Cell adhesion molecules and ischemic stroke

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Objective: To describe the role of adhesion molecules in ischemic stroke.

Methods: A PubMed search of literature pertaining to this study was conducted in April 2008 using specific keyword search terms pertaining to stroke and various listed subtopics related to adhesion molecules.

Results: An important contribution of β2-integrins (CD11/CD18), intercellular adhesion molecule and P-selectin in the recruitment of leukocytes as well as platelets in the post-ischemic cerebral microvasculature has been defined in related studies. Immunoblockade or genetic deletion of these adhesion molecules has been shown to reduce infarct volume, edema, behavioral deficits and/or mortality in different animal models of ischemic stroke. Anti-adhesion agents also appear to widen the therapeutic window for thrombolytic therapy in these experimental models. An emerging role of inflammatory signaling pathways has also been addressed in modulating adhesion properties of post-ischemic cerebral microvasculature. Despite the promising data obtained from animal studies, few clinical trials assessing anti-adhesion therapy in ischemic stroke have failed to show efficacy.

Discussion: Several experiments using cell surface adhesion molecules as targets of stroke therapy are promising yet inadequate. Clinical trials using immune blockade of adhesion molecules by antibodies have failed due to immune reactions of the host. Further clinical trials are needed to test the efficacy of humanized antibodies or non-immunogenic agents that interfere with cell adhesion mechanisms. Adhesion blocking strategies seem to be effective particularly at repertusion and use of these strategies with thrombolytic therapies justifies a continued effort to define the role of adhesion molecules in the pathophysiology of cerebral ischemia–repertusion. [Neurol Res 2008; 30: 783–793]

Keywords: Adhesion; leukocytes; platelets; stroke; cerebral ischemia; inflammation

INTRODUCTION

Stroke is defined as ‘rapidly developing clinical signs of focal or global disturbance of cerebral function with symptoms lasting 24 hours or longer, or leading to death with no apparent cause other than of vascular origin’1. Although this definition includes the hemorrhagic forms of stroke, 80% of stroke cases occur due to the occlusion of arteries carrying blood to the brain and subsequent ischemia. Ischemic stroke is the third leading cause of death in the USA with an estimated cost of 71.8 billion dollars. The mortality rate after an ischemic incident is very high (30%) and survivors almost always face disabilities that require costly long-term care.

Despite the high mortality and morbidity associated with ischemic stroke, current established therapies are limited. To date, the only effective treatment approved for acute ischemic stroke in the USA and Canada is thrombolysis achieved by recombinant tissue plasminogen activators. However, this regime needs to be applied within 3 hours of symptom onset, decreasing the availability of treatment to the majority of patients in need. In addition to thrombolysis, anti-platelet therapies such as aspirin and glycoprotein IIb–IIIa inhibitors (clopidogrel) or anticoagulants (heparin) have been used in the prevention/treatment of acute ischemic stroke. Aspirin treatment is associated with significantly fewer recurrent ischemic strokes and no significant increase in hemorrhagic strokes at day 14. A small but a significant improvement at month 6 has also been observed with aspirin in large-scale clinical studies. Heparin treatment, however, does appear to offer any clinical advantage at month 6, and initial efforts to assess glycoprotein IIb–IIIa directed treatment strategies have not shown promising results.

After an ischemic insult, the neuronal injury around the ischemic core, called the penumbra, continues to develop over several hours. Neuronal tissue within the penumbra is electrically inactive but viable, and considered to represent salvageable tissue that can be targeted with neuroprotective interventions. The slow evolution of ischemic damage within the penumbra provides a window of opportunity for neuroprotective therapies. Attenuating and/or delaying this time-dependent brain injury may improve neurological outcome and facilitate brain recovery from injury.

Experimental interventions that have been used to confer protection to the penumbra include free radical scavengers and synthesis inhibitors, excitotoxicity...
inhibitors, suppressors of neuronal metabolism (e.g., hypothermia), anti-inflammatory agents and membrane stabilizers. While there is substantial experimental evidence demonstrating the beneficial effects of these interventions in animal models, human trials have either failed or proven inadequate.

**ANTI-INFLAMMATION AS A THERAPEUTIC TARGET FOR ISCHEMIC STROKE**

Ischemic stroke frequently results from thromboemboli blocking the blood supply to neuronal tissue. Immediately after cessation of blood flow, due to the high oxygen and nutrient needs of brain tissue, ATP depletion occurs in the neurons. Consequently, the ionic gradients across the cellular membranes cannot be sustained resulting in calcium and water influx and neurotransmitter release. This sequence of events leads to cytotoxic edema, excitotoxicity and activation of intracellular enzymes. The overall impact of blood flow cessation is cellular damage and initiation of an inflammatory response. While the other triggering events for cellular damage occurs rapidly after the stroke, inflammation occurs over hours to days and provides an excellent window of opportunity for new treatment strategies.

Many studies demonstrate that cerebral ischemia is associated with the infiltration of inflammatory cells to the ischemic region. Infiltration of the ischemic brain region by leukocytes is associated with inflammatory activation of cerebral endothelial cells, microglia/macrophages and astrocytes. Activation of these resident cell populations along with immune cells stimulates the production and release of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1 from the ischemic tissue. In this inflammatory environment, cerebral endothelial cells increase their expression of cell surface adhesion molecules that mediate recruitment of leukocytes and platelets to the ischemic region. A role for leukocytes in the pathogenesis of post-ischemic brain injury is supported by three major lines of evidence: (1) leukocytes accumulate in the brain before or during the onset of tissue injury; (2) depletion of neutrophils from the circulation reduces infarct volume and improves neurological outcome; (3) immunoneutralization or genetic deletion of cell adhesion molecules that mediate leukocyte recruitment reduces tissue injury and brain dysfunction in animal models of focal and global cerebral ischemia.

Brain ischemia triggers profound changes in cerebral microvascular endothelium. In microvessels, the endothelial cell activation is accompanied by an up-regulation of adhesion molecules, which promotes the rolling, and firm adhesion of both leukocytes and platelets. The leukocyte recruitment may lead to further injury by releasing reactive oxygen species, proteases and inflammatory mediators. The adhesion process may also trigger signaling cascades in cerebral endothelial cells, leukocytes or both which may contribute to the injury. The accumulation of platelets, either directly attached to endothelial cells or bound to adherent leukocytes, promotes a prothrombotic state that further exaggerates the perfusion deficit and state of 'no-reflow' after cerebral ischemia.

Although there are a large number of reports that implicate leukocytes in the tissue injury associated with ischemic stroke, there are also several reports that do not support this contention. For example, it has been reported that brain necrosis precedes the appearance of neutrophils and that further increases in the number of neutrophils in the infarcted region is not associated with a larger infarct size. Furthermore, some studies have revealed an inability of neutrophil blocking antibodies to protect against neuronal tissue injury following ischemia reperfusion. These observations lead to the conclusion that neutrophils may be by-standers rather than the effectors of the tissue injury induced by cerebral ischemia. This view is also supported by studies that fail to demonstrate protection against ischemic brain injury in adhesion molecule deficient mice. These inconsistent findings may be explained by the differences in experimental models/techniques or by the duration and magnitude of the ischemic insult and reperfusion periods.

**LEUKOCYTE ADHESION IN POST-ISCHEMIC CEREBRAL MICROVESSELS**

The recruitment of leukocytes and platelets in the cerebral microvasculature is widely regarded as a rate-limiting step in the inflammatory response associated with cerebral ischemia. Under normal conditions, (non-inflamed) cerebral endothelium is a poor substrate for the rolling and firm adhesion of circulating cells. Intravital microscopy studies have revealed that the number of rolling leukocytes in normal (non-inflamed) cerebral venules is very low compared to skeletal muscle and mesenteric. However, in response to an ischemic (or other inflammatory) insult, cerebral endothelium is capable of expressing high levels of adhesion molecules and recruiting large numbers of leukocytes.

The recruitment of leukocytes and platelets in cerebral venules exposed to ischemia and reperfusion is a highly coordinated and well-regulated process that involves different adhesion molecules expressed on vascular endothelium and circulating cells. This recruitment process involves at least two different stages, i.e. an initial low affinity binding that is manifested as rolling and a later high affinity interaction that results in firm adhesion. The rolling interaction appears to be largely mediated by P-selectin expressed on endothelial cells that engage with sialyl Leα moieties on circulating cells, while firm adhesion is mediated by an interaction between β2-integrins on leukocytes with intercellular adhesion molecule-1 on cerebral microvascular endothelial cells. Both CD11a/CD18 (lymphocyte function-associated antigen-1) and CD11b/CD18 (macrophage-1 antigen) seem to play a role in the firm adhesion of leukocytes at 24 hours of reperfusion of ischemia since CD11a (lymphocyte...
function-associated antigen-1) or CD11b (macrophage-1 antigen) knockout mice exhibit less accumulation of adherent leukocytes in cerebral venules after 24 hours (but not after 4 hours) of reperfusion following occlusion of the middle cerebral artery

Leukocyte adhesion has been demonstrated in different experimental models of cerebral ischemia and hypoxia. In these experiments, leukocyte adhesion has been observed as early as 30 minutes after reperfusion and as late as 48 hours. This kinetics is consistent with a report describing P-selectin up-regulation as early as 15 minutes following an ischemic insult, with increased E-selectin expression occurring within 2 hours of ischemia.

The intensity of the leukocyte adhesion response appears to depend on the nature of the ischemic–hypoxic insult. For example, asphyxia elicits less leukocyte adhesion compared to focal and global ischemia in brain microvessels. In the early stages of reperfusion after ischemia, neutrophils represent the dominant leukocyte population that accumulates at the cerebral microvasculature. This view is supported by studies showing that rendering mice neutropenic anti-neutrophil serum attenuates the recruitment of leukocytes in cerebral venules 4 hours after ischemia–reperfusion but has no effect on leukocyte adhesion 24 hours after reperfusion. It appears likely that the leukocyte population that accumulates after 24 hours of reperfusion is mainly mononuclear cells rather than neutrophils.

**PLATELET ADHESION IN POST-ISCHEMIC CEREBRAL MICROVESSELS**

Increased leukocyte adhesion after cerebral ischemia–reperfusion is also accompanied by the recruitment of rolling and adherent platelets. Immunoblockade of the platelet adhesion molecule glycoprotein Iib–Illa has no effect on the platelet adhesion elicited by cerebral ischemia–reperfusion. However, adhesion molecule blocking antibodies that inhibit leukocyte adhesion, such as P-selectin, CD18 and intercellular adhesion molecule-1, in post-ischemic cerebral venules, also
blunt the recruitment of adherent platelets\textsuperscript{47,59}. Mice deficient in either endothelial cell or platelet P-selectin exhibit a blunted platelet recruitment response to ischemia–reperfusion\textsuperscript{24,47}. Collectively, the data generated using different blocking antibodies and mutant mice are consistent with a model of platelet recruitment that involves the binding of platelet-associated P-selectin to its ligand P-selectin glycoprotein ligand-1 on leukocytes, which in turn utilize endothelial cell P-selectin and intercellular adhesion molecule-1 to attach to the venular walls. This dependency of platelet adhesion on leukocyte adhesion is consistent with the observation that platelet accumulation lags behind the leukocyte accumulation\textsuperscript{60} in post-ischemic cerebral venules and that a large proportion of platelets bind to already adherent leukocytes\textsuperscript{61}. While the consequences of platelet accumulation to the overall injury response remain poorly understood, there is evidence suggesting the binding of platelets to adherent leukocytes may lead to further activation of both cell types, which may exacerbate the injury response\textsuperscript{54,62}. The results of a recent clinical study of genetic variants of P-selectin glycoprotein ligand-1 and P-selectin genes have revealed an association between some variants and the risk for ischemic stroke; however, it remains unclear if and how this genetic association relates to platelet–leukocyte–endothelium interactions\textsuperscript{63}.

**T-lymphocytes, Blood Cell Adhesion and Stroke**

There is a growing body of evidence in literature that supports a role for T-lymphocytes in the tissue injury following an ischemic stroke\textsuperscript{58,59,65}. In a mouse model of cerebral ischemia–reperfusion, T-lymphocytes appear to be major modulators of the adhesion of leukocytes and platelets in cerebral venules, and infarct size. Mice deficient in either CD4+ and/or CD8+ T-lymphocytes exhibit a reduced infarct size, lower number of adherent leukocytes and platelets in the cerebral venules, and improved neurological outcome 24 hours after reperfusion\textsuperscript{58}. B-lymphocyte deficient mice did not exhibit protection against the inflammation, tissue injury and neurological deficit induced by ischemic stroke. While these observations implicate T (but not B-) lymphocytes in the pathogenesis of experimental stroke, there are no \textit{in vivo} studies to date that directly demonstrate the adhesion of T-lymphocytes to cerebral endothelium after ischemia. However, \textit{in vitro} experiments have revealed that the adhesion of lymphocytes, derived from stroke patients, to cerebral endothelium, is significantly higher than that noted for lymphocytes isolated from healthy subjects. The pathophysiologic relevance of this observation is unclear since it is not known whether T-lymphocytes must directly interact with cerebral endothelial cells to exert their deleterious actions during ischemic stroke.

**Modulation of Blood Cell Adhesion**

Several agents and interventions have been shown to alter the adhesion of platelets and leukocytes in cerebral vessels following ischemia–reperfusion. These include physical factors, pharmacologic agents, interventions targeting the blockade of adhesion molecules and some of the risk factors for cardiovascular diseases. High venular shear rates\textsuperscript{18}, platelet activating factor receptor antagonists\textsuperscript{31}, hypothermia\textsuperscript{52}, hydroxyethyl starch\textsuperscript{57} and estrogen\textsuperscript{66} decrease the leukocyte accumulation, while low venular shear rates\textsuperscript{69} and ovariectomy increase the leukocyte adhesion into cerebral vessels following ischemia–reperfusion.

Some of the risk factors for cardiovascular disease also appear to modulate the adhesion of platelets and leukocytes in the cerebral vessels after ischemia–reperfusion. For example, hypercholesterolemia increases the number of adherent leukocytes and platelets in cerebral venules after ischemia–reperfusion\textsuperscript{59}. Evidence from post-ischemic retinal microvessels suggests that hypertension may also exacerbate the adhesion of leukocytes and platelets following ischemia–reperfusion in the central nervous system\textsuperscript{67}. A recent study has addressed the contribution of angiotensin II type 1 receptor signaling to platelet–leukocyte–endothelial cell interactions in the cerebral microvasculature. Angiotensin II type 1 signaling results in increased adhesion of leukocytes and platelets by a P-selectin and P-selectin glycoprotein ligand-1 mediated mechanism and may represent a mechanism that links hypertension to tissue injury in ischemic stroke\textsuperscript{68}.

**Therapeutic Targeting of Leukocyte–Endothelial Cell Adhesion**

In several experimental studies, adhesion molecules were targeted in order to define the mechanisms of cell recruitment and the related tissue damage after cerebral ischemia–reperfusion (Table 1). These interventions include immunoblockade of adhesion molecules with specific antibodies, inhibition with ligands or antagonists and genetic deletion. The principal targets for these interventions include the selectins, intercellular adhesion molecule-1 and the $\beta$-integrins, lymphocyte function-associated antigen-1 and macrophage-1 antigen.

**Selectin blockade**

Following cerebral ischemia, P- and E-selectins are highly expressed at brain. P-selectin can be detected as early as 15 minutes after reperfusion while E-selectin expression is observed beginning 2 hours after ischemia. The expression of selectins contributes to the early recruitment of circulating cells to the infarct region\textsuperscript{56}. The link between P-selectin expression and injury following ischemic stroke seems to involve a complement-dependent pathway wherein P-selectin up-regulation results from complement activation and can be modulated with a targeted approach to complement receptor 2 without an effect on systemic complement activity\textsuperscript{69}.

The polysaccharide fucoidin, a homopolymer of sulfated L-fucose that competitively inhibits P- and L-selectin, has been shown to attenuate the leukocyte
accumulation during reperfusion of the rat brain following focal ischemia. Fucoidin also reduced infarct size and improved neurological outcome, suggesting a role for selectin-dependent leukocyte-endothelial cell interactions following cerebral ischemia70. However, L-selectin blockade with a humanized anti-L-selectin antibody did not decrease the tissue damage or number of infiltrating leukocytes to the ischemic region in a rabbit model of transient cerebral ischemia71. In another study employing a similar cerebral ischemia–reperfusion model, anti-L-selectin antibodies were found to be effective only when used in combination with tissue plasminogen activators, which addresses the potential involvement of L-selectin in tissue injury following thrombolytic reperfusion of ischemic brain72.

Therapies targeting P-selectin have also been shown to be effective in different experimental settings. In a model of cerebral ischemia–reperfusion, P-selectin knockout mice exhibited a reduction in infarct volume, better functional outcome and a better return of cerebral blood flow after ischemia36. Similarly, blocking antibodies for P-selectin reduced infarct size with a reduced hemorrhagic transition in cerebral ischemia–reperfusion73. In a permanent ischemia model, P-selectin immunoblockade attenuated both infarct size and brain edema, which were associated with a reduction of leukocyte infiltration73. In these studies, the anti-P-selectin antibodies were administered 30 minutes before the ischemic insult, which lessens the therapeutic value of the observed protection.

E-selectin immunoblockade does not need to be done during the pre-ischemic period, thereby enhancing its utility as a potential therapeutic strategy. Blocking E-selectin with specific antibodies as long as 90 minutes after onset of ischemia has been shown to reduce infarct size74. In a rat model of transient ischemia, E-selectin blockade with an analogue of sialyl-LewisX (CY-1503) reduced both infarct size and neutrophil infiltration. The possibility that CY-1503 affords protection by blocking E-selectin on leukocytes or P-selectin on endothelial cells or platelets cannot be discounted74.

A blinded placebo-controlled trial in a non-human primate model of reperfused stroke has been performed to test the combination of blocking of both E- and P-selectins using a humanized monoclonal antibody (HuEP5C7). Administration of the blocking antibody during the occlusion period reduced infarct volume and improved neurological outcome. No immune response

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<tr>
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<td>Anti-E-selectin antibodies</td>
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(complement activation) was noted with the antibody. Despite the promising outcome of this study, there is no evidence supporting the effectiveness HuEPC7 if it is administered after onset of the stroke. Finally, blocking the lectin domain of selectins with a synthetic oligopeptide has been shown to reduce brain injury in a transient, but not in a permanent model of cerebral ischemia, suggesting that reperfusion is necessary for effective anti-selectin therapy.

Induction of mucosal immune tolerance to E-selectin represents another promising strategy for prevention of ischemic and hemorrhagic stroke. Takeda et al. have demonstrated that the induction of mucosal tolerance to E-selectin, by repeated nasal instillation of this antigen, prevents the development of ischemic and hemorrhagic strokes in spontaneously hypertensive stroke-prone rats. In a follow-up study, the protective effects of mucosal tolerance to E-selectin were also demonstrated in a permanent model of cerebral ischemia. In the latter study, the neuroprotective effects of mucosal tolerance to E-selectin was linked to immunomodulation of CD8+ T-cell responses addressing a possible link between T-cell modulation and neuroprotection. A better understanding of this link may provide promising selective therapeutic options in stroke.

**Intercellular adhesion molecule-1 blockade**

The results of several studies have revealed significant intercellular adhesion molecule-1 expression in the post-ischemic brain and a role for this adhesion molecule in the recruitment of inflammatory cells. In vitro experiments confirm an increase in intercellular adhesion molecule-1 in cerebral endothelial cells following ischemia or ischemia-like conditions. Human studies provide further support for an increased intercellular adhesion molecule-1 expression in the ipsilateral cortex and increased circulating levels of soluble intercellular adhesion molecule-1 following ischemic stroke.

Intercellular adhesion molecule-1 expression is an essential step in mediating the firm adhesion of leukocytes in cerebral microvessels after ischemic stroke and there are several studies that address the contribution of intercellular adhesion molecule-1 to cerebral injury after stroke. Intercellular adhesion molecule-1 knockout mice exhibit a reduction in leukocyte adhesion, smaller infarcts, decreased leukocyte adhesion, improved cerebral blood flow and lower mortality after cerebral ischemia and reperfusion. Similarly, intercellular adhesion molecule-1 immunoblockade reduces ischemic brain injury and neutrophil accumulation in both rat and rabbit models of cerebral ischemia. The combined use of anti-intercellular adhesion molecule-1 antibodies with tissue plasminogen activator administration with an extended therapeutic window for thrombolysis.

**Lymphocyte function-associated antigen-1/macrophage-1 antigen blockade**

Lymphocyte function-associated antigen-1 (CD11a/CD18) and macrophage-1 antigen blockade (CD11b/CD18) are β2-integrins that mediate the firm adhesion of leukocytes on vascular endothelium. Lymphocyte function-associated antigen-1 is expressed on all leukocytes where macrophage-1 antigen expression is found on neutrophils, monocytes and natural killer cells. Both adhesion molecules are constitutively expressed and may exhibit increased expression upon stimulation. Intercellular adhesion molecule-1 and -2 on endothelial cells are known targets for lymphocyte function-associated antigen-1 and macrophage-1 antigen. The expression of CD11a and CD18 is up-regulated in stroke patients and patients with transient ischemic attacks for up to 72 hours after the ischemic incident, revealing an association between cerebral ischemia and the cell surface density of these adhesion molecules.

Macrophage-1 antigen and CD18 knockout mice subjected to stroke followed by reperfusion exhibit a reduced infarct volume and lower mortality, compared to their wild type counterparts. In models of transient cerebral ischemia, immunoblockade of either CD11b, CD18 or macrophage-1 antigen also affords protection against tissue injury. Similarly, CD18 immunoneutralization in rats reduces the edema, leukocyte infiltration and infarct size resulting from transient ischemia. In an in vitro study, the enhanced adhesion of lymphocytes to cerebral endothelial cells appears to be dependent on lymphocyte function-associated antigen-1 and intercellular adhesion molecule expression following ischemic stroke. Furthermore, lymphocytes isolated from patients with stroke exhibit increased adhesion to cerebral endothelium when compared to lymphocytes derived from healthy donors. The contributions of lymphocyte function-associated antigen-1 and macrophage-1 antigen to ischemia/reperfusion-induced brain injury and cerebral microvascular dysfunction 4 and 24 hours after reperfusion have been addressed using mice deficient in either leukocyte adhesion molecule. Both mutants exhibited smaller infarcts, improved neurological outcome and less adhesion of leukocytes and platelets in cerebral microvessels. Interestingly, the lack of lymphocyte function-associated antigen-1 or macrophage-1 antigen had no effect on leukocyte adhesion after 4 hours of reperfusion but reduced the number of adherent leukocytes after 24 hours, suggesting a more important role for these adhesion molecules at later times after reperfusion.

Anti-adhesion molecule strategies in ischemic stroke have proven more effective following transient, but not permanent, ischemia. This observation suggests that anti-adhesion interventions may offer more therapeutic benefit in patients receiving tissue plasminogen activators to achieve reperfusion following ischemic...
stroke. Support for this possibility is provided by the observation that a combination of anti-β2-integrin (CD11/CD18) and tissue plasminogen activator extends the therapeutic window of tissue plasminogen activator, with a better outcome than the additive effects of these agents in experimental animals.\(^{99,100}\)

**Leukocyte adhesion and signaling pathways**

Ischemia elicits changes in different signaling pathways in endothelial cells that allow for the recruitment of leukocytes and platelets in cerebral venules. Increased production of reactive oxygen species following ischemia–reperfusion is considered to be one of the major mechanisms that links ischemia–reperfusion and increased adhesion of blood cells in cerebral microvessels. Enhanced superoxide production is associated with cerebral ischemia/reperfusion and this response is exacerbated by hypercholesterolemia and diabetes mellitus.\(^{59}\) NADPH oxidase appears to be a major source of reactive oxygen species production in the post-ischemic cerebral microvasculature.\(^{59,68}\) Reactive oxygen species are known to trigger the expression of endothelial cell adhesion molecules that mediate leukocyte and platelet adhesion in post-ischemic cerebral venules. Superoxide may promote adhesion by inactivating nitric oxide, which is an endogenous inhibitor of leukocyte adherence.\(^{102}\) Nitric oxide donors such as nitroprusside inhibit leukocyte adhesion.\(^{103}\) The opposing actions of superoxide and nitric oxide suggest that the balance between these reactive species in the cerebral microcirculation may be a major determinant of the blood cell adhesion induced by ischemia–reperfusion.

CD40–CD40L signaling also appears to play a major role in modulating the recruitment of blood cells in brain microvessels following stroke.\(^{73}\) CD40 and CD40L knockout mice exhibit a smaller infarct volume and lower numbers of rolling and adherent leukocytes and platelets in post-ischemic venules. While the diminished blood cell recruitment that is observed in CD40−CD40L knockout mice may be a secondary consequence of the smaller infarct volume, a direct effect on blood cell interactions is also a likely explanation for the protection afforded by these mutations.

Notch-1 is a cell surface receptor that controls a broad number of cellular processes such as cell differentiation, proliferation and apoptosis. The Notch-1 signaling pathway also mediates angiogenesis, T-cell activation and proliferation, synaptic plasticity and cell fate decisions in nervous system.\(^{104,105}\) A recent study addressed the contribution of Notch-1 signaling to the inflammatory and tissue injury responses to cerebral ischemia–reperfusion.\(^{106}\) Mice treated with inhibitors of the Notch activating enzyme, gamma-secretase, as well as Notch antisense transgenic mice, exhibit an attenuated brain injury response to ischemia–reperfusion. This protective effect was accompanied by a blunted leukocyte/platelet recruitment response and a reduced number of intercellular adhesion molecule-1 and CD11b positive cells in ischemic brain.

**Clinical trials employing anti-adhesion strategies**

**Interleukin adhesion molecule-1 blockade (Enlimomab)**

The promising data obtained with anti-adhesion molecule strategies in different animal models of ischemic stroke lead to clinical trials that tested the efficacy of anti-intercellular adhesion molecule-1 antibodies in human stroke. The murine monoclonal anti-intercellular adhesion molecule-1, Enlimomab, was administered over 5 days during an observation period of 30 ± 10 days and initially found to be safe for use in humans with ischemic stroke.\(^{107}\) However, in a prospective, randomized, blinded phase III trial, administration of the anti-intercellular adhesion molecule-1 antibody within 6 hours after the onset of symptoms increased mortality, infarct volume and side effects (fever, infection) in patients with a worse functional outcome.\(^{108}\)

One explanation for the failure of anti-intercellular adhesion molecule-1 antibody is the possibility that murine antibody would trigger an immunologic response after administration. This hypothesis was further supported with animal studies showing that anti-intercellular adhesion molecule-1 antibodies can increase selectin expression and activates complement, which in turn activates neutrophils.\(^{109,110}\) In a subsequent in vivo study in rats, anti-intercellular adhesion molecule-1 antibodies administered to cerebral ischemia were found to sensitize the animals, resulting in larger infarcts, activation of complement system, and an upregulation of selectin.\(^{111}\) This experience with Enlimomab underscores the need to consider potentially harmful immune reactions to antibody administration and raises the possibility of improved success if humanized antibodies are employed.

**Macrophage-1 antigen blockade (Leukarest, UK-279,276)**

Leukarest (Hu23F2G) is a humanized, anti-macrophage-1 antigen (CD11b/18) antibody that had been shown to be effective in limiting infarct size and neuronal damage following transient focal cerebral ischemia in rabbits. In a phase III clinical study, Leukarest was tested in stroke patients; however, the trial was halted due to a failure to meet the predefined success criteria.\(^{113,114}\)

UK-279,276, a small recombinant glycoprotein that selectively binds to CD11b integrin of macrophage-1 antigen (CD11b/CD18), has been shown to be effective in reducing infarct size in repurposed animal models of stroke.\(^{100,115}\) In a phase 2 clinical study (acute stroke therapy by inhibition of neutrophils, ASTIN), ~900 patients with ischemic stroke were treated with UK-279,276 within 6 hours of stroke onset. In 204 patients, UK-279,276 was co administered with tissue plasminogen activators. No side effects were reported with UK-279,276. The trial was terminated due to no improvement in outcome of stroke patients with UK-279,276. However, post hoc analysis showed that there was a slight improvement in patients who received a
combination therapy (tissue plasminogen activator + UK-279,276), indicating a possible beneficial effect of UK-279,279 in reperfusion injury related to tissue plasminogen activator administration.

CONCLUSIONS
The data generated from several experiments using cell surface adhesion molecules as targets of stroke therapy are promising yet inadequate. An ideal therapeutic agent that targets adhesion molecules for stroke treatment should not elicit an immune response, narrow the therapeutic window and/or interfere with endogenous restorative processes. Unfortunately, existing reagents do not appear to fulfill all of these requirements.

The diversity of adhesion molecules used by different leukocyte populations to adhere to vascular endothelium warrants more attention as does the relative contributions of these different cell populations to ischemic brain injury. Monocytes and lymphocytes, for example, employ different adhesion glycoproteins from neutrophils to bind to vascular endothelium. While much effort has been devoted to defining the role of neutrophils to brain injury in ischemic stroke, emerging evidence suggests that mononuclear leukocytes play an equal or more important role in this injury process.

Adhesion molecule-directed therapies for stroke are based on the assumption that the recruitment of inflammatory/immune cells in post-ischemic brain is detrimental\(^1^,2^7\). However, some of these recruited cells may contribute to central nervous system regeneration following an ischemic stroke\(^4^,1^1\). For example, the results of a recent animal study indicate that activation of T-lymphocytes is neuroprotective in the setting of cerebral ischemia\(^1^,1^8\). Circulating lymphocytes, monocytes and/or other circulating cells might induce a restorative phenotype following an ischemic insult via mechanisms that involve cell-to-endothelium contact. In a preliminary report\(^1^,1^9\), we have recently described the ability of selectin-specific antibodies to block the recruitment of exogenous bone marrow stromal cells (stem cells) into post-ischemic cerebral venules. Since stem cells may have therapeutic benefit in ischemic stroke, interfering with their recruitment or the trafficking of other restorative cells to the injured brain region may impair or delay the resolution of ischemia-induced tissue injury.

A promising, but inadequately studied aspect of leukocyte adhesion-directed therapies is the combined use of anti-adhesion molecules with thrombolytic agents, such as tissue plasminogen activator. The available evidence suggests the combination of tissue plasminogen activator with a small molecule, non-immunogenic anti-adhesion agent may significantly extend the duration of the therapeutic window of tissue plasminogen activator and confer some protection against brain injury. With improved and more readily available imaging methods for screening stroke patients and the development of novel thrombolytic agents, a larger proportion of stroke patients are likely to receive thrombolytic therapy in the future. A likely consequence of the increased frequency of aggressive intervention in stroke patients is an increased incidence of reperfusion-related brain pathophysiology that may be more responsive to anti-adhesion therapy. This possibility justifies a continued effort to more clearly define the role of adhesion molecules in the pathophysiology of cerebral ischemia–reperfusion and to develop novel reagents that effectively interfere with the adhesion of leukocytes to vascular endothelium.

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