Inflammatory Cell Adhesion Molecules in Ischemic Cerebrovascular Disease

C.J.M. Frijns, MD; L.J. Kappelle, MD

Background—In this review we discuss the role of inflammatory cell adhesion molecules (CAMs) in ischemic stroke and in delayed cerebral ischemia after subarachnoid hemorrhage. Vascular endothelial cells and leukocytes express several inflammatory adhesion receptors, the most important of which are the selectins, immunoglobulin gene superfamily CAMs, and β2 integrins. They mediate the transmigration process of leukocytes to the abluminal side of the endothelium.

Summary of Review—There is ample evidence from animal models of middle cerebral artery occlusion that expression of CAMs is associated with cerebral infarct size. Absence of CAMs in knockout animals resulted in reduced infarct size. When middle cerebral artery occlusion in experimental stroke was followed by reperfusion, administration of anti-CAM antibodies decreased infarct size. Thus far, anti-CAM treatment has not been successful in patients with ischemic stroke. Inflammatory CAM may also play a role in the pathogenesis of delayed cerebral ischemia after subarachnoid hemorrhage. In animal models, increased expression of CAMs has been observed in vasospastic arteries. Increased concentrations of CAMs have also been found in cerebrospinal fluid of patients with subarachnoid hemorrhage.

Conclusions—Further research on the role of inflammatory CAMs in the pathogenesis of ischemic cerebrovascular disorders should lead to new diagnostic and therapeutic strategies. (Stroke. 2002;33:2115–2122.)

Key Words: cell adhesion molecules ■ cerebrovascular disorders

In the past 2 decades, the vascular endothelium has no longer been regarded as an inert vascular lining that can be injured and morphologically changed. It has many different functions that are susceptible to change or dysfunction and that can contribute to cell and tissue injury.1 Inflammatory processes have increasingly been shown to be involved in the process of atherogenesis as well as in the pathogenesis of cerebrovascular diseases.2–4 In this review we focus on the role of inflammatory leukocyte-endothelial cell adhesion molecules (CAMs) in the pathogenesis of ischemic cerebrovascular disease and the possible consequences for clinical practice.

Leukocyte-Endothelial Cell Adhesion
The leukocyte-endothelial adhesion process consists of several steps, beginning with rolling of the leukocyte on the endothelial surface until it has slowed down to such a degree that it sticks to the endothelium. At this point the leukocyte becomes activated and flattens. Firm adherence to the endothelial cells follows. Subsequently, the leukocyte crawls on the endothelium to find an intercellular junction between the endothelial cells for diapedesis to the abluminal side and for transmigration to the target tissue.5

Since 1985, 3 families of leukocyte-endothelial adhesion molecules have been identified: the selectins, the immunoglobulin gene superfamily, and the integrins (Table).6 Selectins mediate rolling of leukocytes on the endothelium of postcapillary venules. E-selectin (CD62E) is synthesized after stimulation by cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) and expressed on the endothelial cell membrane after several hours. P-selectin (CD62P) is constitutively present on granule membranes in endothelial cells and platelets. Therefore, it can be expressed on the outer cell membrane immediately on cell activation by stimulants such as thrombin or histamine. The target cells of both E- and P-selectin are neutrophils and monocytes. Counterreceptors on these white blood cells are carbohydrate structures on membrane glycoproteins7 and L-selectin.8 L-selectin (CD62L) is present on lymphocytes, neutrophils, and monocytes. After cellular activation, it is shed from the cell membrane by proteolytic cleavage.6,9

Firm adhesion of leukocytes to the endothelial cells as well as leukocyte activation is mediated by receptors of the immunoglobulin gene superfamily. To this family belong 5 molecules that are expressed by endothelial cells: intercellular adhesion molecule-1 and -2 (ICAM-1 and ICAM-2), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), and the mucosal addressin (MAdCAM-1) (Table). The CAMs are involved in leukocyte adhesion at relatively low shear forces; they
cause a stronger attachment of leukocytes to the endothelium than the selectins. ICAM-1 (CD54) is continuously present in low amounts on the cell membranes of endothelial cells, leukocytes, epithelial cells, and fibroblasts. Its expression greatly increases on stimulation by cytokines. ICAM-2 (CD102) is a membrane receptor of endothelial cells that does not increase after stimulation, whereas VCAM-1 (CD106) expression on endothelial cells is induced by TNF-α and IL-1. PECAM-1 (CD31) has a role in the attachment of endothelial cells to each other, in leukocyte adhesion, and particularly in transmigration across the endothelium. Its surface expression on endothelial cells is not increased by cytokines.

After rolling of the leukocyte on the endothelial surface has arrested its flow, leukocyte integrins are activated by chemokines, chemotactants, and cytokines. Integrins are transmembrane cell surface proteins. The CD18 or β2 integrins are restricted to leukocytes and bind to their counterreceptors of the immunoglobulin gene superfamily (Table). They share a common β chain and 3 distinct α chains (CD11a, CD11b, or CD11c). Their surface expression is increased by agonists such as TNF-α and after adherence to E-selectin. Leukocyte integrins are involved in the firm adherence of the leukocyte through binding to the endothelial Ig gene superfamily molecules. Leukocytes and monocytes also express the integrin α4β1 (very late antigen-4 [VLA-4], CD49d), which binds to VCAM-1 and to ligands from the subendothelial matrix.

### Ischemic Stroke

**Inflammatory Injury in Acute Stroke**

Experimental studies in animal models of focal ischemic stroke have suggested that polymorphonuclear leukocytes play a role in the development of secondary injury after acute ischemic infarction. Inhibition of accumulation of neutrophils decreased ischemic injury. In humans, an elevated leukocyte count in the acute stage is associated with a relatively poor outcome. There is also evidence of activation of neutrophils in human ischemic stroke in the first days after stroke onset. Still, there is no definite proof of a pathogenic role of neutrophils. Although some studies even contradict the possibility of cerebral neutrophil invasion causing increased infarct volumes, experimental observations have led to the hypothesis that focal cerebral ischemia induces an activated state of leukocytes and cerebral endothelial cells mediated by TNF-α and IL-1. Subsequently, also on
reperfusion, leukocytes may adhere to the activated endothelium, plug capillaries, migrate into brain tissue, and release proinflammatory mediators. This inflammatory reaction could lead to secondary injury of potentially salvageable neurons in the penumbra around the infarct. Evidence for the occurrence of reperfusion injury comes from animal studies of acute focal ischemic stroke. Recently, findings compatible with reperfusion injury in human stroke were reported in a study of perfusion- and diffusion-weighted MRI before and after thrombolysis.

**Inflammatory Adhesion Molecules in Experimental Ischemic Stroke**

In animal models, the role of adhesion molecules has been demonstrated in experiments of permanent or transient focal cerebral ischemia. Considerable variation in these experiments exists with respect to the animal species, the technique of induction of ischemia, the duration of occlusion before reperfusion, the timing of termination of the experimental animals, and the type of CAM that was studied. Additionally, in therapeutic studies, different outcome measures were used, and timing of administration of the therapeutic agent before or after onset of ischemia varied. Increased expression of leukocyte-endothelial adhesion molecules occurs in the ischemic area after both permanent and transient middle cerebral artery occlusion. This finding is in agreement with in vitro experiments with human brain endothelial cells that also showed increased expression of inflammatory adhesion molecules under ischemic conditions. In experiments with knockout animals, absence of adhesion molecules reduced infarct volume after transient focal cerebral ischemia. Animal experiments investigating the effect of anti–adhesion molecule strategies in focal cerebral and spinal cord ischemia showed a beneficial effect in models in which transient focal ischemia was followed by reperfusion, but not in models of permanent ischemia. These positive results of anti-adhesion therapy after reperfusion but not after permanent occlusion, together with the recently proven effectiveness of thrombolytic agents in patients with acute ischemic stroke, have resulted in animal experiments testing combined administration of thrombolytic agents in patients with acute ischemic stroke, although evidence is contradictory. In 9 studies, circulating adhesion molecules were measured in the peripheral blood in samples taken within 12 to 72 hours after stroke onset. Elevated concentrations of soluble (s) ICAM-1 were found in 3 studies of 6–36 of 5 studies. Concentrations of sP-selectin were elevated in 2 studies that assessed the presence of these adhesion molecules. Membrane expression of CD11a was increased in the only study in which it was investigated.

Possible explanations for these contradictory results are differences in control groups, in laboratory assays, or in the timing of blood sampling. Differences in the use of medications influencing expression of adhesion molecules, for instance, aspirin, corticosteroids, or NSAIDs, may be another explanation. Additionally, the presence of other diseases such as infections, diabetes mellitus, or systemic illnesses may have influenced the results. Increased levels of sICAM-1 and sVCAM-1, but normal sE-selectin levels, were reported in patients with diabetes, especially in those with silent cerebral infarcts on MRI.

Pathological data on leukocyte-endothelial adhesion molecules in human ischemic stroke are scant. The first evidence of microvascular expression of ICAM-1 comes from a study in patients with multiple sclerosis: in 4 control patients with cerebral infarcts, increased immunostaining of ICAM-1 was found. In addition, many lymphocyte function-associated antigen-1 (LFA-1) (CD11a)–positive inflammatory cells were found in recent infarcts. In another study in patients who died 5 days to 3 months after stroke onset, an increase of microvessels positively staining with an antibody against VCAM-1 was observed in the infarcted tissue. Increased expression of ICAM-1 has also been found on microvessels of infarcts in patients who survived between 15 hours and 6 days after stroke onset. Moreover, ICAM-1 expression was upregulated in the contralateral hemisphere, though to a lesser degree.

P-selectin expression was limited to areas of platelet deposits and of positive immunostaining for platelet antigen glycoprotein IIb/IIIa.

In summary, inflammatory adhesion molecules are upregulated in patients with ischemic stroke, but interindividual variability is large. Consequently, at this time there are no useful implications for measurement of these molecules in...
clinical practice. However, the findings support the hypothesis of involvement of adhesion molecules in the pathogenesis of inflammatory or reperfusion injury after acute ischemic stroke in humans.

**Anti–Adhesion Molecule Antibodies in Patients With Ischemic Stroke**

Treatment with a murine anti–ICAM-1 antibody (enlimomab) has been investigated in patients with acute ischemic stroke in the Enlimomab Acute Stroke Trial (EAST). Unfortunately, the case fatality rate in this trial was significantly higher in the enlimomab patient group than in the placebo group. Adverse effects of enlimomab may have been caused by immunologic factors, such as a specific humoral or cytotoxic immune response against the medication because of its murine origin or a nonspecific complement activation induced by presentation of Fc fragments. A dose-finding study demonstrated the presence of anti-mouse antibodies after administration of enlimomab in 100% of the treated patients. Addition of enlimomab to whole blood of human volunteers unexpectedly caused activation of neutrophil granulocytes. This finding could not be replicated by F(ab')2 fragments of enlimomab. The occurrence of unwanted immunologic effects of anti–ICAM-1 antibodies was confirmed in recent experiments in rats. Activation of complement, neutrophils, and endothelial cells, as well as the formation of anti-mouse antibodies, was demonstrated.

In retrospect, the choice of the anti–ICAM-1 treatment regimen in the EAST may not have been optimal. Enlimomab is an IgG2a monoclonal anti-human ICAM-1 antibody directed against the binding domain for LFA-1 (CD11a/CD18), whereas the anti–ICAM-1 monoclonal antibody used in most animal experiments (1A29) is an (anti-rat) IgG1 antibody of which the epitope is not known. Moreover, in experimental stroke models, anti–ICAM-1 treatment was given only once, primarily 1 or 2 hours after stroke onset, whereas in the EAST enlimomab was administered during 5 consecutive days.

Probably the most essential difference between ischemic stroke in humans and the experimental strokes in which anti-adhesion therapy was effective is that the early reperfusion that is technically brought about in experimental animals cannot be replicated by F(ab')2 fragments of enlimomab. The occurrence of unwanted immunologic effects of anti–ICAM-1 antibodies was confirmed in recent experiments in rats. Activation of complement, neutrophils, and endothelial cells, as well as the formation of anti-mouse antibodies, was demonstrated.

**Delayed Cerebral Ischemia After Subarachnoid Hemorrhage**

In patients who survive a subarachnoid hemorrhage (SAH) from rupture of an intracranial aneurysm, delayed cerebral ischemia is an important complication, frequently leading to morbidity or death. Although it is most often related to cerebral vasospasm, the pathogenesis of delayed cerebral ischemia is not well understood. angiographically demonstrated vasospasm can occur without cerebral ischemic symptoms, and disappearance of vasospasm during treatment with calcium antagonists does not necessarily lead to a better neurological outcome. The cause of vasospasm of the large, basal intracranial arteries is unexplained. A traditional culprit is the perivascular blood clot in the subarachnoid space, and especially hemoglobin breakdown products (oxyhemoglobin), but the relationship is far from complete. The manner in which perivascular blood products influence vessel wall function and morphology has not been elucidated. Moreover, blood clots associated with nonaneurysmal SAH are not accompanied by ischemic complications, and in a randomized trial of intracisternal tPA, early removal of cisternal blood did not have a significant effect on either angiographically demonstrated vasospasm or clinical outcome. Pathological observations and the presence of fever and leukocytosis in vasospasm suggest that inflammation plays a role in the development of vasospasm. Results from animal experiments support this assumption. Recently, the potential role of mediators of inflammation in experimental and in human SAH has been emphasized in several reports.

**Inflammatory Adhesion Molecules**

In a rat model of SAH, increased expression of ICAM-1 was found on endothelial cells and in the medial layer of basilar arteries 2 to 5 days after SAH, simultaneously with maximal luminal narrowing. In a canine model mRNA levels of inflammatory mediators, including ICAM-1 and VCAM-1, were measured in basilar arteries at 2, 7, or 14 days after SAH. Both the levels of ICAM-1 mRNA and arterial narrowing were maximal on day 7. Treatment with intracisternal anti–ICAM-1 and/or anti-CD18 antibody reduced basal artery vasospasm in a rabbit SAH model. The femoral artery model of vasospasm is another experimental model in which the role of leukocyte adhesion has been investigated. In these studies the femoral arteries of rats were isolated bilaterally and surrounded by a pouch containing clotted blood on one side and saline on the control side. Blood-exposed vessels showed a significant increase in ICAM-1 immunoreactivity, peaking at 24 hours after blood exposure and reaching the baseline again by 48 hours. Transient narrowing of the femoral artery was demonstrated within the first 12 hours. Subsequently, the diameter decreased again, with maximal narrowing at 12 days after blood exposure. Wall thickness and arterial collagen were also maximal on day 12. However, from this experiment, no causal relation between increased ICAM-1 expression and vasospasm could be inferred. A second study in the same rat model investigated the influence of intraperitoneal administration of an anti–ICAM-1 antibody. In treated rats, a significant reduction of femoral artery narrowing at 12 days was reported, as well as a significant decrease of leukocyte infiltration in the region of the periadventitial blood clot measured 24 hours after blood exposure.

The anti-inflammatory agent ibuprofen can inhibit leukocyte-endothelial adhesion through suppression of IL-
leukocytes would transmigrate to the periadventitial region of arteries that are exposed to blood. Subsequently, increased expression of adhesion molecules on endothelial cells is achieved that post-SAH vasospasm is primarily a result of transmigration of leukocytes, inferred from significantly reduced periadventitial leukocyte counts. The authors hypothesized that post-SAH vasospasm is primarily a result of increased expression of adhesion molecules on endothelial cells of arteries that are exposed to blood. Subsequently, leukocytes would transmigrate to the periadventitial region and infiltrate in the subarachnoid space, where they produce toxins and endothelins that cause vasospasm.

Evidence for a role of leukocytes and inflammatory adhesion molecules in the pathogenesis of cerebral ischemia after SAH in humans is scant. In 1 study significantly increased concentrations of sE-selectin, sICAM-1, and sVCAM-1, but not of L-selectin, were detected in cerebrospinal fluid obtained within 3 days after aneurysm rupture. Concentrations in patients with SAH ranged from control levels, in approximately half of the patients, to very high values. Levels of sE-selectin and sL-selectin in the upper range were found in the 3 patients who later developed vasospasm. However, in cisternal cerebrospinal fluid samples of patients who were operated within 72 hours or after 10 days, levels of sE-selectin were below the threshold level in all patients. A publication of serum concentrations of adhesion molecules in patients with SAH reported an increase in sP-selectin and a decrease in sL-selectin concentrations in patients with compared with patients without delayed cerebral ischemia. Mean concentrations of sE-selectin, sPECAM-1, sICAM-1, and sVCAM-1 did not significantly differ between the 2 patient groups. In another study, an association of serum sICAM-1 concentrations with clinical outcome was found in patients with SAH.

Reperfusion Injury

At the moment of aneurysm rupture, patients often lose consciousness, presumably because of diffuse cerebral hypoperfusion caused by increased intracranial pressure and acute vasocostriction. Measurements at the time of a recurrent bleed after SAH showed intracerebral circulatory arrest lasting up to several minutes. To our knowledge, reperfusion injury after the initial circulatory arrest has not yet been considered as a contributory factor in the pathogenesis of delayed cerebral ischemia. One could speculate that inflammatory changes in the microcirculation caused by transient ischemia or by reperfusion contribute to delayed cerebral ischemia, especially in conjunction with superimposed vasospasm or other local factors such as manipulation of blood vessels during diagnostic or therapeutic interventions. Up to the present time, expression of adhesion molecules in the microcirculation of ischemic brain areas after experimental or clinical SAH has not been investigated.

Future Options

The growing amount of evidence on the role of leukocyte-endothelial adhesion molecules in ischemic cerebrovascular disease offers substantial opportunities for further experimental and clinical research. However, there are many pitfalls on the way from hypothesis to clinical trial, as is illustrated by the results of the EAST. Another clinical trial with an anti-adhesion approach at this time should probably not be recommended. More proof is necessary to increase a priori chances of a positive trial result.

The role of neutrophils still has to be elucidated, both in stroke after permanent occlusion and in cerebral reperfusion injury. For example, in the latest rat experiments to explain the failure of the EAST, the invasion of leukocytes in infarcted brain was decreased by anti–ICAM-1 (IgG1A29), but the infarct volume was not influenced by anti–ICAM-1 treatment. We also need to prevent or at least minimize the immunogenicity of anti-adhesion antibodies, for example, by using f(ab’), fragments or humanized antibodies. Effects of anti-adhesion antibodies on different components of the immune system should be monitored, first in animal experiments and after that in phase I trials, to screen for potential toxic effects. Phase IIa and IIb trials should not be omitted and should be conducted in large enough groups of patients.

Experimental studies of anti-adhesion blocking agents have shown that this treatment will work only if there is early reperfusion. Therefore, the combination of anti-adhesion strategies and thrombolytic therapy deserves further investigation. Ideally, anti-adhesion therapy would be started in the hyperacute stage even before the administration of thrombolytic agents. To find the optimal treatment duration and dosage for patients with ischemic stroke, more insight is needed into the therapeutic window. We need to establish how long it takes after reperfusion has been achieved until ischemic brain tissue becomes irreversibly damaged and how the time window depends on the delay from onset of symptoms until partial or complete reperfusion. Serial diffusion- and perfusion-weighted MRI studies in patients treated with thrombolysis may answer these questions. The combination of these techniques with serial single-photon emission CT scans of radiolabeled neutrophils may show whether neutrophil invasion precedes increase of infarct volume after reperfusion.

Some of the problems that exist in the treatment of acute ischemic stroke with anti–adhesion molecule antibodies are not pertinent to delayed cerebral ischemia after SAH. Reperfusion does not have to be effected but may be an inherent consequence of SAH and may play a role in the development of delayed cerebral ischemia. Treatment can be started early, even before the onset of symptoms of ischemia. As is the case for acute ischemic stroke, the pathogenetic role of leukocytes must be proven. The subtype of leukocytes may be mononuclear leukocytes rather than neutrophils. Therefore, the most effective anti–adhesion molecule treatment in patients with SAH may not be the same as in acute stroke patients. Obviously, all necessary laboratory and clinical investigational steps in the development of nontoxic drugs, as described for acute stroke treatment, pertain to treatment of delayed cerebral ischemia as well.
References


7. Somers WS, Tang J, Shaw GD, Camphausen RT. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLE(X) and PSLG-1. Cell. 2000;103:467–479.


61. Frijns J, Kappelle Cell Adhesion Molecules in Cerebrovascular Disease 2011


Inflammatory Cell Adhesion Molecules in Ischemic Cerebrovascular Disease
C.J.M. Frijns and L.J. Kappelle

Stroke. 2002;33:2115-2122
doi: 10.1161/01.STR.0000021902.33129.69
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/33/8/2115

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/