlling leukocyte–endothelial interactions

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RECEIVED JULY 31, 2010; REVISED NOVEMBER 16, 2010; ACCEPTED NOVEMBER 18, 2010. DOI: 10.1189/jlb.0710432

## ABSTRACT

Leukocyte trafficking from the blood into the tissues represents a key process during inflammation and requires multiple steps mediated by adhesion molecules and chemoattractants. Inflammation has a detrimental role in several diseases, and in such cases, the molecular mechanisms controlling leukocyte migration are potential therapeutic targets. Over the past 20 years, leukocyte migration in the CNS has been investigated almost exclusively in the context of stroke and MS. Experimental models of ischemic stroke have led to the characterization of adhesion molecules controlling leukocyte migration during acute inflammation, whereas EAE, the animal model of MS, has provided similar data for chronic inflammation. Such experiments have led to clinical trials of antileukocyte adhesion therapy, with consistently positive outcomes in human subjects with MS, showing that interference with leukocyte adhesion can ameliorate chronic inflammatory CNS diseases. This review summarizes our current understanding of the roles of adhesion molecules controlling leukocyte–endothelial interactions in stroke and MS, focusing on recently discovered, novel migration mechanisms. We also discuss the growing evidence suggesting a role for vascular inflammation and leukocyte trafficking in neurodegenerative diseases such as AD. Moreover, we highlight recent findings suggesting a role for leukocyte–endothelial interactions in the pathogenesis of seizures and epilepsy, thus linking endothelial activation and leukocyte trafficking to neuronal electrical hyperactivity. These emerging roles for leukocytes and leukocyte ad-

hesion mechanisms in CNS diseases provide insight into the mechanisms of brain damage and may contribute to the development of novel therapeutic strategies. *J. Leukoc. Biol.* **89**: 000–000; 2011.

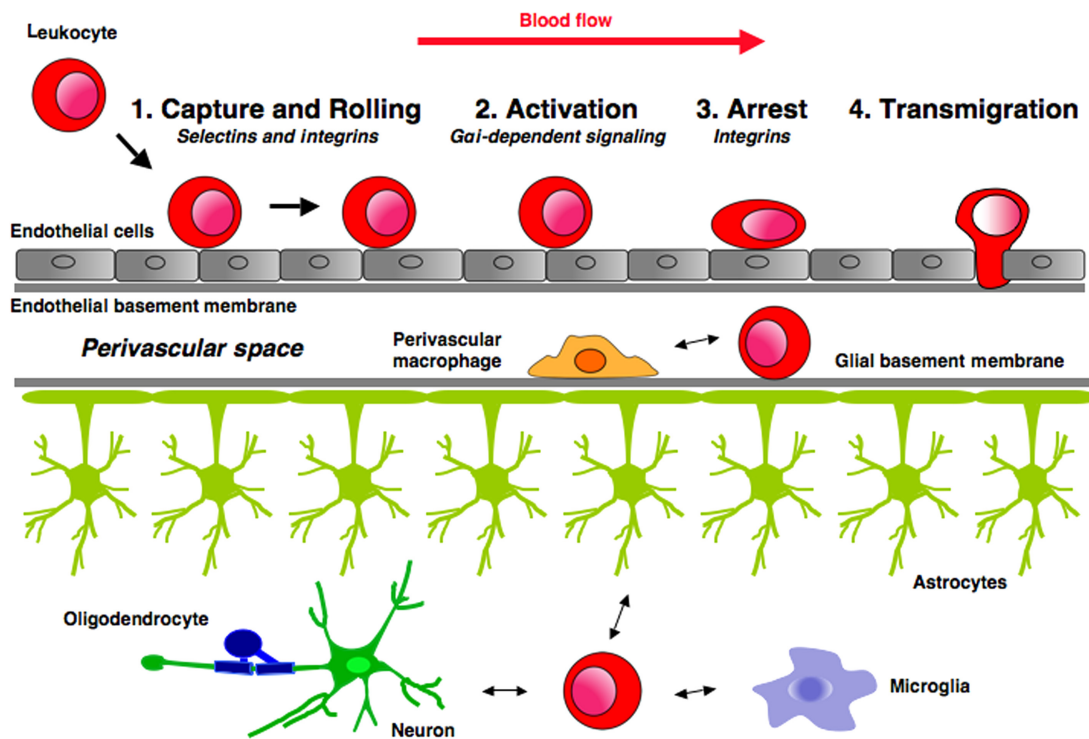
## Introduction

The migration of leukocytes from blood vessels into the CNS is a key event in the pathogenesis of neurological diseases involving acute and chronic inflammation. Leukocyte extravasation is a finely regulated sequence of events controlled by adhesion molecules and activating factors [1, 2]. It is often described in terms of the following four “classical” steps: 1) capture (tethering) and rolling, which are mediated by interactions between selectins and mucins and between integrins and members of the Ig superfamily; 2) activation, during which signaling through the  $G\alpha_i$  pathway is induced by chemoattractants and leads to the activation of integrins; 3) arrest, which is mediated by leukocyte integrins and their endothelial counter-ligands; and 4) diapedesis/transmigration (**Fig. 1**) [3]. More recently, several additional steps have been defined, including slow rolling, adhesion strengthening and spreading, intravascular crawling, and finally, transcellular and paracellular transmigration [4].

Central to the role of leukocyte migration in inflammatory CNS diseases is the concept of the BBB, the specialized lining of capillaries that restricts the passage of certain types of molecules into the CNS. Early research linked the BBB concept to leukocyte migration and led to the misconception that leukocyte extravasation and the diffusion of soluble molecules are regulated at the same site in the vascular tree, resulting in the imprecise evaluation of data [5]. Leukocyte trafficking from the blood into the CNS occurs at postcapillary venules, and the “barrier” function of the CNS endothelium is a result of specialized microvascular endothelial cells that have intercellular tight junctions and are associated with a basement mem-

Abbreviations: A $\beta$ =amyloid  $\beta$  peptide, AD=Alzheimer disease, ALCAM=activated leukocyte CAM, BBB=blood-brain barrier, CAA=cerebral amyloid angiopathy, CSF=cerebrospinal fluid, EAE=experimental autoimmune encephalomyelitis, FucT=fucosyltransferase, HEV=high endothelial venule, I/R=ischemia-reperfusion, LT=leukotriene, Mac-1=macrophage antigen-1, MAdCAM-1=mucosal vascular addressin CAM-1, MS=multiple sclerosis, PAF=platelet-activating factor, PD=Parkinson disease, PML=progressive multifocal leukoencephalopathy, PSGL-1=P-selectin glycoprotein ligand-1, S1P=sphingosine 1-phosphate, SAS=subarachnoid space, Treg=regulatory T cell

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**Figure 1. The steps of leukocyte migration through the endothelium in CNS parenchymal venules.** Endothelial cells in CNS parenchymal venules have tight intercellular junctions and are associated with inner and outer basement membranes. End feet (projected mostly by astrocytes) form the glia limitans, which is also associated with a glial basement membrane. The endothelial and glial basement membranes delimit the perivascular space. The steps of a “simplified” model of leukocyte migration through the endothelium are presented above: 1) capture and rolling, which are mediated by selectins/mucins and integrins/members of the Ig superfamily; 2) activation, in which chemokines and other chemoattractants induce intracellular signaling through the G $\alpha_i$  pathway, leading to integrin activation; 3) arrest, which is mediated by activated integrins and their endothelial Ig superfamily counter-ligands; and 4) diapedesis or transmigration. To reach the parenchyma and potentially interact with neural cells, leukocytes must cross the endothelial basement membranes, interacting with cells in the perivascular space, and then cross the glia limitans. Notably, CNS venules in the pial area lack the glia limitans and perivascular space. Once the leukocytes reach the brain parenchyma, they can physically or chemically interact with neurons and glial cells.

brane and ECM [5–8]. In addition, astrocytes and microglial foot processes form the glia limitans, which together with its basement membrane, constitutes the parenchymal side of the vascular wall (Fig. 1). Leukocyte migration from postcapillary venules in the CNS parenchyma therefore involves crossing the endothelium and endothelial basement membranes into Virchow-Robin spaces, followed by migration through the glia limitans with its distinct basement membrane and dense, albeit discontinuous, layer of astrocytic and some microglial end feet [5]. The complexity of leukocyte migration in the CNS parenchyma suggests that leukocytes require increased migration capacity and/or a more-permeable vascular wall and glia limitans to reach the neuropil. Finally, the motility behavior of extravasated leukocytes in the neuropil is largely unknown, and the molecular mechanisms controlling leukocyte trafficking inside CNS parenchyma await elucidation.

This review first summarizes the roles of selectins, integrins, and their ligands in leukocyte migration and then focuses on the adhesion receptors controlling leukocyte rolling and arrest in CNS vessels during acute and chronic CNS inflammation. We emphasize novel aspects of leukocyte migration in stroke and MS and discuss the emerging role of leukocyte trafficking in new contexts such as AD and epilepsy.

## ADHESION MOLECULES CONTROLLING LEUKOCYTE-ENDOTHELIAL INTERACTIONS

### Selectins and mucins

Selectins are adhesion molecules involved in the tethering (capture) and rolling of leukocytes in the blood vessels of lymphoid and nonlymphoid tissues under physiological and pathological conditions. All three of the known selectins (L, P, and E) are type I transmembrane glycoproteins that bind sialylated carbohydrate structures in a Ca<sup>2+</sup>-dependent manner. Each selectin has several consensus repeat domains with homology to lectins and complement regulatory proteins, resulting in subtle differences in carbohydrate binding that confer selectin specificity [9].

L-selectin is expressed on most circulating leukocytes and controls homing to secondary lymphoid organs and migration to sites of inflammation (see Table 1) [9–11]. It binds to peripheral node addressins and MAdCAM-1 in the HEVs and can mediate secondary tethering by interacting with the mucin PSGL-1 expressed by adhered leukocytes or leukocyte fragments or directly expressed by inflamed endothelium, suggesting it helps to deliver leukocytes expressing L-selectin to sites

TABLE 1. Selectins and Integrins Controlling Leukocyte Trafficking

Adhesion molecule	Expression pattern	Ligands involved in leukocyte trafficking	Function
L-selectin	Myeloid cells, naïve and central memory lymphocytes [9]	Peripheral node addressins [9–11] MAdCAM-I [9–11] PSGL-1 [9, 13]	Lymphocyte capture and rolling in the (HEVs) of peripheral LNs and Peyer's patches [9–11] Leukocyte secondary tethering in inflamed vessels [9, 12–14]
P-selectin	Constitutive expression on the microvessel endothelial cells in the lung and choroid plexus, on peritoneal macrophages, on platelet-derived microparticles [9, 15, 16] Up-regulated on activated platelets and inflamed endothelium [9, 18, 19]	PSGL-1 [9, 20]	Lymphocyte migration in the normal lung and CNS [9, 15, 16] Leukocyte migration in sites of inflammation [9, 18, 19]
E-selectin	Constitutively expressed in normal skin vessels [17] Up-regulated on inflamed endothelium [9, 18, 19]	PSGL-1 [9, 20] CD44 [21, 22]	Lymphocyte homing in the normal skin [9, 17] Leukocyte migration in sites of inflammation [9, 18, 19]
$\alpha$ L $\beta$ 2 integrin	All leukocytes [3, 4]	ICAM-1 [3, 4] ICAM-2 [3, 4]	Lymphocyte arrest in HEVs (homing to lymphoid organs) [1–4] Leukocyte rolling and arrest in inflamed venules [3, 4, 23]
$\alpha$ M $\beta$ 2 integrin	Innate immunity cells and some T cells [3, 4]	ICAM-1 [3, 4]	Leukocyte migration to sites of inflammation [3, 4]
$\alpha$ 4 $\beta$ 1 integrin	Monocytes, T cells, eosinophils [3, 4] Neutrophils under inflammatory conditions [24]	VCAM-1 [4]	Lymphocyte, monocyte, and eosinophil rolling and arrest in inflamed venules [3, 4] Neutrophil rolling in sites of inflammation [24]
$\alpha$ 4 $\beta$ 7 integrin	T cells [3]	MAdCAM-1 [3]	Lymphocyte homing in mucosal tissues [3, 25]

of inflammation (see Table 1) [9, 12–14]. P-selectin and E-selectin are constitutively expressed on endothelial as well as nonendothelial cells or cell fragments and are also up-regulated by inflamed endothelium in most organs during inflammatory diseases (Table 1) [9, 15–19]. Several glycosyltransferases have a role in the synthesis of selectin ligands, including the FucTs, FucT-IV and FucT-VII, the *O*-linked branching enzyme core-2- $\beta$ 1,6-glucosaminyltransferase-I, a  $\beta$ 1,4-galactosyltransferase-I, and at least two sialyltransferases of the ST3Gal family—tyrosine sulfotransferases and sulfotransferases [4, 9, 26].

PSGL-1 is a mucin-type glycoprotein that binds all three selectins in vivo [9, 20]. It is considered the main ligand for P-selectin and is expressed on the surface of myeloid cells, activated lymphocytes, monocyte-derived microparticles, and inflamed endothelial cells [4, 9, 14]. The expression of cutaneous lymphocyte antigen, a modified version of PSGL-1 generated by FucT-VII, has been implicated in the interactions with E-selectin and in lymphocyte traffic to the brain in the context of early EAE, the animal model of MS [27, 28].

CD44 is an additional ligand for E-selectin that controls rolling velocity in neutrophils [21, 22]. It can also initiate the rolling of activated T cells by binding to endothelial hyaluronic acid, followed by firm adhesion mediated by the integrin VLA-4 [29], which associates with CD44, forming a bimolecular complex between rolling and firm adhesion, which requires an intact CD44 cytoplasmic tail [30].

### Integrins and their ligands

The integrins are a large family of heterodimeric transmembrane glycoproteins that attach cells to ECM proteins or to ligands on other cells. Integrins comprise a large (120–170 kDa)  $\alpha$ -subunit and a small (90–100 kDa)  $\beta$ -subunit.

The most prominent member of the  $\beta_2$  integrin family is leukocyte  $\alpha_L\beta_2$  integrin (CD11a/CD18), also known as LFA-1 [4], which participates in rolling interactions but predominantly mediates the firm adhesion/arrest of leukocytes in the blood vessels of lymphoid organs or at sites of inflammation by binding the Ig superfamily ligands ICAM-1 (CD54) and ICAM-2 (CD102), expressed by the vascular endothelium [3, 4, 23]. Another important member of the  $\beta_2$  integrin family is  $\alpha_M\beta_2$  (CD11b/CD18), also known as Mac-1 or complement receptor 3, which mediates interactions with vascular ICAM-1 [3]. Interestingly,  $\alpha_L\beta_2$  and  $\alpha_M\beta_2$  have recently been shown to be activated by intracellular signaling generated when PSGL-1 is cross-linked by P-selectin, suggesting that the PSGL-1 signaling pathway is a key regulator of integrin-mediated firm adhesion in the control of leukocyte recruitment [31].

The most important  $\beta_1$  integrin expressed on leukocytes is VLA-4 ( $\alpha_4\beta_1$  integrin, CD49d/CD29). This binds to VCAM-1 (CD106) expressed on endothelial cells and is implicated in the control of leukocyte rolling and firm arrest in inflamed vessels [4, 24]. VLA-4 can associate with molecules that regu-

late its ability to bind VCAM-1, such as CD47, CD81, and CD44 [32]. Recently, a further VLA-4 ligand was identified as osteopontin, a member of the Sibling protein family (small integrin-binding ligand, *N*-linked glycoproteins), but a role for this molecule in leukocyte trafficking has yet to be demonstrated [33]. Interactions between  $\alpha_4\beta_7$  integrin and its vascular ligand MAdCAM-1 play a specific role in lymphocyte homing by acting as a brake during naive lymphocyte interactions in the HEVs of Peyer's patches [25].

The avidity of adhesion mediated by LFA-1 and VLA-4 is regulated by two integrin activation mechanisms—i.e., binding affinity and the valency of ligand binding [4]. Higher affinity results from conformational changes of individual integrin heterodimers, which leads to increased ligand-binding energy and a decrease in the rate of ligand dissociation. In contrast, valency reflects the density of integrin heterodimers within the plasma membrane region involved in cell adhesion and can depend on the abundance of individual integrins and their lateral mobility [4].

Endothelial chemokines bind to GPCRs, exposed by leukocytes, and trigger integrin-dependent arrest to endothelial cells. The following chemokines are able to induce activation of  $\beta_1$ ,  $\beta_2$ , and  $\beta_7$  integrin-dependent adhesion: CCL2, CCL3, CCL4, CCL5, CCL17, CCL19, CCL21, CCL20, CCL22, CXCL8, CXCL9, CXCL10, and CXCL12 [4, 34, 35]. In addition to chemokines, lipid mediators, such as LTs, PAF, and S1P, were shown to trigger integrin-dependent adhesion in leukocytes through GPCRs [36–39].

## ADHESION RECEPTORS CONTROLLING LEUKOCYTE-ENDOTHELIAL INTERACTIONS IN CNS DISEASES

### Ischemic stroke

Counter-ligands for leukocyte adhesion molecules are up-regulated on damaged endothelial tissue soon after the onset of ischemic stroke [40], and brain endothelium releases cytokines and chemokines that recruit and activate platelets and leukocytes [40, 41]. This leads to the migration of neutrophils and monocytes through the brain endothelium during the acute phase of stroke, which is also characterized by the secretion of inflammatory mediators, the activation of microglia, and neuronal damage [41–43]. Lymphocyte migration in the CNS has also been observed during stroke and has been implicated in the progression of cerebral I/R injury. Recently, it has been shown that blocking T cell infiltration into the brain using the immunosuppressant FTY720 reduces I/R-induced brain damage [44]. Although the role of specific lymphocyte subpopulations in stroke pathogenesis is not clear, recent data have shown that  $\gamma\delta$  T cells producing IL-17 are recruited in the brain and have a central, pathogenic role in the delayed phase of experimental ischemic brain injury [44].

Selectins, mucins, integrins, and Ig superfamily proteins have been implicated in the induction of neural damage in experimental animal models of ischemic stroke. The up-regulation of endothelial E- and P-selectin expression is required for the development of postischemic inflammatory responses

and the amplification of brain injury [45–47]. Most studies have also shown the presence of elevated, soluble selectins in the serum and corticospinal fluid of human ischemic stroke patients in the acute phase, suggesting vascular inflammation and endothelial selectin expression [48–51]. Interestingly, recent reports suggest that elevated levels of soluble P-selectin in mice can modulate cerebral vascular function and thus, exacerbate the effects of stroke [52]. These results support recent data obtained in patients with peripheral arterial occlusive disease, showing that higher levels of soluble P-selectin in the plasma enhances neutrophil adhesion in a PSGL-1-dependent manner [53]. Elevated levels of P-selectin may indeed favor leukocyte adhesion to inflamed brain endothelium, as PSGL-1 binding to P-selectin promotes leukocyte integrin activation through a complex signaling cascade involving Src kinases, phosphorylated Nef-associated factor-1 and phosphoinositide-3-OH kinase [31]. Taken together, these data suggest that P-selectin is a crucial molecule in stroke pathogenesis.

Mice deficient for P-selectin and animals treated with antibodies that inhibit P-selectin present a lower infarct volume, lower mortality, and faster cerebral flux recovery after reperfusion [54, 55]. Similar results were achieved in mouse and rat models of ischemic stroke treated with sialyl-Lewis<sup>x</sup> analogs that block E- and P-selectin or E-selectin alone [56, 57]. The administration of a humanized anti-E/P-selectin mAb also reduced the cerebral infarct area and ameliorated the neurological outcome in an experimental study of stroke performed on nonhuman primates [58]. Interestingly, mucosal tolerance to E-selectin conferred cell-mediated protection against ischemic brain injury by immunomodulation targeted to activated blood vessel segments [59–61]. In contrast to the anti-E- and -P-selectin approaches described above, antibodies specific for L-selectin did not affect stroke outcome in animal models [62]. The effect of blocking PSGL-1 in animal models of ischemic stroke has yet to be investigated, but in other noncerebral, experimental models of ischemia, soluble rPSGL-1 or anti-PSGL-1 antibodies can reduce reperfusion-associated damage [63].

The expression of endothelial Ig superfamily adhesion molecules has correlated with I/R injury in a variety of *in vivo* studies. ICAM-1 expression increased soon after stroke with a peak 12–24 h after onset and preceded leukocyte migration into the brain [64, 65]. Neutralization of ICAM-1, with mAb or anti-sense oligonucleotides, improved the neurological outcome in several animal models of I/R injury [66–70]. Moreover, ICAM-1-deficient mice presented with a smaller infarct volume and fewer infiltrated leukocytes after reperfusion following stroke [71–73]. The role of VCAM-1 in animal models of reperfusion injury appears to be less significant, as the treatment of experimental stroke models with an anti-VCAM-1 antibody did not ameliorate the neurological outcome [74].

The role of leukocyte  $\beta_2$  integrins in animal stroke models has been studied extensively. In agreement with the results obtained by blocking ICAM-1, the inhibition of CD11b, CD18, and CD11a activity (by gene knockout or by administering specific antibodies) led to a reduction in stroke-associated injury and was related to low-level neutrophil infiltration [67, 75–85]. Importantly, the inhibition of  $\beta_2$  integrins appeared to selectively improve the outcome of stroke associated with reperfu-



sion, supporting the hypothesis that leukocyte trafficking is involved in injuries associated with secondary reperfusion [83]. Although the role of VCAM-1 in ischemic stroke appears less important, the inhibition of VLA-4 using a mAb led to a significant reduction in the infarct size in a rat model of transient focal cerebral ischemia, supporting a role for  $\beta_1$  integrins in ischemic brain injury [86].

Expression of endothelial chemokines potentially able to activate leukocyte integrins and favor leukocyte trafficking has been described in animal models of ischemic stroke. Vascular expression of IL-8 was documented in animal models of stroke and was implicated in neutrophil recruitment and cerebral edema, whereas an anti-IL-8 antibody significantly reduced brain edema and infarct size [87]. Expression of CCL2 in stroke was studied by several laboratories, and the presence of CCL2 on brain endothelium following focal ischemia is thought to have a deleterious effect by increasing monocyte infiltration [88]. In addition, deficiency of CCL2 or of its receptor CCR2 is protective in experimental models of stroke [89, 90]. Monocyte infiltration is thought to be mediated also by CCL3, and the neutralization of this chemokine inhibits cell migration in vivo [91]. Expression of the CXCL12 chemokine was documented on brain endothelium in the peri-infarct and infarct regions, and CXCR4, the CXCL12R, was implicated in the migration of leukocytes in the ischemic brain [92]. CCL4, CCL5, and CXCL10 expression was also correlated to leukocyte infiltration, but their presence on endothelium needs to be clarified by further studies [88, 93, 94].

In addition to chemokines, lipid intermediates have been suggested to mediate leukocyte adhesion in inflamed brain vessels in experimental animal models with relevance to stroke. LTB<sub>4</sub> has been shown to mediate integrin-dependent neutrophil adhesion in vitro and was suggested to mediate neutrophil trafficking after cerebral ischemia [36, 95, 96]. In addition, cysteinyl LTs are able to activate integrin-dependent neutrophil adhesion and have been shown to induce neutrophil arrest in intravital microscopy settings with relevance to stroke [97–99]. PAF is a classical activator of neutrophil integrins, and its inhibition has therapeutic effects in animal models of stroke [36, 100, 101]. Furthermore, it was shown recently that S1P is able to trigger  $\beta_2$  integrin-dependent adhesion of myeloid cells, and S1P analog FTY720 is neuroprotective in mouse and rat models of ischemic stroke [102–104].

Novel mechanisms of leukocyte migration have been discovered in recent investigations using ischemic stroke models. For example, leukocyte surface apyrase CD39 reduces neutrophil and monocyte trafficking as well as platelet reactivity by phosphohydrolyzation of adenine nucleotides and modulation of the ambient vascular nucleotide milieu and integrin expression [105]. Although no clear role in leukocyte adhesion has been found for the VLA-4 ligand osteopontin, its expression in endothelial cells is induced by impairing the BBB in hypertensive rats, whereas the nasal administration of osteopontin peptide mimetics confers neuroprotection in stroke models, suggesting that osteopontin inhibitors may be useful therapeutic leads [106, 107].

In support of the data from animal models, the expression of leukocyte adhesion molecules can also help to predict the clinical outcome in human ischemic stroke patients. On admission, patients with higher levels of PSGL-1 in neutrophils may present a higher risk for early neurologic deterioration, and the severity of acute ischemic stroke corresponds to the level of Mac-1 expressed in monocytes [108]. Stroke patients also express more endothelial ICAM-1 in the infarcted zone than nonischemic areas [109]. The levels of soluble ICAM-1 and VCAM-1 in the CSF and plasma of patients following cerebral ischemia may also reflect the severity of the stroke, although these results are controversial [48–51, 110–113].

Surprisingly, attempts to replicate the successful antiadhesion molecule therapy achieved in animal models have largely failed in the clinic. A phase-III, randomized, controlled trial using murine monoclonal anti-ICAM-1 (enlimomab) was unsuccessful, as the nonhuman antibody was cleared rapidly by the immune system [114, 115]. A phase-II trial of a humanized CD11/CD18 antibody (rovelizumab) failed to meet predefined criteria for improvement in myocardial infarction and in acute stroke patients [116, 117], and a phase-II trial of recombinant neutrophil-inhibiting factor, a nonantibody inhibitor of Mac-1, was curtailed as a result of the lack of positive results (acute stroke therapy by inhibition of neutrophils) [118]. These discrepancies may reflect differences in the inflammation mechanisms during I/R in humans and experimental animals. The evaluation of treatment duration and the type of pathology (transient vs. permanent ischemia) may also be critical for the design of successful clinical trials. Finally, the inhibition of  $\beta_2$  integrins may reduce the activity of Tregs, which are key cerebroprotective immunomodulators in experimental stroke models [119–121].

## MS

MS is considered a T cell-mediated, inflammatory, demyelinating, autoimmune disease of the CNS, characterized by multifocal, perivascular infiltrates, predominantly of lymphocytes and macrophages. It is accepted that vascular breakdown and T cell migration into the CNS initiate an immune response against myelin antigens that contributes to disease pathogenesis [122, 123]. EAE is a model used to study the pathogenic mechanisms in demyelinating disorders, and MS is the most common of these in humans. The disease model can be induced by immunization with myelin components (active EAE) or by transfer of encephalitogenic T cells (adoptive-transfer EAE).

T cell migration into the CNS is a critical event in EAE/MS pathogenesis. Early experiments suggested that inflammatory cells migrate first into the SAS [124–126], and more recent data suggest that T cells must cross the blood-CSF barrier in choroid plexus vessels [16, 127, 128] or pial vessels [16, 129] to achieve this. Elegant studies using two-photon microscopy showed recently that transferred, myelin-specific T cells migrate first in the pial zone of spinal cord venules, which approach the CNS surface from deeper parenchymal layers [130]. Pial vessels provide an easier route for inflammatory cells; they have different tight junction composition, a different endothelial barrier antigen expression, and they also lack

the astrocyte sheathes found in the parenchymal vessels [5, 131]. Choroid plexus venules also provide a route for leukocyte migration, as they lack tight junctions between endothelial cells and thus, represent an early point for T cell entry into the CNS in MS models [16, 127, 128]. The above data confirm that T cell infiltration and reactivation involve migration into the SAS [132].

EAE can be induced by adoptive transfer of myelin-specific Th1 cells, which produce IFN- $\gamma$ , IL-2, and TNF, or Th17 cells, which produce IL-17A, IL-17F, and IL-22 [132]. Recent data show that Th17 cells have a greater migration capacity than Th1 cells *in vitro* and contribute to brain endothelium damage, suggesting that Th17 cells migrate more aggressively [133]. Although the adhesion molecules involved in Th1–endothelium interactions in different vascular districts (including the brain) are well characterized, the molecular mechanisms controlling Th17 adhesion in the inflamed brain are unknown, and their characterization will provide further insight into EAE/MS pathogenesis. CD8<sup>+</sup> T cells are also emerging as important players in MS and EAE, although their adhesion mechanisms are poorly characterized, and their contribution to tissue damage is still debated. Myelin-specific CD8<sup>+</sup> T cells can induce EAE, and CD8<sup>+</sup> T cells migrating into the CNS outnumbered the CD4<sup>+</sup> T cells in some studies [134–136]. Moreover, single-cell analysis of active MS brain lesions revealed that CD8<sup>+</sup> T cells are far more numerous than the more heterogeneous, extravasated CD4<sup>+</sup> T cells and are also capable of oligoclonal expansion [137]. In support of these results, we have shown that CD8<sup>+</sup> T cells isolated from untreated patients with relapsing-remitting disease in the acute phase display more rolling and arrest behavior than CD4<sup>+</sup> T cells in inflamed murine brain pial venules, suggesting a potential role for CD8<sup>+</sup> T cells in relapses [138]. CD4<sup>+</sup>CD25<sup>+</sup> Tregs can also penetrate the CNS of mice with preclinical EAE, and they accumulate after the onset of disease [139]. However, the adhesion mechanisms controlling Treg–endothelium interactions in CNS venules are completely uncharacterized.

Under physiological conditions, the CNS strictly regulates the immigration of immune cells and performs immunosurveillance in the perivascular and SASs, leaving the parenchyma untouched. P-selectin, E-selectin, and ICAM-1 immunoreactivity was detected in noninflamed human pial and choroid plexus venules but not in parenchymal microvessels, whereas activated memory T cells in normal CSF expressed high levels of PSGL-1 [16]. Based on these important findings, it has been proposed that activated T cells are recruited to the CSF under normal conditions through interactions between P-selectin/P-selectin ligands and ICAM-1/LFA-1 [16].

Recent investigations have identified the critical factors affecting lymphocyte migration in the CNS during EAE, providing new insights into the mechanisms that control neuroinflammation. We have previously used intravital microscopy, which allowed the visualization of pial and (to a limited extent) parenchymal vessels, to show that activated lymphocytes but not resting T cells can roll and firmly adhere in the mouse brain microcirculation when inflammation has been induced with LPS or TNF [27]. Moreover, experiments with TCR trans-

genic mice showed that transgenic T cells specific for myelin basic protein and the irrelevant antigen OVA accumulated in the same quantity in the CNS, suggesting that antigen specificity has no impact on lymphocyte extravasation in the CNS, and it must instead be controlled by T cell activation [140].

Endothelial selectins and their leukocyte counter-ligand PSGL-1 may also play a role in MS and EAE. E-selectin expression was detected in the endothelium of MS lesions in the brain, whereas plasma levels of soluble P- and E-selectin were associated with relapsing-remitting disease [141, 142]. In addition, peripheral blood CD4<sup>+</sup> T cells from MS patients express more PSGL-1 and have a greater transmigration capacity across human brain endothelial cells [143]. However, intravital microscopy in murine cerebral pial vessels showed that CD8<sup>+</sup> T cells from MS patients at the beginning of a new relapse undergo more PSGL-1-dependent rolling on P-selectin than CD4<sup>+</sup> T cells [138]. We have also shown previously that the activation of cerebral microvessels with LPS or TNF induces P- and E-selectin expression in cerebral endothelial cells, mimicking early endothelial activation during EAE [27]. Furthermore, PSGL-1 expression in Th1 cells, together with the FucT activity responsible for PSGL-1 glycosylation, induced efficient tethering and rolling of the T cells in inflamed brain vessels [28]. P-selectin mediates the tethering and rolling of leukocytes in mice with preclinical and clinical EAE, and the inhibition of P-selectin reduces the ability of activated T cells to migrate into the brains of mice with EAE [144–146]. Interactions between endothelial E-selectin and hyaluronan and CD44 expressed on the surface of activated T cells may also contribute to initial adhesive interactions in inflamed brain vessels [147].

Despite consistent data supporting the role of mucins and selectins in leukocyte–endothelial interactions in inflamed brain venules, experiments involving the inhibition of selectins and PSGL-1 in EAE have produced inconsistent results. Antibodies against P-selectin improved the outcome of EAE when coadministered with an antibody against the  $\alpha 4$  integrin [145], but other studies have shown no effect [148–151], and some have even shown that mice deficient in PSGL-1 develop more severe symptoms, supporting recent results obtained in other experimental models of autoimmune diseases [145, 152] (unpublished results). A possible explanation for these contradictory results was offered in a recent study that revealed a correlation between the migration behavior of CD4<sup>+</sup>CD25<sup>+</sup> Tregs and their ability to bind E- and P-selectins, hinting at a protective role for PSGL-1 in EAE [153, 154]. It has also been shown that the stimulation of DCs through PSGL-1 with P-selectin enhanced their ability to generate CD4<sup>+</sup>CD25<sup>+</sup> forkhead box p3<sup>+</sup> Tregs, suggesting a regulatory role for PSGL-1 and selectins during EAE [155].

Endothelial integrin ligands, such as ICAM-1 and VCAM-1, are expressed in the venules of MS/EAE lesions [27, 141, 156, 157]. Elevated levels of soluble endothelial adhesion molecules have been detected in MS patients during relapses and have been associated with disease severity and the spreading lesions observed by magnetic resonance imaging [158–162]. The counter-ligands LFA-1 and VLA-4 have been shown to mediate the rolling and arrest of activated T cells on the endothelium of inflamed cerebral pial vessels in EAE models investigated by

intravital microscopy [27, 144, 145]. In contrast, myelin-specific T cells showed no evidence of rolling on spinal cord pial vessel endothelium (apparently expressing constitutive VCAM, an integrin ligand normally absent in healthy, nonlymphoid inflammatory sites), instead undergoing a unique, immediate arrest event mediated by VLA-4/VCAM-1 [163]. However, more recent two-photon microscopy experiments dispute this finding, showing that adoptively transferred, myelin-specific T cells roll before undergoing arrest in spinal cord pial vessels [130]. The importance of VLA-4–VCAM-1 adhesive interactions has been confirmed in several studies using antibodies against the  $\alpha 4\beta 1$  integrin, which have inhibited or reversed EAE [164–167]. In addition, it has been shown that antibodies against VLA-4 and P-selectin block EAE more efficiently than antibodies against VLA-4 alone [145]. However, treatment with antibodies against VLA-4 after the onset of relapsing EAE (at the peak of acute disease or during remission) exacerbated disease relapses and increased the accumulation of CD4<sup>+</sup> T cells in the CNS [168]. Inhibiting LFA-1/ICAM-1 was less effective than inhibiting VLA-4/VCAM-1, suggesting a key role for VLA-4 in the pathogenesis of EAE [169–171]. There may also be a role for  $\alpha M\beta 2$  in the induction of EAE, as gene knockout and antibody inhibition experiments reduce leukocyte infiltration in the CNS and improve the clinical outcome [171, 172]. The inhibition of MAdCAM-1 and  $\alpha 4\beta 7$  integrin has also been shown to inhibit EAE in some studies, but these results conflict with *in vitro* data showing that MAdCAM-1 is not expressed in the CNS endothelium during EAE, and Stamper-Woodruff studies suggesting that MAdCAM-1 does not mediate T cell adhesion on inflamed CNS endothelium [156, 173, 174]. Taken together, these results suggest that  $\alpha 4$  integrins, LFA-1 and Mac-1 do play a role in the control of leukocyte trafficking in the brain during inflammation and that inhibiting them improves the clinical course of EAE.

Current data suggest that GPCR-dependent signaling is required *in situ* for stable integrin-dependent adhesion of lymphocytes in inflamed brain venules [27, 175]. Lymphoid chemokines CCL19 and CCL21, previously shown to mediate arrest of naïve lymphocytes in HEVs, are also expressed in inflamed brain venules, whereas CCR7<sup>+</sup> cells accumulate in inflammatory lesions during EAE, suggesting that CCL19 and CCL21 are also involved in T lymphocyte migration into chronically inflamed CNS [176, 177]. These results are in agreement with recent data showing that CCL19 is constitutively expressed in the CNS and up-regulated in MS lesions, suggesting that CCR7 may have a role in lymphocyte traffic in the human brain [178]. CXCL12 is expressed on CNS endothelial cells in a normal spinal cord and at the onset of EAE [179]. CXCL12 is also constitutively expressed in human CNS parenchyma on blood vessel walls and is elevated in MS lesions, suggesting a role of this chemokine in leukocyte extravasation in the human brain [180]. Intravital microscopy performed at EAE onset has shown that CCL2 and CCL5 chemokines are involved in leukocyte arrest in inflamed brain venules [181]. CCL2 and CCL5 expression is also abundant within MS lesions, suggesting a role for CCL5 in integrin-dependent arrest in inflamed CNS vessels [182, 183]. The chemokine receptor CXCR3, previously shown to trigger

rapid adhesion, was found on CSF T cells of healthy subjects and patients with MS, whereas active MS lesions have a high frequency of T cells expressing CXCR3, suggesting that CXCR3 may be involved in T cell adhesion in CNS venules [182, 184].

LTB4 has been shown recently to be not only a potent chemoattractant and adhesion activator in neutrophils but also mediate  $\beta 1$ - and  $\beta 2$ -integrin-dependent adhesion in monocytes *in vitro* and *in vivo* under flow condition [185]. Moreover, intravital microscopy experiments showed that LTB4R BLT1 mediates effector CD4 and CD8 rapid integrin-dependent arrest in postcapillary venules [37, 38]. In support of these results, recent studies suggested that LTB4R BLT1 is required for the induction of EAE [186]. SIP was shown recently to activate the interactions of the integrin LFA-1 with its ligand ICAM-1 and of the integrin VLA-4 with its ligand VCAM-1 of polarized T cells at the basal surface of lymphatic but not blood vessel endothelium, suggesting that SIP causes sequestration of lymphocytes in secondary lymphoid organs [39]. Involvement of SIP in lymphocyte adhesion through GPCRs has been identified by previous work with FTY720 (fingolimod), a microbe-derived immunosuppressive agent that acts as an agonist for the S1PRs and increases  $\beta 2$  and  $\beta 7$  integrin-dependent adhesion and homing of lymphocytes in HEVs [187–189]. The administration of fingolimod in animal models of EAE had a preventive and therapeutic effect by reducing T cell infiltration in the CNS as well as by exerting effects on the BBB and resident neural cells [190–194]. Therapeutic efficacy observed in animal studies has been substantiated in Phase 2 and 3 trials involving patients with relapsing or relapsing-remitting MS, and oral treatment with FTY720 was approved by the U.S. Food and Drug Administration in September 2010 [195].

The prominent role of VLA-4 in EAE suggests that the inhibition of  $\alpha 4$  integrins might improve the prognosis of MS patients. Indeed,  $\alpha 4$  integrins have already been targeted successfully with natalizumab, although its success in the treatment of MS has been marred by occasional cases of the opportunistic viral infection PML, which occurs in ~1:1000 patients [196–198]. The induction of PML during natalizumab therapy appears not to be a case of virus reactivation but rather, a modification of the JC virus-specific cellular immune response and the mobilization of JC virus-infected pre-B cells from bone marrow stores [199–201]. For this reason, clinical administration of the drug is restricted to patients with more aggressive disease. Recent studies have demonstrated remarkable efficacy in patients with higher disease activity, including rapid improvement of disability status and ambulation after the failure of previous therapies in relapsing-remitting MS [202, 203]. Overall, the therapeutic efficacy of natalizumab is proof-of-principle that the inhibition of leukocyte trafficking can be used to treat neurological diseases in which leukocyte extravasation has a detrimental role [204].

Although researchers studying leukocyte trafficking during EAE/MS have focused on T cells, monocytes also play a significant role, but their migration is poorly understood. Circulating monocytes that transmigrate into the CNS parenchyma play an active role in exacerbating inflammatory



disease as tissue macrophages by providing a source of proinflammatory cytokines. The presence of monocytes and macrophages in early MS lesions has been correlated with demyelination, and their elimination has been shown to ameliorate EAE [205, 206]. VLA-4 promotes the adhesion of monocytes in inflamed brain endothelium *in vitro* and *in vivo*, but whether the known therapeutic effect of inhibiting VLA-4 includes the repression of monocyte trafficking is not yet clear [164, 204, 207, 208]. In addition to classical adhesion mechanisms, the interaction between signal regulatory protein- $\alpha$  and the integrin-associated protein (CD47) has been shown to contribute to the adhesion step when monocytes migrate into the brain [209].

ALCAM-1 (CD166) was characterized originally as a ligand for CD6 and has been shown recently to regulate leukocyte extravasation in the inflamed CNS [210, 211]. Although it is a member of the Ig superfamily and mediates leukocyte adhesion, it is not considered an integrin ligand. It is expressed on activated CD4<sup>+</sup> T cells, B cells, monocytes, and endothelial cells [210, 211]. ALCAM-1 is up-regulated in the endothelial cells of active MS/EAE lesions. Inhibiting the molecule limits the transmigration of CD4<sup>+</sup> lymphocytes and monocytes across the BBB *in vitro* and *in vivo* and reduces the severity and delays the onset of EAE [211]. Further *in vivo* studies are necessary to clarify its involvement in the multistep process of leukocyte extravasation.

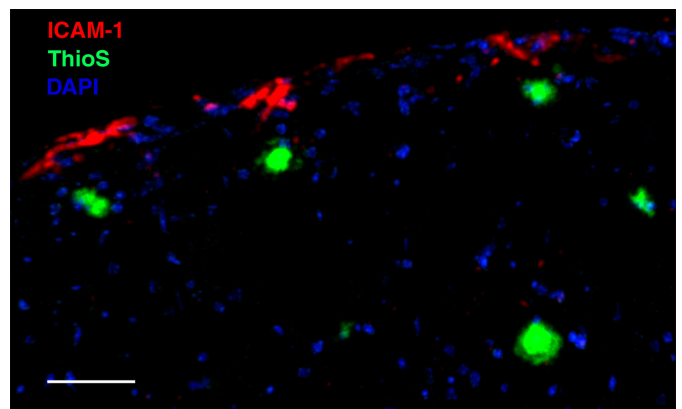
## AD

AD is the most common neurodegenerative disorder and the leading cause of senile dementia worldwide [212]. Clinically, the cognitive and behavioral symptoms of AD are concordant with neuronal loss and atrophy in brain regions linked to learning and memory [213]. Neuropathologically, the hallmarks of the disease are intracellular, neurofibrillary tangles, derived from the cytoskeletal protein  $\tau$ , neuronal degeneration, and extracellular senile plaques composed primarily of A $\beta$  and surrounded by activated microglia, reactive astrocytes, and dystrophic neurites [214, 215]. In addition, a more recently established hallmark of AD is brain inflammation, but whether its role in disease pathogenesis is beneficial or detrimental has yet to be determined [216]. Studies of AD pathology, genetics, and therapy suggest that inflammatory mechanisms are most likely involved in the early steps of the pathological cascade [217].

Investigations of AD patients have revealed evidence of a dysfunctional BBB and impaired perivascular flow, suggesting that the initiation and progression of AD may involve vascular pathology and hemodynamic changes [218, 219]. Ryu and McLarnon [220] have recently provided morphological data indicating a loss of BBB integrity in AD tissues, with extensive areas of fibrinogen immunoreactivity in association with microglial reactivity; this morphology is not present in normal brain samples. The direct injection of A $\beta$ (1–42) into rat hippocampus induced a time-dependent increase in BBB leakage and microgliosis, whereas *i.v.* infusion with A $\beta$ 1–40 produced extensive vascular disruption including endothelial and smooth muscle damage [220, 221]. A $\beta$  is also deposited in the vascular wall of intracerebral and leptomeningeal vessels causing CAA,

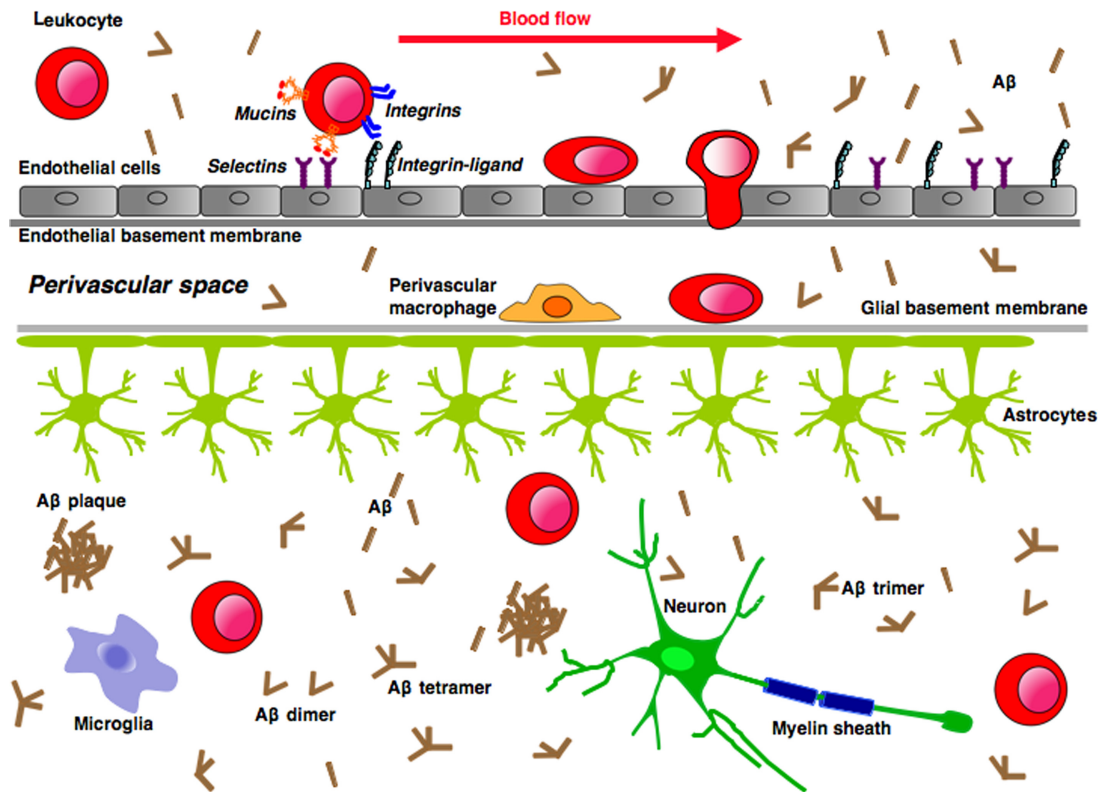
which is present in ~80% of AD brains [222–224]. A $\beta$ -CAA is associated with the degeneration of smooth muscle cells, pericytes, endothelial cells, and loss of BBB integrity [225, 226]. Overall, the human data show that neurovascular dysfunction is present in AD and suggest a pathogenic link between cerebrovascular disease and AD [227].

Several studies have demonstrated that the vascular deposition of A $\beta$  induces oxidative stress and apoptosis in the cerebral vasculature, promotes the expression of adhesion molecules, alters the expression of tight junction proteins, and changes mechanical properties of the endothelial membranes in a manner favoring the transmigration of immune cells [228]. A $\beta$  also stimulates the secretion of inflammatory cytokines and up-regulates the expression of CAMs in human vascular cells cultured *in vitro*, suggesting it can function as an inflammatory stimulator that activates vascular cells [229, 230]. Importantly, leukocyte adhesion and spreading on the endothelium of mesenteric vessels were observed by intravital microscopy minutes after A $\beta$  infusion in rats, and a consistent number of adherent leukocytes underwent transmigration through the vascular wall [221]. Similar results were obtained recently in cerebral vessels after *i.v.* injection of A $\beta$ 1–40 and A $\beta$ 1–42 using a cranial window preparation in rats [231]. A $\beta$  deposition in the cerebral vessels of AD brains correlated with the accumulation of monocytes in the vessel walls and activated microglia in the adjacent parenchyma [232, 233]. In support of these results, *in vitro* studies have shown that exposure to A $\beta$  enhances monocyte adhesion to endothelial monolayers and subsequent transendothelial migration, indicating that A $\beta$  deposition induces the brain endothelium to promote leukocyte adhesion and transmigration (Figs. 2 and 3) [234, 235]. *In vivo* studies using transgenic mouse models of AD have suggested that many microglial cells in the cores of amy-



**Figure 2.** ICAM-1 expression in brain vessels near amyloid plaques in the cortex of an 8-month-old 5XFAD mouse. The 5XFAD mouse is a transgenic model of AD, expressing recombinant human amino precursor protein and Presenilin-1 proteins carrying several pro-AD mutations. The confocal image shows ICAM-1 immunoreactivity (red fluorescence) on cerebrovascular endothelial cells in the cortex upper layer in the proximity of A $\beta$  plaques [thioflavin-S staining (ThioS), green fluorescence]. DAPI staining was used to highlight the cell nucleus (blue fluorescence; original scale bar=50  $\mu$ m). The results represent original data obtained by the authors.





**Figure 3. Leukocyte trafficking mechanisms in AD, potentially induced by A $\beta$ .** Oligomeric A $\beta$  assemblies, commonly known as amyloid-derived diffusible ligands, are found in the blood, perivascular spaces, and brain parenchyma. A $\beta$  stimulates the secretion of inflammatory cytokines and up-regulates the expression of vascular adhesion molecules, leading to adhesion and transendothelial migration of leukocytes, which can accumulate in brain parenchyma and interact with glial cells and neurons. The adhesion molecules and receptors are explained in more detail in Fig. 1 and Table 1.

loid plaques originate from bone marrow and are recruited from the blood [236]. These cells have a beneficial effect, as they can eliminate amyloid deposits by cell-specific phagocytosis. In addition to monocytes, T cell infiltration has been observed in AD patients [237–240]. Togo et al. [238] showed that most T cells in postmortem AD brain tissue are located in the hippocampus and other limbic structures, which are among those regions most heavily affected in AD. In a study investigating the immune response associated with CAA, CD4<sup>+</sup> and CD8<sup>+</sup> T cells were observed in addition to monocytes and macrophages in leptomeningeal and cortical vessels [241].

Interestingly, A $\beta$  induces adaptive immune responses in the peripheral nervous system. A $\beta$ -reactive T cells become more abundant in the elderly and in patients with AD, and T cell priming becomes less frequent with aging [242, 243]. Man et al. [244] showed that peripheral T cells in AD patients overexpress MIP-1 $\alpha$ , which binds to CCR5 on the surface of brain endothelial cells, promoting T cell migration through endothelial tight junctions. Furthermore, the injection of A $\beta$  into the rat hippocampus induces an interaction with receptor for advanced glycation end products expressed on the surface of brain endothelial cells, which up-regulates CCR5 expression and triggers T cell infiltration in the brain [245]. Peripheral T

cells in AD patients also overexpress CXCR2, potentially promoting transendothelial migration [246]. However, it is not clear how circulating T cells penetrate the BBB and infiltrate the AD cerebral parenchyma or how the local inflammatory milieu influences T cell emigration or survival, resulting in the accumulation of T cells in the brain. It is important to determine whether T cell trafficking is involved in the pathogenesis of AD or is solely an epiphenomenon and whether T cells are beneficial or deleterious in AD.

Little is known about the molecular mechanisms controlling leukocyte trafficking in the AD brain. The treatment of cerebral endothelial cells with oligomeric A $\beta$  in vitro results in the expression of P-selectin and increased endothelial stiffness [228]. However, the expression of transmembrane or soluble P-selectin has not been reported in vivo or in AD brains. Increased levels of E-selectin in the plasma, together with the up-regulation of ICAM-1 and VCAM-1, are associated with an increased risk of diabetes, a condition that is commonly associated with cardiovascular disease and AD [247–249]. Although the expression of E-selectin in AD cerebral vessels has yet to be shown, there are significant increases in plasma E-selectin levels in patients with late-onset AD [250]. In addition, a clinical and epidemiological study, aiming to demonstrate that vascular risk factors may be involved in AD, clearly showed a sig-

nificant increase in soluble E-selectin levels in AD patients compared with controls, suggesting that plasma E-selectin could be used as a marker of endothelial dysfunction contributing to disease progression [251]. However, a smaller study found that serum E-selectin levels did not increase in AD patients any more significantly than in other noninflammatory neurological diseases [252].

PECAM-1 may also contribute to the pathogenesis of A $\beta$ -related cerebral vascular disorders, such as AD. Giri et al. [229] demonstrated that interactions between A $\beta$ 1–40 and a monolayer of human brain endothelial cells promote the adhesion and transendothelial migration of monocytes, but this could be blocked with an antibody against PECAM-1.

Several studies have reported the endothelial expression of Ig superfamily integrin ligands in AD as well as increased levels of soluble endothelial integrin receptors, strongly indicating that endothelium in the AD brain can mediate the integrin-dependent adhesion of leukocytes. Microvascular endothelial cells showed higher levels of ICAM-1, a marker of cytokine-activated endothelial cells, in transgenic mouse models of AD and human patients with the disease (Fig. 2) [253–255]. ICAM-1 was found inside senile plaques containing fibrillar or nonfibrillar A $\beta$ , in structures containing low levels of A $\beta$ , and around plaques [256–258]. Frohman and colleagues [256] showed that the higher levels of ICAM-1 expression were restricted to the cerebrovascular endothelium, whereas its ligand LFA-1 was present on microglial cells and infiltrating leukocytes, strongly suggesting a role for leukocyte trafficking mechanisms in the pathogenesis of AD. Whereas ICAM-1 can be expressed on a variety of cells under inflammatory conditions, including neural cells, the expression of VCAM-1 is restricted to endothelial cells. However, despite several indirect indications suggesting that VCAM-1 expression in the brain endothelium is linked to AD, there have been no neuropathological studies showing vascular VCAM-1 expression in the disease.

Elevated levels of soluble integrin ligands provide further signs of vascular inflammation in AD. Increased serum levels of ICAM-1 were observed in AD patients, but this was thought to originate in neural cells rather than activated endothelial cells [259]. Zuliani and colleagues [250] reported higher levels of soluble VCAM-1 in the plasma of 60 patients with late-onset AD and 80 patients with vascular dementia, compared with healthy elderly controls. In addition, another important study involving 260 AD patients showed a strong correlation between the disease and the presence of soluble forms of ICAM-1, VCAM-1, and PECAM-1 in comparison with age and gender-matched, nondemented controls [260].

Soluble adhesion receptors have also been proposed as AD markers or as markers of aging. ICAM-1 was one of 18 plasma proteins creating a signature that can be used to classify blinded samples from AD and control subjects with ~90% accuracy and to identify patients whose mild cognitive impairment progressed to AD 2–6 years later [261]. However, the levels of soluble ICAM-1 and VCAM-1 were not associated with an increased risk of AD in another study, using 727 randomly selected subjects [262]. A recent study looking for markers of systemic inflammation and endothelial dysfunction in an el-

derly population of 679 volunteers showed higher plasma levels of circulating C-reactive protein, fibrinogen, ICAM-1, and VCAM-1, suggesting that inflammatory changes, including vascular inflammation, are associated with aging [263]. Moreover, another study has shown that circulating VCAM-1, but not ICAM-1 or E-selectin, is an age-dependent parameter, independent of cardiovascular risk [264]. These results support the idea that circulating VCAM-1 could be used as a marker for biological aging and to strengthen further the link between inflammatory responses and AD, which has an increased age-dependent susceptibility.

## PD

PD is the second-most common neurodegenerative disorder, superseded only by AD [265]. The cause of PD is unknown, but most experts share the opinion that PD is a result of a combination of genetic and environmental factors that induce a profound loss of dopaminergic neurons [266]. It has been shown in human pathology, animal models, and in vitro studies that PD shares common pathogenic mechanisms with AD, including the aggregation and deposition of misfolded proteins and chronic inflammation. There is increasing evidence that chronic inflammation, BBB dysfunction, and immune cell migration play a significant role in PD [267]. For example, one previous study showed substantial infiltration of CD44<sup>+</sup> T cells into the substantia nigra and striatum in a mouse model of PD [268], and another showed a significant increase in the number of LFA-1<sup>+</sup> leukocytes in the substantia nigra and its dorsal extension, associated with capillaries in PD and intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in monkeys [269]. Other reports have shown higher levels of CD4<sup>+</sup>CD45RO<sup>+</sup> T cells expressing Fas in the peripheral blood of PD patients [270, 271]. Notably, it has been suggested recently that dopaminergic neuron loss may be driven by neutrophil infiltration, leading to neuronal damage in the substantia nigra, reduced astrocyte density, and an increase in BBB permeability [272]. However, further studies are needed to determine how vascular inflammation and leukocyte trafficking mechanisms contribute to PD pathogenesis.

## Epilepsy

A seizure is a paroxysmal hypersynchronous discharge from CNS neurons. Repeated seizures can lead by unknown mechanisms to epilepsy, a chronic neurological disorder that affects 1% of the world's population. Experimental and clinical data indicate that repeated seizure activity can lead to chronic recurrent epileptic seizures, but the mechanisms responsible for this transition remain unclear. Lymphocyte accumulation has been observed in the brain parenchyma of patients with refractory epilepsy, along with a leaky BBB [273]. Perivascular and parenchymal T cells (mainly CD8<sup>+</sup> cytotoxic T cells) were observed in gray and white matter in samples obtained from patients with a tuberous sclerosis complex, which associates with epilepsy [274]. Cells of the microglia/macrophage cell system and CD3<sup>+</sup> lymphocytes were also shown to accumulate in brain samples obtained from patients with temporal lobe epi-

lepsy and hippocampal sclerosis [275]. Finally, our recent data show that a number of leukocytes in the brain parenchyma of patients with epilepsy increase independently of disease etiology [276].

Early preclinical studies showed that the *in vivo* administration of the proconvulsant agent kainic acid up-regulates ICAM-1 and VCAM-1 on the surface of brain endothelial cells [277]. More recent *in vitro* studies showed that epileptiform activity induced by bicuculline can rapidly induce the expression of adhesion molecules on the brain endothelium [278]. We have shown recently that seizures induce the expression of VCAMs such as VCAM-1, ICAM-1, and E- and P-selectin [276] and that seizures promote leukocyte rolling and arrest in cortical vessels, mediated by PSGL-1 and leukocyte integrins VLA-4 and LFA-1 [276]. Interestingly, our recent data show that Th1, but not Th2, cells are preferentially recruited in inflamed brain venules after seizures, suggesting that cells from the adaptive immune system may contribute to the progression of epilepsy. The inhibition of leukocyte–vascular interactions with antibodies dramatically reduced the number of seizures, and fewer seizures were also observed in knockout mice lacking functional PSGL-1 [276]. Vascular leakage, which is known to enhance neuronal excitability, was induced by acute seizure activity but prevented by inhibiting leukocyte–vascular adhesion, suggesting a pathogenic link between leukocyte–vascular interactions, vascular damage, and seizures [276]. In support of these results, recent studies using a model of viral meningitis showed that myelomonocytic cell recruitment leads to vascular leakage and acute, lethal seizures [279]. Taken together, these data suggest that leukocyte–endothelial interactions could be targeted by drugs to prevent and/or treat epilepsy, as shown by the recent, successful treatment of epilepsy with natalizumab in a patient with MS and severe refractory epilepsy [280].

Despite growing evidence suggesting a role for leukocytes in the induction of seizures, limited information is available about the role of vascular chemokines in promoting leukocyte trafficking and epilepsy. However, CCL2 expression was documented in the blood vessels at late time-points after pilocarpine-induced status epilepticus in mice, potentially related to changes in permeability of the BBB and leukocyte recruitment during epileptogenesis [281]. CCL2 up-regulation has also been shown following kainate-induced seizure in the rat hippocampus and has been correlated to the temporal profile of BBB permeability and immune cell trafficking at the site of injury [282]. Importantly, a recent study by Louboutin and colleagues [283] demonstrated a key role for the leukocyte chemokine receptor CCR5 in the induction of seizures in a rat model based on direct neural stimulation by kainic acid. In this paper, the authors found that seizures induce elevated expression of CCR5 ligands, CCL3 and CCL5, in the microvasculature, and increased BBB leakage and CCR5<sup>+</sup> cell migration into the CNS. The decrease in leukocyte CCR5 strongly protected from acute seizure induction, BBB leakage, CNS injury, and inflammation, suggesting a role for CCR5 expression on circulating leukocytes and endothelial expression of CCL3 and CCL5 in the control of vascular inflammation and leuko-

cyte trafficking required for acute seizure generation by excitotoxic agents [283].

## CONCLUDING REMARKS AND FUTURE PERSPECTIVES

It is clear that distinct leukocyte populations migrate at selective CNS sites under physiological conditions and during acute and chronic vascular inflammation, according to local patterns of adhesion molecule expression. The results obtained in experimental models of ischemic stroke and MS demonstrate that inhibiting adhesion molecules can have therapeutic effects in brain diseases involving detrimental leukocyte trafficking. The efficacy of natalizumab is greater than most conventional MS therapies, and recent results suggest that patients with other neurological diseases, such as epilepsy, may also benefit from drugs that block the activity of VLA-4 [276, 280]. The challenge for the future is to determine which adhesion mechanisms are critical for the control of leukocyte subsets that promote or exacerbate CNS diseases and to identify leukocyte subpopulations and their trafficking molecules that have a beneficial role. This will help to prevent collateral effects and adventitious infections and will leave protective leukocyte extravasation and immune surveillance unaffected.

Preliminary studies have also shown links between inflammation and disease progression in AD and epilepsy, and a greater understanding of the role of leukocyte trafficking in these diseases will provide more insight into the mechanisms underlying neurodegeneration and may help to identify novel therapeutic approaches. The role of inflammation and leukocyte trafficking in AD and PD still remains to be clarified, but recent AD research suggests that neuroinflammation in AD brains is linked to endothelial changes mediated by A $\beta$ , greater endothelial permeability causing neurotoxic blood plasma components to leak into the neuropil, and enhanced adhesion and transendothelial migration of leukocytes. A key research goal in AD is to determine which inflammatory pathways have useful, restorative, or scavenging roles and which are detrimental, as this will reveal potential new drug targets and leads. Currently, neurovascular dysfunction is thought to contribute to the pathogenesis of dementias in the elderly, so a greater understanding of this process is required for the development of novel therapies aiming to normalize vascular and neuronal dysfunction.

Recent studies have identified an inflammatory component in the pathogenesis of epilepsy, but current antiepileptic drugs aim to depress aberrant neuronal excitation and do nothing to prevent inflammation. Interfering with the adhesion of immune cells to the cerebral vasculature could open new avenues for epilepsy treatment, particularly in refractory cases [284]. Recent clinical data suggest the antiadhesion drug natalizumab may be safer and cases of PML less frequent than understood previously [285]. The use of natalizumab to treat refractory epilepsy and epilepsy following MS or proinflammatory events such as trauma, stroke, and infection may therefore be a useful therapeutic strategy.



## AUTHORSHIP

All authors contributed equally to the writing of this review article.

## ACKNOWLEDGMENTS

This work was supported in part by grants from the National Multiple Sclerosis Society (NMSS; New York, NY, USA), Fondazione Cariverona, Italian Ministry of Education and Research (MIUR), Fondazione Italiana Sclerosi Multipla (FISM), and the European Research Council Grant 261079-NEUROTRAFFICKING (to G.C.).

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**KEY WORDS:**

stroke · multiple sclerosis · Alzheimer's disease · epilepsy · integrins · selectins