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## Adhesion Molecules in CNS Disorders: Biomarker and Therapeutic Targets

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### Abstract

Mounting evidence has been provided regarding the crucial role of leukocyte extravasation and subsequent inflammatory response in several central nervous system (CNS) disorders. The infiltrated leukocytes release pro-inflammatory mediators and activate resident cells, leading to tissue injury. Leukocyte-endothelia interaction is critical for leukocyte extravasation and migration from the intravascular space into the tissue during inflammation. The basic physiology of leukocyte-endothelia interaction has been investigated extensively. Traditionally, three kinds of adhesion molecules, selectin, integrin, and immunoglobulin families, are responsible for this multiple-step interaction. Furthermore, blocking adhesion molecule function by genetic knockout, antagonizing antibodies, or inhibitory pharmacological drugs provides neuroprotection, which is associated with a reduction in leukocyte accumulation within the tissue. Detection of the soluble form of adhesion molecules has also been proven to predict outcomes in CNS disorders. Lately, vascular adhesion protein-1 (VAP-1), a novel adhesion molecule and endothelial cell surface enzyme, has been implicated as a brake during leukocyte extravasation. In this review, we summarize the functions of traditional adhesion molecules as well as VAP-1 in the leukocyte adhesion cascade. We also discuss the diagnostic and therapeutic potential of adhesion molecules in CNS disorders.

### Keywords

adhesion molecule; CNS disorders; leukocyte adhesion cascade; vascular adhesion protein-1

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## 1, Introduction

Inflammation is a common response in the progression of central nervous system (CNS) diseases, including stroke, neurodegenerative diseases, traumatic brain injury (TBI), etc. For decades, it was assumed that the CNS enjoyed the privilege of being protected by the blood-brain barrier (BBB) [1]. Under normal physiological conditions, an intact BBB prevents cell trafficking into the CNS. Trans- and paracellular movement of molecules and cells across the BBB are limited by endothelial cells [2]. Unfortunately, under most pathophysiological conditions, structural integrity of the BBB collapses and leukocytes accumulate into CNS [3], which is a key stage in neuroinflammation development.

Initially, adhesion molecules were attributed to being cell surface structures that mediate cell–cell and cell-extracellular matrix (ECM) interactions. They are also the key inflammatory mediators by promoting blood-borne leukocyte crossing of the BBB and aggregating in the injury site [4, 5]. Infiltration of systemic immune cells into the injured sites by sequential interactions between leukocytes and endothelial cells is an initial step of the inflammatory response. After tissue injury, circulating immune cells will release inflammatory chemokines, recognize endothelial cells around the injured tissue, stop on the luminal surface of the blood vessel, transmigrate paracellularly across the endothelial layer, and reach the injured site [6–8]. This whole process is called the leukocyte adhesion cascade, which has multiple steps, including tethering, rolling, activation, firm adhesion, and transmigration. Different adhesion molecule superfamilies mediate each step (Figure 1). Chemokines stimulate leukocyte and endothelial cell activation, selectin promotes tethering and rolling, integrin mediates firm adhesion, and integrin and immunoglobulin facilitate transmigration.[9] In addition, some special adhesion molecules also assist in rolling, firm adhesion, and transmigration during leukocyte recruitment, such as vascular adhesion protein 1 (VAP-1), a cell surface molecule displaying amine oxidase activity [10, 11]. All steps are required for effective leukocyte recruitment, therefore, blocking any step may reduce leukocyte aggregation in the inflamed sites. To date, numerous preclinical models and clinical trials have determined the pathophysiological role of leukocyte accumulation during neuroinflammation in CNS disorders [12–17]. Better understanding of adhesion molecule function for modulating leukocyte trafficking into the brain may provide further insight into novel or alternative therapeutic approaches to treat inflammatory CNS disorders.

This review focuses on adhesion molecule mediated leukocyte-endothelial cell interaction during inflammation in multiple forms of CNS diseases in both preclinical models and clinical trials. Understanding the role of adhesion molecules in the leukocyte adhesion cascade will elucidate their involvement in neuroinflammation and present exciting therapeutic and diagnostic opportunities for all kinds of CNS diseases.

## 2, Adhesion Molecules

### 2.1 Selectin

Selectins are a group of transmembrane molecules expressed on the outer cell surfaces of leukocytes and endothelial cells following activation [18]. There are three kinds of selectins, P, E, and L. P-selectin and E-selectin are expressed on activated endothelial cells, whereas

L-selectin is mainly expressed on leukocytes. P-selectin is also expressed by activated platelets.

P-selectin, also known as CD62P, is a membrane-bound glycoprotein identified primarily in the secretory granules of platelets and Weibel-Palade bodies of endothelial cells [19, 20]. P-selectin is the most important selectin for modulating leukocyte rolling by interacting with P-selectin glycoprotein ligand 1 (PSGL1) [21] through binding to its amino terminal region [22]. Under inflammatory conditions, P-selectin translocates from the storage granules to the outer surface of the cell and interacts with leukocytes. Pro-inflammatory agents stimulate P-selectin translocation, including lipopolysaccharide (LPS), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1 (IL-1), and thrombin by phosphorylating tyrosine, threonine, and serine residues on P-selectin's cytoplasmic tail [23]. P-selectin is also shed from the endothelial cell membrane and released into blood. Blood concentrations of soluble P-selectin are high in many pathophysiological conditions, such as thrombotic thrombocytopenic purpura, hemolytic-uremic syndrome, chronic obstructive pulmonary disease, myocardial infarction, hypercholesterolemia, and rheumatoid arthritis [20]. High soluble P-selectin blood concentration may be associated with excessive leukocyte infiltration in those pathophysiological conditions. E-selectin (CD62E), also called endothelial leukocyte adhesion molecule-1 (ELAM-1), is restrictedly expressed by activated endothelial cells and mediates slow leukocyte rolling. Similar to P-selectin, E-selectin is induced by pro-inflammatory agents, such as LPS, IL-1 and TNF- $\alpha$ . E-selectin plays a major role in leukocyte recruitment to inflamed sites through its close collaboration with P-selectin. E-selectin binds to glycosylated CD44 and E-selectin ligand 1 (ESL1) [24]. Additionally, E-selectin interacts with PSGL1, which is not only expressed by endothelial cell but also leukocytes [9]. L-selectin, also known as CD62L or leukocyte endothelial cell adhesion molecule-1 (LECAM-1), is a glycoprotein constitutively expressed by all leukocytes [25, 26]. It interacts reversibly with its endothelial cell ligands and mediates neutrophil rolling on the endothelium during inflammation [27]. Its ligands are mostly expressed on the endothelial cell surface, including GLYCAM-1, MAdCAM-1, CD34 [28], and PSGL-1 [29]. When leukocytes are activated by pro-inflammatory agents, the conformation of L-selectin will change to rapidly and transiently enhance its binding affinity to its ligands [30, 31]. Because of the proteolytic cleavage of membrane-binding L-selectin, soluble L-selectin is observed in the plasma after L-selectin mediated rolling of leukocytes [32, 33].

## 2.2 Integrins

Integrins are a family of adhesion molecules responsible for cellular attachment to extracellular matrix (ECM) and cell-cell interactions. They are cell-surface heterodimeric glycoproteins and consist of  $\alpha$  and  $\beta$  subunits [34, 35]. The sub-classification of integrins is denominated by the  $\beta$  subunit. Ligand specificity is also determined by the complex of  $\alpha$  and  $\beta$  subunits [8]. The  $\beta 2$  (CD18) integrin families are regarded as the most important for leukocyte migration during inflammation. The  $\beta 2$  integrins (CD11/CD18), including CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), and CD11c/CD18, mainly modulate firm adhesion during leukocyte transmigration. LFA-1 is expressed by leukocytes, while Mac-1 is mainly expressed by neutrophils, monocytes, and natural killer cells [36]. The  $\beta 2$  integrins are

critical in innate immunity. An encoding gene mutation will lead to defective synthesis of LFA-1, Mac-1, and CD11c/CD18 causing leukocyte adhesion deficiency (LAD). LAD patients often suffer from repeated bacterial infections because of the ineffective transmigration of leukocytes [37]. LFA-1 plays a critical role in the leukocyte adhesion cascade by interacting with ICAM-1 and ICAM-2 on the endothelial cell surface [38, 39]. Generally, integrins only mediate leukocyte firm adhesion, but  $\beta 2$  integrins may also be necessary for slow rolling of the leukocytes [40]. Leukocyte rolling velocities were increased in LFA-1 or Mac-1 deficient mice, but they were still lower than in CD18 deficient mice [41].

### 2.3 Immunoglobulin

Some members of the immunoglobulin superfamily are adhesion molecules that mediate interaction between leukocytes and endothelial cells during the inflammatory response. They are ICAM-1, ICAM-2, VCAM-1, and PECAM-1. They have similar structural and genetic features, including two to seven immunoglobulin domains that interact with leukocyte integrins [16]. ICAMs are homodimers mainly expressed by endothelial cells, and they bind to  $\beta 2$  integrins, LFA-1, and Mac-1. ICAM-1 (CD54) is one of the most important ligands for  $\beta 2$  integrins [42]. It is a glycoprotein composed of a transmembrane domain and a short cytoplasmic sequence. ICAM-1 mediates leukocyte passage across the vascular endothelial cell layer to the sites of tissue injury by promoting leukocyte firm adhesion and transmigration [43]. Under normal conditions, ICAM-1 is expressed at low concentrations on the resting endothelium. In addition, ICAM-1 is also expressed on Schwann cells and plays a role during inflammation of the peripheral nerves. ICAM-1 expression increases rapidly following stimulation by pro-inflammatory cytokines, such as IL-8, IL-1, and TNF- $\alpha$ , and by other pro-inflammatory agents, such as LPS. Mice with deficient ICAM-1 expression have impaired leukocyte migration during inflammation. A soluble form of ICAM-1 (sICAM-1) plays a role in neutrophil adhesion [44]. Similar to ICAM-1, ICAM-2 is a homodimeric molecule, but it does not respond to pro-inflammatory factors [16, 39]. VCAM-1 is expressed at very low levels by resting endothelial cells, but it can be upregulated under inflammatory conditions [45, 46]. It promotes leukocyte adhesion by interacting with very late antigen-4 (VLA-4) [16].

### 2.4 VAP-1, a Dual Role Adhesion Molecule

In 1992, VAP-1 was first discovered as a 90-kD endothelial cell protein in synovial vessels from arthritis patients using a monoclonal antibody, 1B2 [47]. The antibody markedly reduced lymphocyte binding to high endothelial venules in both frozen section adhesion assays and flow chamber assays. One year later, the localization of VAP-1 expression was determined by the same group. Their results showed that, in addition to abundant expression in lymphatic organs, VAP-1 is also extensively expressed on endothelial cells in several non-lymphatic tissues, including skin, brain, kidney, liver, and heart [48].

Further studies discovered the enzymatic nature of VAP-1 (Figure 1). VAP-1 belongs to the semicarbazide-sensitive monoamine oxidase (SSAO) family. This characteristic was identified by cDNA cloning that encoded a type II transmembrane protein with similar identity to the copper-containing amine oxidase family. VAP-1 also possessed amine

oxidase activity [49] that can oxidize primary amines and release biologically active products, including aldehydes, hydrogen peroxide, and ammonium. Coinciding with the discovery of VAP-1's enzymatic nature, its adhesion molecule function was observed by Smith and colleagues. In their experiment, they conducted a VAP-1 cDNA transfection into endothelial cells and found that VAP-1 led to lymphocyte binding, which was partially inhibited with anti-VAP-1 mAbs[49].

Mounting evidence suggests that VAP-1 is an endothelial glycoprotein induced by inflammation. Studies with human samples using confocal microscopes showed that VAP-1 exists both on the luminal surface and in the intracellular granules of human endothelial vessels (HEVs). In samples from patients with inflammatory bowel diseases or chronic dermatoses, VAP-1 levels were also found to be upregulated at inflamed sites[48]. Furthermore, the mechanism controlling VAP-1 function has been explored. In normal endothelial cells, VAP-1 is stored in intracellular granules within the cytoplasm. Under inflammatory conditions, VAP-1 was upregulated and translocated to the endothelial surface [11]. The translocation of VAP-1 to the cell surface was observed directly after inflammation in experimental canine and pig inflammatory models. VAP-1 increased on the endothelial cell surface 60 minutes after induction by inflammation, peaked at 8 hours, and lasted until 48 hours [50]. Similar to P-selectin, VAP-1 translocates from intracellular granules to the vascular luminal surface at the site of inflammation. However, the underlying mechanism and mediators inducing VAP-1 expression and translocation remains unclear. Thirteen inflammatory factors, including TNF- $\alpha$  and thrombin, were used to determine the mediators leading to the VAP-1 induction in human endothelial cells. Unfortunately, none increased cell surface expression of VAP-1 [48]. It is very rare to have only a single mediator involved in inflammation *in vivo*, therefore, this study suggested VAP-1 might be induced by a combination of multiple mediators.

The signaling functions of VAP-1's catalytic activity have been investigated. The potent biological products of VAP-1, such as aldehyde, H<sub>2</sub>O<sub>2</sub>, and NH<sub>3</sub>, may induce expression of other adhesion molecules or pro-inflammatory cytokines. They are cytotoxic at high concentrations and potentially trigger VAP-1 downstream signals [11, 51]. Reactive oxygen species and H<sub>2</sub>O<sub>2</sub> help regulate gene expression in vascular endothelial cells [52]. Mounting evidence showed H<sub>2</sub>O<sub>2</sub> formation is associated with leukocyte infiltration by inducing P-selectin expression and leukocyte rolling [53]. In human endothelial cells, both P- and E-selectin are induced on transcriptional and translational levels by VAP-1 enzymatic activity. In VAP-1 transgenic mice, P-selectin induction is dependent on VAP-1 enzymatic activity [54]. In an age-related mouse macular degeneration (AMD) model, VAP-1 suppression diminished expression of pro-inflammatory cytokines, such as TNF- $\alpha$  and MCP-1, as well as adhesion molecules, such as ICAM-1 [55]. Lalor and colleagues provided specific substrates of VAP-1 directly to liver endothelial cells, which lead to endothelial cell activation. They also found VAP-1 mediated activation was dependent on NF $\kappa$ B, PI3K, and MAP kinase pathway. Additionally VAP-1 activation upregulated E-selectin, ICAM-1, VCAM-1, as well as CXCL8, and intensified the inflammatory response [56]. Taken together, these findings suggest VAP-1 is a novel type of adhesion molecule with SSAO enzymatic activity that may also have signaling functions.

### 3, Adhesion Cascade in CNS Disorders

#### 3.1 Stroke

**3.1.1 Ischemic Stroke**—The inflammatory response is initiated in the vascular system after brain ischemia and reperfusion (I/R) occurs. Mounting evidence indicates an accumulation of systemic immune cells, specifically blood-borne leukocytes, occurs in the ischemic region and is partly associated with ischemic tissue injury [57–59]. The infiltrated leukocytes activate resident cells in the brain, including endothelial cells, microglia, and astrocytes [17, 60]. As a consequence, blood-borne leukocytes, in addition to the aforementioned cells, amplify leukocyte recruitment signals by producing adhesion molecules and releasing pro-inflammatory cytokines [17, 36, 61]. As described above, adhesion molecules mediate leukocyte transmigration into the brain tissue after stroke and regulate the transmigration speed [36]. Therefore, restriction of adhesion molecule function may have potential therapeutic effects on cerebral injury after ischemia/reperfusion [62].

P- and E-selectin expression profiles have been determined in suture, thrombotic, and embolic middle cerebral artery (MCA) occlusion models of stroke. The results indicated P-selectin was upregulated as early as 15 min while E-selectin increased at 2 hours after the MCA occlusion. Both peaked at 6 hours after MCA occlusion [63]. Accordingly, P-selectin  $-/-$  or P-selectin monoclonal antibody (mAb) reduced recruitment of leukocytes and platelets after cerebral I/R [64]. Pharmacological compounds have also been used to inhibit early leukocyte interaction with endothelial cells. Fucoidin (FCN), a competitive inhibitor of P- and L-selectin, reduced cerebral infarction size and improved neurological function in a focal ischemia model [65]. However, another report showed HuDREG200, a humanized antibody to L-selectin, did not effectively reduce infarction size, neutrophil accumulation, or cerebral blood flow in a rabbit model of cerebral I/R, although this antibody reduced myocardial neutrophil infiltration in a feline model of myocardial I/R [66]. This evidence indicates L-selectin might not be crucial in the leukocyte adhesion cascade. CY-1503, an analog of sialylLewis(x) (SLe(x)), significantly reduced infarct volume and myeloperoxidase positive cells in the ischemic area by blocking E-selectin function [67]. Additionally, in a transient intraluminal middle cerebral artery occlusion (MCAO) model, anti-E-selectin monoclonal antibody was applied by intravenous injection [68], which attenuated cerebral neutrophil accumulation and provided dose-dependent neuroprotection. Therefore, blockage of selectin-mediated adhesion may limit immune cell transmigration and reduce reperfusion injury in the brain.

The significance of  $\beta$ 2 integrins in ischemic injury has been demonstrated. In a cerebral I/R model, leukocyte adhesion to cerebral venules was reduced in both LFA-1 and Mac-1 deficient mice 24 hours after reperfusion. In line with this result, LFA-1 or Mac-1-deficient animals also presented significantly reduced infarct volume and improved neurological functions [14]. Prior work also showed Hu23F2G, an antibody of the CD11/CD18 integrin, reduced neutrophil infiltration as well as ischemic injury in a rabbit model of transient focal ischemia [69]. Similarly, anti-Mac-1 antibody lead to reductions in intraparenchymal neutrophil accumulation and ischemic brain injury [70]. Furthermore, individual inhibition of CD11b, CD18, or ICAM-1 also reduced brain injury by decreasing leukocyte

accumulation after ischemic stroke [71–73]. In addition to  $\beta$ 2 integrins,  $\alpha$  integrins may also affect leukocyte infiltration following ischemia. Antibodies to  $\alpha$ 4 integrins attenuated infarction and improved neurological function by blocking lymphocyte and monocyte transmigration in a transient focal cerebral ischemia rat model [74].

ICAM-1 and VCAM-1 have been broadly studied in ischemia and hypoxia. ICAM-1 levels were highly increased in the brain at 3 hours following ischemic stroke, peaked between 6–12 hours, and remained high until 5 days later [75]. Whether VCAM-1 levels are upregulated after cerebral ischemia is still controversial. Blann et al found intense expression of VCAM-1 by astrocytes and endothelial cells in the infarcted area from a stroke patient [76]. However, a study from Vemuganti et al showed VCAM-1 mRNA expression was never changed in the ipsilateral cortex between 3 and 72 hours after reperfusion [77]. ICAM-1 is one of the most important targets in anti-adhesion strategies. In ICAM-1-knockout mice, the size of ischemic lesions was reduced [78], and they had a lower mortality rate compared to wild-type [79]. Other treatment approaches, such as ICAM-1 antibodies, also reduced infarction volume and improved neurological outcomes after ischemia [80, 81]. ICAM-1 antisense oligonucleotides were also applied in an ischemic stroke model, since ICAM-1 mRNA levels were upregulated within hours after ischemia. Their results showed ICAM-1 knockdown reduced infarct volume and improved neurological function [77].

Because anti-adhesion treatments were effective in preclinical studies, they have been applied in clinical trials. A recombinant neutrophil inhibiting factor (rNIF) was administered to patients within 6 hours after symptom onset (Acute Stroke Therapy by Inhibition of Neutrophils) [82]. In addition, an anti-CD11/CD18 antibody was employed within 12 hours after symptom onset [12, 83] in ischemia patients. However, both clinical trials failed, although they inhibited neutrophil aggregation and improved functional outcomes in animal studies [69, 84, 85]. The different expression profiles of integrin between human and animal stroke models may explain these failures. Enlimomab, a murine anti-ICAM-1 antibody, has been investigated in patients with ischemic stroke [86]. Unfortunately, more adverse effects were observed with enlimomab treatment than placebo, including infections and fever. Treated patients also had a higher fatality rate than in the placebo group. The murine origin of this antibody may induce an immune response in patients [87], which may explain its failure. It has also been reported enlimomab activates neutrophilic granulocytes in the whole blood of human volunteers [88].

**3.1.2 Hemorrhagic Stroke**—The inflammatory response plays an important role in brain injury after intracerebral hemorrhage (ICH). Mounting evidence indicates systemic immune cells, specifically blood-derived leukocytes, accumulating at the injured site are the primary orchestrators of brain injury following ICH [89].

Neutrophils are a major type of leukocyte that mediates secondary brain injury following ICH. In a collagenase-injection mouse model, Wang and colleague found neutrophils infiltrated into and around the hematoma 4 hours after injury and peaked at 3 days [90]. Other studies conducted using an autologous blood-injection model showed neutrophils appeared within 1 day and disappeared at 3 to 7 days [91–93]. Microglia are a type of

macrophage in the CNS and account for 5–20% of the total glial population [94]. Under normal conditions, they exist in a resting state. When activated by an inflammatory response following brain injury, they undergo a series of morphological changes, such as enlargement in size with stout processes, activation of phagocytic function, and upregulation of specific genes [95]. In a collagenase-injection model, studies showed microglia activation occurs earlier than neutrophil infiltration. Microglia activation occurred in the peri-hematoma region around 1–2 hours after injection, markedly increased at 1 day, peaked at 7 days, and declined to base level at 3 weeks [96, 97]. A similar time course was observed in an autologous blood model. Microglia activation appeared 1–4 hours after injection, peaked 3–7 days, and lasted as long as 4 weeks [91, 92, 98]. Prior studies suggested leukocytes, particularly neutrophils, might be major contributors to the inflammatory response and brain injury following ICH. However, a few studies addressed the effects of adhesion molecules in hemorrhagic stroke. Gong and colleagues found that ICAM-1 immunoreactivity was observed in blood vessels around the hematoma in a rat blood-injection ICH model [91]. In a collagenase model, CD18(–/–) knockout mice had reduced brain edema and mortality, which were associated with decreased MPO immunoreactivity in their brains [99].

Few studies explored the role of VAP-1 in the stroke-induced inflammatory response. VAP-1 may be involved in immune cell infiltration after stroke since leukocyte accumulation has been observed at the injured site. In myocardial samples from ischemic hearts of human patients, VAP-1 expression was markedly upregulated on endothelial cells, and VAP-1 neutralizing antibody reduced the number of adherent granulocytes by 60% [50]. In a transient forebrain ischemic rat model, a small molecule VAP-1 inhibitor was administered either at onset or 6 hours after reperfusion. The VAP-1 inhibitor limited neutrophil adhesion and infiltration, even when treated 6 hours after reperfusion, as well as provided subsequent neuroprotection [100]. We studied the role of VAP-1 in ICH and found VAP-1 inhibition can reduce brain edema and improve neurological function in both collagenase and blood-injection mouse models. Accordingly, P-selectin, E-selectin, and ICAM levels as well as infiltrated neutrophils were diminished [101].

Soluble VAP-1 (sVAP-1) may also play an important role after stroke. Airas and colleagues found sVAP-1 was markedly increased in the serum of acute stroke patients less than 6 hours after ischemia and may promote vasculopathy [102]. Hernandez-Guillamon and colleagues reported the baseline VAP-1/SSAO activity is a predictor for intracranial bleeding after tissue plasminogen activator (tPA) treatment [103]. They found elevated plasma VAP-1 activity in patients who suffered from a hemorrhage and had subsequent deleterious neurological outcomes. Additionally, in a rat model, they found the VAP-1 inhibitor prevented the side effects associated with delayed tPA treatment. The authors also elucidated potential mechanisms. *In vitro*, tPA promoted neutrophil degranulation and MMP-9 release, which mediate BBB disruption. VAP-1 is an adhesion molecule that mediates leukocyte infiltration. Therefore, VAP-1 inhibition may protect against tPA-induced vascular damage by inhibiting leukocyte infiltration. Recently, the same group found plasma VAP-1/SSAO activity is increased in hemorrhagic stroke patients and may be a predictor for neurological outcome after ICH [104]. All of these clinical reports suggest VAP-1 is a potential therapeutic target for ICH-induced brain injury.



Cerebral vasospasm is a major complication in subarachnoid hemorrhage (SAH) patients, and its mechanisms have not been elucidated. Increasing evidence showed inflammation, especially leukocyte-endothelial interaction, plays a crucial role in the pathogenesis of vasospasm following SAH. It has been reported increased leukocyte levels increase the risk of vasospasm [105–108] and were associated with a 90% rate of poor outcome [105].

In an SAH canine model, Aihara et al quantitatively measured the expression levels of inflammatory genes and found ICAM-1 mRNA levels were highest at day 7, which may be associated with the persistent contraction of the basilar artery [109]. Before this study, in an SAH rat model, Handa et al observed increased ICAM-1 expression in both the endothelial and medial layer of the basilar artery following SAH [110]. To examine the role of ICAM-1 and CD18 in vasospasm after SAH, Bavbek et al administered anti-ICAM-1 antibody or anti-CD18 antibody, alone or combination, in a rabbit SAH model. They found both antibodies alone inhibit vasospasm, and their effects are enhanced when used in combination [111]. Recently, in a blood-injection SAH rat model, ICAM-1 and E-selectin, but not VCAM-1, were significantly elevated in the basilar arteries at 72 hours after SAH, while 6-mercaptopurine administration repeated at 24 and 48 hours markedly reduced their expression levels compared to the control group [112]. Adhesion molecule expression has also been examined in a femoral artery model of vasospasm. Sills et al found exposing large intracranial arteries to blood extensively increased ICAM-1 immunoreactivity in endothelial cells starting at 3 hours after blood exposure and lasting until 24 hours [113]. Using an anti-ICAM-1 antibody in this model significantly inhibited vasospasm, which was associated with decreased inflammatory cell infiltration, including macrophages and granulocytes, into the peri-adventitial region of blood-exposed arteries [114]. Similar results were also found when administering anti-LFA-1 monoclonal antibody systemically in the rat femoral artery model [115]. Lin et al examined the efficacy of anti-E-selectin monoclonal antibody on cerebral vasospasm in a murine SAH model. Results showed diameters of anterior cerebral arteries were reduced at 24 hours after anti-E-selectin antibody treatment in addition to a reduction of infiltrated leukocytes [116].

In patients, the levels of soluble adhesion molecules were detected in cerebrospinal fluid (CSF) after subarachnoid hemorrhage (SAH). Polin et al found soluble forms of E-selectin, ICAM-1, and VCAM-1, but not L-selectin, were markedly increased in the CSF of patients suffering from recent aneurysmal SAH [117]. However, the levels of adhesion molecules in SAH patients are not consistent. In a report from Nissen et al, they observed serum concentrations of P- and L-selectin, but not E-selectin, ICAM-1, VCAM-1 and PECAM, were altered in patients with and without ischemic neurological deficit (DIND) after aneurysmal subarachnoid haemorrhage [118, 119]. Statins are a class of drugs that induce anti-inflammatory effects by interacting with LFA-1 and Mac-1 [120, 121]. In a clinical study, statins were used to treat vasospasm after SAH. Kramer et al found statin treatment was not associated with any reduced vasospasm development or improved outcomes after aneurysmal SAH [122]. However, Tseng et al reported acute pravastatin therapy improved vasospasm-related delayed ischemic deficits and functional outcomes after aneurysmal SAH [123, 124].

Based on the aforementioned evidence, therapeutic targeting of adhesion molecules may protect against inflammation and reverse vasospasm as well as improve functional outcomes in SAH patients.

### 3.2 Brain Trauma

Traumatic brain injury (TBI) is a fatal condition and most common cause of death worldwide, especially in children and young adults below 40 years old [125]. Traumatic brain injury (TBI) occurs when sudden trauma damages the brain. The injury is divided into primary and secondary injury [126]. The primary injury is the direct insult that may lead to blood vessel disruption and bleeding into or around brain tissue. The secondary injury is an indirect result of the traumatic insult, which is a series of complex cellular and/or biochemical events leading to delayed brain damage, mostly by inflammation and reactive oxygen species (ROS) [126, 127].

TBI-induced disturbances in the cerebrovasculature may cause endothelial cell activation that alters expression of cell surface markers. Balabanov and colleagues observed E-selectin and ICAM-1 were upregulated as early as 2 hours and was sustained through 24 hours, but VCAM-1 was not induced, in a percussion model of moderate TBI in rats [128]. Similarly, ICAM-1 levels were significantly upregulated on the endothelium of the traumatized hemisphere at 4 hours and remained elevated until 72 hours, while PECAM-1 was not changed. Administration of an ICAM-1 monoclonal antibody significantly reduced neutrophil accumulation (about 40%) at 24 hours following TBI [129]. Another study observed ICAM-1 levels were significantly increased in the choroid plexus and cerebral endothelium at 24 hours after TBI, but ICAM-1 deficient mice did not reduce neutrophil accumulation compared to the wild-type group [130]. Knoblach and colleagues found anti-ICAM-1 antibody administration with multiple treatments at 1, 10, and 24 hours significantly improved motor function in a rat TBI model, which was associated with reduced MPO activity, an indicator of neutrophil invasion [13]. These results indicated ICAM-1 blockage might reduce leukocyte accumulation and improve neurological function after TBI.

In TBI patients, sICAM-1 levels in their CSF correlate with tissue injury as well as BBB disruption and maybe a predictor for TBI outcomes. Patients with elevated sICAM-1 levels in their CSF had severe cerebral injuries and BBB impairment while patients with normal sICAM-1 levels had minor injuries [131, 132]. Similarly, both P-selectin and ICAM-1 CSF levels increased in children with severe TBI and were associated with the greater risk for poor outcomes [133]. As a novel marker in neurological disorders, sVAP-1 has also been investigated following TBI. Lin and colleagues found sVAP-1 activity was significantly reduced in TBI patients compared to control groups, which negatively correlated with circulating leucocytes. Patients with high plasma sVAP-1 activity have a much higher mortality rate than patients with lower sVAP-1 activity [134].

### 3.3 Neurodegenerative Disease

**3.3.1 Alzheimer Disease**—Alzheimer's disease (AD) is the most common neurodegenerative disease that worsens as it progresses and eventually leads to death. There

is still no effective cure for this disease. The pathology of AD involves formation of plaques and tangles in the brain composed of amyloid and tau proteins. In particular, inflammation has been shown to directly affect the pathogenesis of AD [135].

Adhesion molecules have been associated with AD. Vascular functions are altered in the brains of AD patients, which indicate a common genetic link between acute myocardial infarction and AD [136]. Several brain and peripheral blood vessel abnormalities in AD have been described. Previous studies showed platelets could release amyloid- $\beta$  in the circulatory system and play a critically important pro-inflammatory role. Upon activation, platelets secrete inflammatory mediators and adhere to leukocytes and endothelial cells by means of adhesive proteins, such as P-selectin, platelet endothelial cell adhesion molecule-1 (PECAM), and intercellular adhesion molecule-1 and -2 (ICAM-1 and -2)[137]. Endothelial markers change during the pre-dementia stage of mild cognitive impairment and mild AD [138]. Interestingly, in a preliminary clinical study, P-selectin and L-selectin plasma levels were decreased in 12 AD patients, and L-selectin was also very low in AD patients with the highest cognitive deterioration [139]. The reduction in P-selectin and, particularly, L-selectin levels in AD patients reflected regulatory impairment of both endothelial function and leukocyte migration [140]. However, E-selectin was not significant different between AD patients and control subjects in a small sample from a case-control study[141]. Determination of selectin blood concentration could be useful in diagnosing and monitoring of the activity of AD. Other adhesion molecules, such as soluble PECAM and ICAM-1, were also increased in AD patient plasma [142, 143]. More importantly, in brain senile plaques from AD patients, ICAM-1 and integrin accumulation was observed by immunostaining[144]. In one study, AD patients had higher serum levels of sICAM-1 than control subjects [141]. In AD patient CSF, sICAM and sVCAM-1 were strongly correlated with each other and with BBB impairment [142]. Zuliani and colleagues found increased plasma VCAM-1 levels in 60 patients with AD as well as 80 patients with vascular dementia when compared to normal elderly controls independent of small or large vessel disease [145]. In summary, data derived from the above studies suggest the promise of using those adhesion molecules as biomarkers for early diagnosis and assessment of AD progression. Future study will further establish the sensitivity and specificity of adhesion molecules for early detection and diagnosis of AD.

**3.3.2 Multiple Sclerosis**—Multiple sclerosis (MS) is an immune-mediated disorder of the CNS. MS consists of multifocal areas of leukocyte infiltration into the CNS parenchyma, causing inflammation, BBB breakdown, and demyelination, which develop into the clinical manifestations of this disabling disease. Adhesion molecules regulate adhesive interactions between leukocytes and endothelial cells. Circulating immune cells gain access to the CNS and induce inflammation in MS. The animal model for MS, experimental autoimmune encephalomyelitis (EAE), implicates adhesion molecules as critical for EAE pathogenesis. The initial goal for MS research, regarding adhesion molecules, was to identify CNS-specific adhesion molecule expression. Studies were initially performed on brain microvessel endothelial cells because adhesion molecules have an indisputable role in leukocyte extravasation. It has been well documented that adhesion molecules, such as E-selectin, ICAM-1, and VCAM-1, are present on brain microvessels in active lesions of MS

patients[146]. Washington et al found temporal and topographical expression of ICAM-1 is correlated with leukocyte infiltration [147]. Accumulating data shows glial cells, astrocytes, and microglia are sources of those adhesion molecules when activated [148]. Moreover, soluble forms of adhesion molecules have been found in the serum and CSF of MS patients, and they are considered useful diagnostically tools. Doerck et al addressed the important question of temporal ICAM-1 and VCAM-1 expression in a serial manner. They found brain ICAM-1 and VCAM-1 upregulation (quantitative analysis by immunocytochemistry) parallels disease onset and remains elevated in the clinical remission phase in the rat model of adoptive transfer experimental autoimmune encephalomyelitis[149]. The important role for P-selectin glycoprotein ligand-1(PSGL-1) or its endothelial ligand, P-selectin, in MS pathogenesis has been largely discussed in EAE models ([150]). Blocking PSGL-1 or P-selectin did not have any impact on disease development.[151]. Furthermore, E-/P-selectin-deficient [152] or PSGL-1-deficient C57BL/6 mice [153, 154] failed to demonstrate slowed development of actively induced EAE by immunization with myelin peptides in multiple laboratories. Finategrast, a novel antagonist of  $\alpha 4\beta$  integrins, reduced inflammatory immune cell trafficking into the nervous system in patients with relapsing-remitting MS[155]. Piraino and colleagues concluded  $\alpha 4\beta 1$ -integrin mediates leukocyte entry through the BBB into the CNS during EAE[156]. Subsequent studies confirmed the predominant role of  $\alpha 4$ -integrin and vascular cell adhesion molecule (VCAM)-1 in inflammatory cell accumulation in the brain using various EAE animal models [15]. Natalizumab inhibits the influx of leukocytes into the CNS via blockade of the 3–4 subunit of very late activation antigen (VLA)-4. Recently, Natalizumab effectively inhibited EAE development and clearly reduced inflammatory cell recruitment into the CNS [157]. The development of a humanized anti- $\alpha 4$ -integrin antibody might be a novel treatment for relapsing-remitting multiple sclerosis [158]. Although accurate function of VAP-1 is still unknown, it is supposed to bind to its putative ligand on the leukocyte surface to hinder infiltration into brain tissues. In a case-controlled study, Airas L et al reported significantly higher VAP-1 serum concentrations in multiple sclerosis patients with ongoing inflammatory activity when compared to MS patients without inflammatory activity [159]. Their study indicated soluble VAP-1 is crucial for MS pathogenesis.

The collective data lends credence to a potential association between adhesion molecules and MS. On a global perspective, many further studies remain to be done to address the therapeutic effects of inhibiting adhesion molecules for MS. Because poor outcomes of the disease are still unacceptably high, prospective, randomized, double-blind, placebo-controlled, and crossover methods must be employed.

#### 4. Concluding perspectives

Inflammation is one of the key contributors exacerbating and driving pathological mechanisms behind several forms of CNS disorders. Adhesion molecules are groups of cell surface molecules controlling the multiple-step leukocyte adhesion cascade. Due to their critical role in leukocyte-endothelial interaction, adhesion molecules, especially novel ectoenzyme, VAP-1, have been extensively investigated in preclinical research and in clinical trials. Therapeutic targeting of adhesion molecules prevented the inflammatory response in multiple forms of CNS disorders. Our review discussed one of the ectoenzymes,

VAP-1 in CNS disorders since it is a dual function molecule regulating leukocyte extravasation and a promising target for reducing inflammation in CNS disorders.

Although understanding of the leukocyte adhesion cascade has advanced over the past several decades, several gaps exist in our knowledge that slow successful translation of anti-adhesion strategies in clinic. First, inflammatory response differences between patients and animal models need more attention. Further study should discern the optimal adhesion molecule intervention time in both animal models and patients. Additionally, inflammation is a protective defense system against pathogens, but it is also involved in tissue repair and functional recovery from CNS disorders. Therefore, whether inflammation is detrimental or protective depends on the phase or severity of CNS disorders. Furthermore, the extent of the effects from blockage of a single adhesion molecule on the entire leukocyte adhesion cascade must be determined. Prior studies reported some novel multiple-function cell surface enzymes [10, 160, 161], such as VAP-1, that act as adhesion cascade brakes by regulating other adhesion molecules and cytokine expression [55, 162]. Future study should enhance understanding of ectoenzyme functioning in leukocyte extravasation and identify more possible ectoenzyme mediators. Concentrating on discerning new ectoenzyme functions involved in leukocyte extravasation may help develop new therapeutic strategies for treating neurological diseases.

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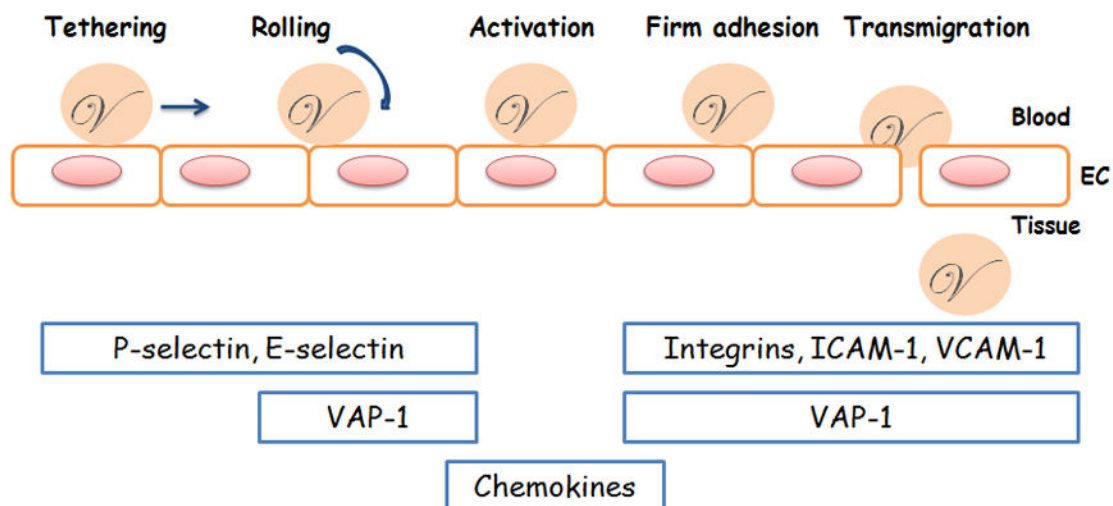
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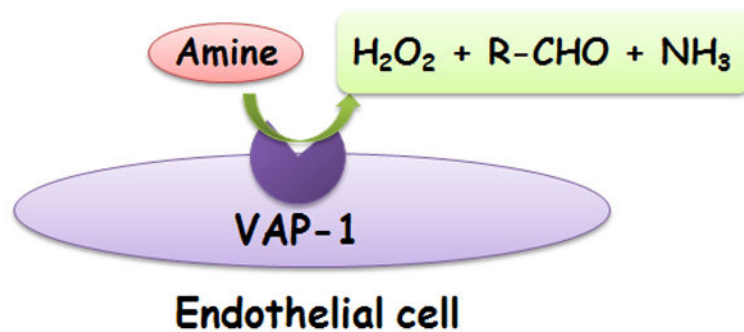
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**Figure 1. Adhesion molecules and the leukocyte adhesion cascade**

The leukocyte adhesion cascade is a multi-step process that includes tethering, rolling, activation, firm adhesion, and transmigration steps. Classical adhesion molecules, including selectin, integrin, and immunoglobulin, are involved in different phases by supporting leukocyte crossing of the endothelial layer from blood into tissue. Chemokines stimulate leukocyte activation. One novel ectoenzyme, VAP-1, contributes to the rolling, firm adhesion, and transmigration steps. EC: endothelial cell.



**Figure 2. Enzymatic function of VAP-1**

The figure shows VAP-1 is an endothelial cell semi-carbazide sensitive amine oxidase (SSAO). VAP-1 catalyzes oxidative deamination of primary amines and releases biologically active products, including  $NH_3$ , aldehydes, and  $H_2O_2$ .