**Comments, Opinions, and Reviews**

Polymorphonuclear Leukocytes and Monocytes/Macrophages in the Pathogenesis of Cerebral Ischemia and Stroke

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**Background:** The extent to which polymorphonuclear leukocytes and monocytes/macrophages contribute to the pathobiology of cerebral ischemia and stroke is an issue of long-standing contradiction and controversy. Recent developments in the ability to selectively modify leukocyte adhesion with antiadhesion antibodies and the potential clinical application of this therapeutic approach have spurred a resurgence of experimental studies examining the role of leukocytes in cerebral ischemia and stroke.

**Summary of Review:** We review studies examining leukocyte accumulation, initiation of thrombosis, and exacerbation of ischemic brain injury in stroke, and we examine other proposed contributions of leukocytes to cerebrovascular pathophysiology.

**Conclusions:** The importance of specific characteristics of a given ischemia model and of underlying stroke risk factors in determining the degree of leukocyte involvement and effectiveness of therapies directed against these cells is discussed. (Stroke 1992;23:1367-1379)

**KEY WORDS** • cerebral ischemia • leukocytes • neutrophils

Since the late 1960s, leukocytes, both polymorphonuclear leukocytes (PMNL) and monocytes/macrophages (but not lymphocytes), have been implicated in the pathogenesis of cerebral ischemia and stroke. Their actual role in these processes, however, remains undefined. Work in the 1970s demonstrated delayed accumulation of PMNL and monocytes after both clinical and experimental stroke. Such findings were interpreted as support for the then-popular notion that leukocytes contributed only to phagocyte-mediated tissue debridement and scar formation that occurred days to weeks after stroke.1,2 Epidemiological studies performed in the early 1980s suggested that leukocytes, particularly PMNL, contributed to the initiation of stroke, through either a rheological effect or possibly participation in thrombosis.3 Soon afterward, experimental work with cerebral air embolism in dogs demonstrated granulocyte and platelet accumulation in areas of low cerebral blood flow (CBF) during the early postischemic reperfusion period.4-6 Subsequent studies using PMNL depletion demonstrated a contribution of leukocytes to postischemic hypoperfusion and neuronal dysfunction.7 During the late 1980s, other sporadic reports supported a role of leukocytes in the pathogenesis of ischemic brain injury.8-14 These reports were largely overshadowed by the volumes of work and resultant fundamental recent advances in mechanistic understanding of some aspects of the pathobiology of ischemic neuronal death. The apparent lack of a vascular distribution to the selectively vulnerable zones (such as the CA1) where delayed neuronal death is observed after transient ischemic insults shifted the focus away from microcirculatory (and thus leukocyte) contributions to ischemic brain injury. This shift in focus extended beyond the transient global ischemia models to influence studies of focal ischemia and stroke as well.

Recently, tremendous advances have been made in our understanding of the mechanisms of leukocyte adhesion and migration. Most notably, PMNL and monocyte adhesion has been shown to occur via highly specific receptor–ligand interactions with endothelium and the extravascular matrix. Three families of adhesion molecules mediate these functions: 1) the integrin family, 2) the selectins, and 3) the immunoglobulin superfamily.15,16 Key determinants for leukocyte adhesion to endothelium or the extracellular matrix are proteins on the leukocyte cell surface known as integrins. The integrins contain CD11 and CD18 noncovalently associated subunits. The most important integrins are LFA-1 and Mac-1, both of which contain a common CD18 protein as their β subunit.15 The counterreceptors for these integrins are the intercellular adhesion molecules (ICAM-1 and ICAM-2) from the immunoglobulin superfamily.15,17 ICAMs are expressed on a wide variety of cells including endothelium, and their expression is modulated by a wide spectrum of inflammatory mediators (such as interleukin-1 [IL-1]) that appear to promote leukocyte adhesion via effects on these receptors.15,17 The brain has traditionally been deemed as being immunologically priv-
ilegled, and limited investigation of leukocyte adhesion and migration across the cerebral microvascular endothelium has been performed; nevertheless, brain endothelial cells do express ICAM-1 and ICAM-2. The third class of adhesion receptors are the selectins, which are glycoproteins that modulate leukocyte endothelial adhesion via interaction of carbohydrate residues. The best-described selectin is the endothelial–leukocyte adhesion molecule (ELAM-1) expressed on endothelial cells. The activity and number of molecules of this selectin on the cell surface are again regulated by inflammatory mediators. Unlike the ICAMs, ELAM-1 does not interact with the CD11/CD18 integrin moiety but, rather, binds to another selectin moiety on the leukocyte surface—the sialyl-Lewis carbohydrate residue. Thus, CD18-dependent and -independent mechanisms for PMNL and monocyte adhesion exist. The contribution of selectins to leukocyte adhesion in the cerebral microcirculation has not been defined; however, lymphocytes have been shown to attach to myelin via L-selectin. Although numerous additional endothelial–leukocyte adhesion molecules exist, approximately 80% of PMNL–endothelial adhesion (stimulated by IL-1) is blocked by the simultaneous inhibition of ICAM-1 and ELAM-1 receptors.

As a consequence of the aforementioned breakthroughs in leukocyte biology, selective and relatively nontoxic antibodies inhibiting PMNL and monocyte adhesion to vascular endothelium (with the resultant inability of leukocytes to migrate into tissue in the inflammatory process) have begun to be applied to numerous disease states exhibiting hypoperfusion or ischemia, such as myocardial ischemia and infarction, hemorrhagic shock, and, most recently, spinal cord ischemia. In addition, there have been significant advances in the ability to modify oxygen radical-induced tissue injury, and the contribution of leukocytes to this mode of injury may also be important in cerebral ischemia/reperfusion. Improved techniques for the detection of leukocytes in brain tissue, such as the tissue myeloperoxidase (MPO) assay, and immunocytochemical techniques have contributed to additional work in this area. Also, recent studies have demonstrated a pivotal role for monocytes/macrophages in the response of numerous organs and tissues, particularly the lung and liver, to disease processes involving ischemia or shock states. Monocyte/macrophage involvement after cerebral injury may similarly occur. This idea has rejuvenated interest and stimulated a reappraisal of the possible role of leukocytes in the pathogenesis of cerebral ischemia and stroke.

In this review, we examine the evidence surrounding five key questions: 1) Do PMNL and monocytes accumulate in brain after cerebral ischemia, and what factors influence the time course and extent of accumulation? 2) What is the contribution of such leukocytes to the ischemic and postischemic injury processes in the brain and to the initiation of stroke? 3) What is the relation between the various experimental models of cerebral ischemia and the effects of antileukocyte interventions? 4) What is the relation between stroke risk factors (chronic underlying microcirculatory disturbances from atherosclerosis, hypertension, diabetes, or advancing age) and leukocyte involvement in cerebral ischemia? and 5) Do PMNL or monocytes contribute any beneficial effects after ischemia in the brain? Although we cannot provide definitive answers to these questions, we summarize and discuss recent advances in this area and suggest potentially important areas for future work.

Leukocyte Accumulation in Cerebral Ischemia and Stroke

Polymorphonuclear Leukocytes

The accumulation of PMNL after stroke in humans and after permanent middle cerebral artery (MCA) occlusion in animal models was examined in classical histopathologic studies of these processes. Although PMNL accumulation is observed in the "reactive zone," described as a rim of tissue located at the periphery of the central infarcted core, and accumulation is maximal at 48–72 hours. By 7 days, some PMNL have even entered the core of the infarct. In these studies, PMNL accumulation was described as having occurred during a delayed "inflammatory stage" of cerebral ischemia and was suggested as contributing to phagocytosis of degradation products of nervous tissue or erythrocytes, associated with the initiation of scar formation or of "a healing process." In most histopathologic studies of experimental cerebral infarction, leukocyte accumulation is assessed even though tissue specimens were perfusion-fixed. Such studies may have underestimated the intravascular component of leukocyte involvement.

In 1972, Sörnäis et al. presented one of the first studies specifically focusing on leukocyte accumulation after stroke. Cytological examination of serial cerebrospinal fluid (CSF) samples from 125 patients revealed that the involvement of leukocytes, particularly PMNL, depended on the type of infarction, being greatest in hemorrhagic and embolic infarcts. Pale infarcts with no collateral blood flow showed the least CSF pleocytosis and hemorrhagic infarcts with collateral flow and embolic infarcts showed moderate CSF pleocytosis, whereas intracerebral lobar hematoma was associated with a marked PMNL pleocytosis. As in the histopathologic studies, peak PMNL pleocytosis in CSF occurred 48–72 hours after the onset of symptoms. Consequently, hemorrhage was thought to be an important contributor to PMNL participation in cerebral ischemia. Supporting delayed PMNL accumulation after clinical stroke, Pozzilli et al. used autologous indium-111–labeled leukocytes (a mixture of PMNL and mononuclear cells) and gamma camera imaging in patients and again showed delayed leukocyte accumulation (2–14 days after the onset of stroke symptoms).

In 1986, using indium-111–labeled, autologous PMNL in a model of air embolism–induced cerebral ischemia in dogs, Hallenbeck and coworkers demonstrated that PMNL accumulate progressively during the first 4 hours of reperfusion. In this model, PMNL accumulation was intense in brain regions with low blood flow and correlated significantly with the severity of ischemia defined by the extent of cortical somatosensory evoked potential (CSEP) amplitude reduction after embolization. Although the exact anatomic location of PMNL was not determined, the rapidity of accumulation suggested that a large component represented...
PMNL–endothelial adhesion, PMNL aggregate formation in the cerebral microcirculation, or both. This work was the first demonstration of early PMNL accumulation in cerebral ischemia. Previous work in myocardial ischemia had already shown early and progressive PMNL accumulation after ischemia. A criticism of these air embolism studies has been that it is unclear how these results relate to a vascular occlusion model or to clinical thrombotic stroke. Air embolism is a unique form of ischemic insult that, in addition to parenchymal ischemia, produces a primary endothelial insult and leukocyte involvement at the blood–bubble interface. This would probably lead to more (and possibly more rapid) PMNL accumulation than that observed in a vascular ligation model.

Similar studies demonstrating early PMNL accumulation during the first 8 hours after cerebral trauma do not resolve this controversy. Although it is associated with ischemia, trauma is also accompanied by direct disruption of vascular endothelium and with hemorrhage, both of which could contribute to PMNL accumulation independent of ischemia. Recently, del Zoppo et al used a primate model of 3 hours of MCA occlusion and 1 hour of reperfusion and demonstrated that approximately 8% of brain microvessels (≤10 μm inner lumen diameter) were completely or partially occluded by PMNL in the hypoperfused regions of the MCA distribution. In addition, no PMNL were identified in the perivascular parenchyma, suggesting that PMNL participation was intravascular during this early period of reperfusion. Barone et al recently developed a MPO assay modified for use in brain tissue. MPO is an enzyme that is found exclusively in PMNL and monocytes but not lymphocytes. Brain tissue MPO activity and PMNL infiltration assessed histologically had increased almost sixfold at 24 hours after MCA occlusion in spontaneously hypertensive rats (SHR). Although earlier time points were not specifically addressed, the magnitude of the accumulation at 24 hours is consistent with progressive PMNL accumulation in a more traditional vascular occlusion model of focal cerebral ischemia. Barone et al used this same ischemia model (MCA occlusion in SHR) and MPO technique to study the impact of reperfusion on PMNL accumulation. They demonstrated that PMNL accumulation in the ipsilateral cortex was increased about threefold after 160 minutes of ischemia plus 24 hours of reperfusion compared with that following permanent MCA occlusion for 24 hours. These studies begin to address comprehensively the question of PMNL accumulation in a traditional vascular occlusion model of focal cerebral ischemia. It may be important, particularly in the work of Barone et al, that PMNL accumulation was demonstrated specifically in SHR. The potential critical influence of stroke risk factors on leukocyte involvement is discussed later.

In addition to these studies in focal ischemia, Anderson et al recently demonstrated early PMNL accumulation after incomplete global cerebral ischemia produced by bilateral carotid artery occlusion plus hypotension in dogs. Histology revealed PMNL accumulation in the microcirculation and parenchyma at 3 hours of reperfusion, and PMNL accumulation correlated significantly with severity of the ischemic insult as quantified by phosphorus magnetic resonance spectroscopy. However, ischemia for up to 40 minutes produced little or no PMNL infiltration, and accumulation occurred only after a very prolonged insult (400 minutes). Practically speaking, these data suggest that early PMNL infiltration is more likely to occur in focal ischemia, which is often prolonged and likely to produce severe tissue injury. In contrast, PMNL accumulation after global ischemic insults is unlikely to be clinically important if 40 minutes of ischemia really produces little accumulation.

Monocytes

Monocyte accumulation after experimental focal cerebral ischemia in primates and after stroke in humans was also evaluated in the previously described studies of brain histopathology and CSF analysis. In contrast to the maximal PMNL accumulation at 24–72 hours after permanent MCA occlusion in primates, Garcia and Kamijo described large numbers of monocytes and macrophages at 7 and 16 days after occlusion. Like PMNL, these mononuclear cells were again located in the "reactive zone" at the boundary of the infarct. In contrast, Sönnsä et al demonstrated that peak monocyte and macrophage accumulation in CSF occurred somewhat earlier—between 3 and 7 days after stroke in humans. Pleocytosis was again noted to be maximal after hemorrhagic infarction. Recently, extensive investigation of monocyte and macrophage accumulation after central nervous system (CNS) injury has been focused on models of spinal cord ischemia and penetrating cerebral trauma. Giulian and Robertson used two immunohistochemical markers of mononuclear phagocyte infiltration (nonspecific esterase and the uptake of Di-I-acetylated low density lipoprotein [Di-I-Ac-LDL]) and found mononuclear phagocyte accumulation beginning at 16 hours and peaking at 48 hours after spinal cord ischemia and reperfusion in rabbits. The mononuclear phagocytes identified in this investigation represented both monocyte-derived macrophages and ameboid microglia because both cell types contain nonspecific esterase activity and the Di-I-Ac-LDL receptor. These two cell types were differentiated by examining peroxidase activity (positive only in monocyte-derived macrophages) and morphology. Studies of penetrating cerebral trauma in rats suggested both intrinsic and extrinsic mononuclear phagocytic inflammatory responses, with monocyte-derived macrophages appearing adjacent to the wound site as early as 5 hours after trauma and brain-derived ameboid microglia proliferating at regions more distant from the immediate injury site. In addition, astrocytes may also be capable of transforming into macrophage-like cells. The time course and magnitude of postischemic monocyte accumulation in the brain have not been specifically addressed in stroke models.

In addition to parenchymal infiltration during postischemic reperfusion, monocyte adhesion to the endothelium and accumulation in the subendothelial space as a consequence of preexisting atherosclerosis may be important to initiating or propagating thrombosis in stroke. A 50-fold increase in vascular adhesion and infiltration of monocytes is present during the process of atherosclerotic plaque formation in rats maintained on an atherogenic diet. Simultaneous subendothelial macrophage infiltration is observed in human atherosclerotic
lesions.58–59 In light of the importance of atherosclerosis as a stroke risk factor, chronic perivascular monocyte accumulation before stroke and intravascular and parenchymal monocyte accumulation after ischemia may both play significant pathophysiological roles.

Some future directions for the study of leukocyte accumulation in stroke are suggested by voids in the current literature. The time course and extent of PMNL and monocyte accumulation and their interactions have not been determined simultaneously in a given model. In addition, leukocyte accumulation has not been quantified by identical techniques in different experimental ischemia models in a single laboratory. Vascular occlusion models and models with a true thrombotic event initiating the ischemia should be compared. A model such as the photochemically initiated thrombosis described by Watson et al59 could be compared with vascular occlusion resulting in a similar infarct volume in the same species. The impact of thrombolysis-mediated restoration of reperfusion on leukocyte accumulation could also be addressed in such a model. The specific impact of individual and combined stroke risk factors on the accumulation of leukocytes also remains to be addressed. Recent studies suggest that the underlying level of leukocyte activation before ischemia or shock is extremely important to outcome and is quite variable even in controlled experimental animal models.60 The importance of the underlying level of leukocyte activity to outcome after cerebral ischemia remains to be investigated. Finally, further investigation of the time course and extent of leukocyte accumulation in clinical stroke is needed, particularly in concert with the investigation of treatments directed against leukocyte accumulation. Recently, Benavides et al61 demonstrated that macrophage infiltration/proliferation can be quantified in human brain (ex vivo) using a radiolabeled ligand for the peripheral benzodiazepine binding site (ω3). The availability of position-specific ligands for the ω3 receptor, or the development of other novel ligands, may allow in vivo quantification of macrophage infiltration after stroke.

**Contribution of Polymorphonuclear Leukocytes and Monocytes/Macrophages to Injury Process**

The postulated effects of PMNL and monocytes to the pathogenesis of cerebral ischemia and stroke include 1) limitation of CBF by vessel plugging or vasoconstrictive mediator release, 2) exacerbation of blood-brain barrier or parenchymal injury via hydrolytic enzyme release, lipid mediator production, or oxygen radical production, and 3) initiation of thrombosis. Clinical studies support a role for activated leukocytes in the injury process and in the initiation of stroke. Prentice et al62 reported that biannual leukocyte counts of >10,000/μl increased the incidence of stroke by about twofold. This effect seemed to be explained by an elevated absolute count of PMNL but not monocytes. Similarly, Pozzilli et al63 found that an elevated peripheral leukocyte count at 72 hours after stroke was significantly associated with larger infarct size, impaired level of consciousness, and poor clinical outcome. An association between subsequent stroke risk and an elevated leukocyte count has also been shown for patients with transient ischemic attack.63 It is unclear, however, if these associations are related to a specific contribution of leukocytes to the injury process, the initiation of stroke, or both. Alternatively, much (but not all) of the association between an elevated PMNL count and stroke risk can be explained by covariance between tobacco smoking and the PMNL count. Similarly, the correlation between an elevated leukocyte count and poor prognosis after stroke may relate to factors other than direct leukocyte participation in cerebral injury, factors such as greater stress-induced leukocytosis or likelihood of systemic infection in patients with large infarcts.64 Although extensive direct and indirect support for a role of leukocytes exists in numerous pathological states including ischemia and reperfusion outside of the CNS,65–67 the quantitative contribution of leukocytes to ischemic brain injury and stroke remains to be defined.

Supporting an important role for vascular obstruction by leukocytes in the cerebral circulation, several laboratories have demonstrated that leukocyte filterability is decreased in humans after stroke, both during the first 24 hours13 and for at least 3–4 months.11 Recently, Grau and coworkers73 showed that decreased leukocyte filterability after stroke is produced by increased PMNL adhesion to the endothelial components laminin and fibronectin and not by decreased deformability. The authors suggested that circulating PMNL exhibit abnormal adhesive properties in patients with stroke and that this may further impair microvascular blood flow, particularly where flow is sluggish and where rheology favors adhesion.64

Supporting a vasoconstrictor effect of activated leukocytes, Migue et al74 combined human leukocytes with femoral artery rings from normal or atherosclerotic monkeys in an elegant in vitro study and showed that thrombin- or complement-activated leukocytes produce endothelium-independent vasoconstriction (about 25% of maximal constriction). Stimulation of either PMNL or mononuclear cells (a mixture of monocytes and lymphocytes) produced a similar response in normal arteries. The vasoconstrictor response to activated mononuclear cells (but not PMNL) was markedly greater in atherosclerotic arteries. Vasocostriction was mediated by hydroxyl radical (in PMNL only) and by an unidentified mediator of <1,000 molecular weight (in PMNL and mononuclear cells). Cyclooxygenase and lipoxygenase products did not appear to be involved.74 Monocyte/macrophage elaboration of the potent vasoconstrictors endothelin 1 and endothelin 3 may be important in this setting.75 In contrast, intracoronary infusion of formyl-methionyl-leucyl-phenylalanine (fMLP) in normal rabbits produced profound coronary constriction and ST segment depression and dysrhythmias,72 and cyclooxygenase and lipoxygenase products were implicated in this process.76 It is not clear if these vasoconstrictor effects would be identical in large cerebral vessels or in the cerebral microcirculation.

**Effect of Activated Leukocytes on Cerebral Blood Flow**

PMNL and monocytes can be activated in vivo or in vitro by a variety of stimulants including both the croton oil derivative phorbol myristate acetate (PMA) and the chemotactic tripeptide fMLP. These drugs have been used to investigate the vascular effects of PMNL and monocyte activation. PMA simultaneously activates leu-
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vasoconstriction. This has been demonstrated in the brain, where a transient vasodilation accompanies acute brain ischemia or after cerebral infarction, and the impact of stroke risk factors on this effect has not been determined.

Effect of Activated Leukocytes on Blood–Brain Barrier Permeability and on Brain Parenchyma

Independent of their specific effects on blood flow, activated leukocytes are important mediators of vascular and parenchymal injury outside of the CNS. Many investigations have demonstrated important roles for both PMNL and monocytes in numerous models, including burns, cutaneous trauma, acute lung injury, and sepsis. Leukocytes contribute to the increased vascular permeability evoked by the intradermal injection of inflammatory mediators such as leukotriene B₄, platelet-activating factor, activated complement, and endothelin (but not histamine).75,87,71 Although the role of leukocytes in the pulmonary vasconstrictor response in sepsis and ARDS is variable,77,92 mounting evidence suggests that PMNL, monocytes/macrophages, or both may increase pulmonary vascular permeability during acute lung injury.77–79,92 Oxygen radicals, lipid-derived mediators, and proteases have been implicated in this process. The specific contribution of activated leukocytes to increased vascular permeability in the CNS in trauma, ischemia, and infection is controversial. Scoettle et al47 demonstrated a high correlation between PMNL accumulation and edema after percutaneous cerebral trauma in rats, which is similar to that observed in cutaneous trauma.58 However, PMNL depletion produced by vinblastine sulfate before trauma did not attenuate the development of edema in the percutaneous cerebral trauma model.13 Similarly, PMNL depletion with anti-PMNL antibody after cortical freezing in rats actually increased the amount of edema measured at 24 hours after injury.84 In other models, however, global immunosuppression with total body irradiation attenuated cerebral edema after either MCA occlusion or intracranial hemorrhage in rats,92 and PMNL depletion with anti-PMNL antiserum blunted the rise in intracranial pressure (ICP) after experimental thromboembolic stroke in rabbits.96 In the latter study, it is unclear if the decrease in ICP resulted from a specific effect on the blood–brain barrier or from improved CBF with a resultant decrease in infarct size. Brain edema was not reduced by prior PMNL depletion in an experimental model of meningitis in rabbits.97 Prior PMNL depletion did not attenuate the increase in blood–brain barrier permeability produced by topical application of arachidonic acid to the cerebral cortex of rats.98

As is discussed later, several (but not all) studies using leukocyte depletion in experimental cerebral ischemia models support the proposed contribution of leukocytes to CBF reduction in cerebral ischemia and stroke. Also supporting this hypothesis, studies of experimental myocardial infarction in rabbits have shown PMNL infiltration at 1 day after ischemia and monocyte infiltration at 4 days.76 Infusion of fMLP to further activate these accumulated leukocytes pharmacologically at either 1 or 4 days resulted in coronary vasoconstriction and coincident release of large amounts of eicosanoids (thromboxane B₂, prostaglandin E₂, and leukotrienes B₄, C₄, and D₄). The effect on CBF (or other outcome variables) of pharmacological activation of accumulated leukocytes is undetermined during cerebral ischemia or after cerebral infarction, and the impact of stroke risk factors on this effect has not been determined.

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Despite the importance of leukocytes to the increase in vascular permeability in the inflammatory process,99 the effect of activation of endogenous leukocytes in the cerebral circulation on blood–brain barrier permeability has only recently begun to be examined, and data are conflicting. Faustmann and Dermietzel100 demonstrated that PMNL migrate across the blood–brain barrier after complement activation with topical application of α-bungarotoxin in cats. Although the location of PMNL migration (the postcapillary venule) is similar to that observed in the extracerebral circulation, the mechanism of PMNL migration across the blood–brain barrier appeared unique. In the extracerebral circulation PMNL migrate through interendothelial junctions. In
contrast, migration across the blood–brain barrier in this acute inflammatory condition occurred via a transendothelial route, with resultant disruption of the barrier. In contrast, movement of PMNL across a blood–brain barrier system of cultured bovine brain microvessel endothelial cells (in which tight junctions are present) occurred without disruption of the barrier. The specific effects of PMNL or monocyte depletion on blood–brain barrier permeability after cerebral ischemia remain to be studied. Both are important topics for future research.

Leukocyte-mediated injury to neurons and glia is also possible in cerebral ischemia and stroke. Activated human PMNL kill rat alveolar epithelial cells in culture. This cytotoxic effect appears not to involve oxidative processes but does depend on intimate PMNL–target cell contact and protease release. The effect of activated leukocytes on brain parenchyma has been studied very little. In brain homogenates, PMNL augment the lipid peroxidation induced by added iron. Although the specific effects of activated PMNL on neurons or glia have not been investigated, Giulian et al demonstrated that human immunodeficiency virus–infected human macrophages and microglia produce neurototoxic factors that kill neuronal cells in culture. These neurotoxins were heat-stable, protease-resistant molecules that act via N-methyl-D-aspartate receptors. This suggests that unique or additional mechanisms of leukocyte-mediated injury may operate in the CNS.

**Initiation of Thrombosis**

Although acute pharmacological activation of leukocytes does not initiate ischemia or even hyperperfusion in the cerebral circulation of normal experimental animals, several lines of evidence suggest that PMNL and monocytes can contribute to the initiation of stroke. Histopathologic examination of coronary or cerebral thrombosis revealed leukocyte–platelet aggregates similar to the time course of PMNL and platelet accumulation in areas of low CBF observed by Hallenbeck et al and Obrenovitch and Hallenbeck after cerebral air embolism. Numerous leukocyte–platelet interactions have been reported and platelets, PMNL, and monocytes have many activators and products in common.

Syrränen et al showed that infection (with fever) was found during the month before the development of stroke in young patients significantly more often than in control subjects matched for age and sex. In addition, the same group of researchers found increased antibody titers against bacteria (streptococci, staphylococci, and enteric Gram-negative bacteria) in 34% of young (<45 years of age) adults with cerebral infarction but in only 9% of the control subjects. When one or more stroke risk factors are present, active infection with marked leukocytosis and leukocyte activation may not be necessary to initiate stroke; rather, an interaction between inflammation and the coagulation system may activate the coagulation cascade and trigger a local thrombotic event. Segments of the extracranial and intracranial vasculature may undergo subtle activation of their endothelium by the stroke risk factors, leading to augmented expression of receptors for monocytes. These cells can then undergo transendothelial migration to occupy a subendothelial perivascular position. Through the periodic release of cytokins, such as tumor necrosis factor-α, these cells may convert the endothelium from an anticoagulant to a procoagulant surface and predispose to local thrombosis or hemorrhage, perhaps through a process analogous to the local Shwartzman reaction. A brain stem infarct in rats can be produced by such a process, namely, a modified local Shwartzman reaction. In this process, an increased frequency of brain stem thrombohemorrhagic lesions is produced by the intracisternal injection of endotoxin into rats with known stroke risk factors (hypertension, diabetes, advancing age) compared with risk factor–free control rats.

In addition to an interaction between leukocytes and the coagulation system, hemodynamic effects such as an enhanced vasoconstrictor response to leukocyte activators in the cerebral circulation and compromised CBF from carotid stenosis with slowing of leukocyte passage may also contribute to the initiation of stroke in the appropriate setting.

Despite the experimental studies showing a relation between PMNL and platelets in vascular thrombosis and despite the apparent relation among infection, circulating PMNL, and clinical stroke, the precise role of leukocytes in the initiation of thrombotic stroke remains undefined. Specific studies of the effect of PMNL depletion on infarct size and functional outcome in an experimental model of cerebral thrombosis, rather than vascular occlusion, would begin to address this question. This issue will be discussed further in the section on stroke risk factors.

**Antileukocyte Interventions in Experimental Models of Cerebral Ischemia and Stroke**

Therapy directed specifically at attenuating leukocyte (PMNL or monocyte) accumulation or function in experimental models of cerebral ischemia have produced apparently conflicting results. Careful inspection of the experimental models involved and the presence or absence of underlying stroke risk factors may, however, help explain the variable effects of modifying leukocyte accumulation or function.

**Embolic Ischemia**

The ischemia model in which beneficial effects of therapies directed against leukocytes have been most reproducible is experimental cerebral embolism. Dutka et al described a beneficial effect of mechlor-ethamine-induced depletion of PMNL (but not mononuclear cell) achieved after air embolism–induced cerebral ischemia in dogs. In this model, PMNL depletion improved both CBF and CSEP amplitude at 1 hour after ischemia. PMNL depletion also seemed to increase the requirement for air administration during the induction of ischemia in this model, in which the amount of air is governed by the degree of CSEP amplitude suppression produced. Thus, intraschismic and postschismic mechanisms producing a beneficial effect of PMNL depletion might be operating. A weakness of this study is the extremely brief follow-up (1 hour) and the relatively toxic means of depleting PMNL. Recently, Helps and Gorman performed a similar study in rabbits and demonstrated that mechlor-ethamine-induced leukocyte depletion completely eliminated postschismic hypoperfusion and CSEP amplitu-
The authors concluded that leukocytes are essential to the postischemic deterioration of CBF and neuronal function that occurs after cerebral air embolism.

Supporting the hypothesis that PMNL are detrimental in cerebral ischemia produced by thromboembolism, Bednar et al demonstrated a beneficial effect of antineutrophil antiserum administered 20 minutes before autologous clot embolization and limited hypotension (45 minutes of a mean arterial blood pressure of 30 mm Hg) in rabbits. CBF, infarct size, and ICP were all improved at 4 hours after embolization by pretreatment with antineutrophil antibody but not antiplatelet antibody. In contrast, Clark et al studied the effect of pretreatment with antileukocyte adhesion antibody on neurological recovery after administering multiple microsphere cerebral emboli in rabbits. Antibody to the CD18 subunit common to the integrin adhesion receptors on leukocytes was used. This antibody inhibits integrin-dependent endothelial adhesion and, thus, adhesion-dependent leukocyte function of both PMNL and monocytes but does not decrease the circulating number of these cells. Contrasting with the aforementioned studies in embolism models, no beneficial effect on neurological outcome at 18 hours after the insult was observed. However, the embolism technique used in this study produced a model of occlusion without reperfusion, and a relatively crude neurological scoring system was used as the sole outcome variable. On balance, leukocytes appear to play a potentially important role in cerebral embolism, but additional laboratory studies are needed, particularly studies with careful evaluation of long-term functional outcome.

**Global Cerebral Ischemia**

Grotta et al studied the effect of PMNL depletion (anti-PMNL antibody resulting in 95% reduction in number of circulating PMNL and 50% reduction in mononuclear cells) on CBF after incomplete forebrain ischemia in rats. Incomplete forebrain ischemia was produced using bilateral common carotid artery occlusion plus controlled hemorrhage to a mean arterial blood pressure of 50 mm Hg for 15 minutes. Induced before ischemia, leukocyte depletion produced a significant attenuation of the postischemic hyperperfusion that occurs at 1 hour of reperfusion in this model. Treatment with anti-PMNL antibody beginning at 2 minutes of recirculation, however, did not improve CBF at 1 hour after ischemia despite a marked reduction in the number of circulating PMNL by 15 minutes of reperfusion. These data suggest the possibility of a detrimental effect of leukocytes during ischemia or very early reperfusion in this model. A beneficial effect of simultaneous leukocyte and platelet depletion on CSEP amplitude during ischemia and early reperfusion was also shown by Vasthare et al in this model. The use of an incomplete ischemia model in these studies allows for a potentially critical improvement in CBF during ischemia via either decreased sludging of leukocytes in the microcirculation or reduced release of leukocyte-derived vasoconstrictors.

Although a beneficial effect of prior PMNL depletion on CBF after global ischemia has thus been demonstrated in these two studies, Aspey et al examined the effect of leukocyte depletion (induced with cyclophosphamide) on the occurrence of the no-reflow phenomenon observed after 30 minutes of bilateral carotid artery occlusion in gerbils. An 85% reduction in the circulating leukocyte count did not decrease the incidence or severity of reflow failure assessed by carbon black perfusion at 10 minutes of reperfusion. In a more extensive study, Schott et al found no beneficial effect of anti-PMNL antibody treatment on neurological recovery during the first 24 hours after a 10-minute cardiac arrest in dogs, suggesting that PMNL contribute little to clinically relevant global cerebral ischemia, namely, cardiac arrest. Indeed, PMNL depletion increased mortality in this model. These data are somewhat questionable, however, because of the high mortality rate observed after a 10-minute cardiac arrest even without PMNL depletion (42%) and the relatively incomplete level of PMNL depletion achieved. The lack of a vascular distribution to selectively vulnerable zones, the importance of excitotoxic neuronal injury to the pathobiology of global ischemic insults, the inability to treat either before or during the initial extracorporeal resuscitation in the clinical setting, and the lack of PMNL accumulation unless extremely prolonged ischemia is involved would make this an unlikely area for important effects of antileukocyte therapy. Similarly, PMNL accumulation is not observed in zones of neuronal necrosis produced by local injection of the excitatory amino acid kainic acid. Nevertheless, therapies targeted at improving early postischemic or intras ischemic CBF probably deserve further study in global ischemia models. Examination of the effect of leukocyte depletion or antileukocyte adhesion treatment on CBF during cardiopulmonary resuscitation might be worthy of future study. Recently interest has been directed to the use of extracorporeal support immediately after cardiac arrest. This would allow for prompt leukocyte removal via filtering systems or inhibition of leukocyte function (without hemodynamic instability) and probably merits further laboratory investigation.

**Focal Cerebral Ischemia**

Takeshima et al studied the effect of antileukocyte adhesion antibody on recovery after MCA occlusion in cats, again using a monoclonal antibody (60.3) directed against the CD18 adhesion moiety on PMNL and monocytes. In this study 90 minutes of cerebral ischemia produced by bilateral common carotid artery occlusion and unilateral MCA occlusion was followed by 180 minutes of reperfusion. Antileukocyte adhesion antibody was given between 40 and 50 minutes of ischemia. No effect of treatment was observed on the recovery of CBF, CSEP amplitude, and infarct volume as quantified by tetrazolium staining. However, it is unclear if any therapeutic strategy applied after 40 minutes of ischemia could reduce infarct size in this model. In addition, the degree of leukocyte accumulation in the brain was not evaluated, nor was inhibition of postischemic leukocyte accumulation by anti-CD18 therapy shown in this model. As previously discussed, CD18-dependent and -independent mechanisms of leukocyte adhesion to vascular endothelium exist, and the relative importance of these mechanisms to leukocyte accumulation in the brain and participation in stroke have not been adequately addressed.
Nevertheless, this was a well-conducted outcome study in which multiple end points were carefully evaluated in a relatively standard model. It certainly suggests that leukocyte participation mediated by the integrin adhesion receptor system plays a major role in this setting, that is, cerebral vascular occlusion in an otherwise normal animal. This result in the cerebral circulation is in contrast to those observed in other vascular beds (such as the coronary circulation) where anti-CD18 therapy is protective in ischemia/reperfusion. 

Spinal Cord Ischemia

Some of the most dramatic beneficial effects of therapies directed against leukocyte accumulation and function have been seen in models of spinal cord ischemia. In 1983, Means and Anderson reported early PMNL infiltration after spinal cord compression in cats. Large numbers of PMNL were seen by 8 hours after the insult, and neuronophagia by PMNL was observed at 8–24 hours. Using a rabbit model of spinal cord ischemia produced by aortic occlusion below the renal arteries, Clark et al demonstrated a significant reduction in neurological dysfunction with anti-CD18 antibody treatment. Again, this treatment inhibits adhesion of both PMNL and monocytes. In this study, treatment was initiated 30 minutes before ischemia, and neurological outcome was assessed 18 hours after ischemia. In addition to the improvement in neurological function with treatment, a marked reduction in leukocyte accumulation was noted histologically in treated animals. Lindberg et al recently reported a beneficial effect of anti-CD18 antibody treatment on motor function in the same model. In their study, benefit was seen with treatment initiated as late as 30 minutes after the onset of reperfusion.

In a similar model of spinal cord ischemia in rabbits, Giulian and Robertson demonstrated that treatment directed against mononuclear phagocyte accumulation and function improves neurological function at 3 days after ischemia. Mononuclear leukocyte accumulation and function was most effectively inhibited both in vitro and ex vivo with the combination of chloroquine and colchicine. This treatment markedly improved motor function at 3 days compared with either no treatment or dexamethasone treatment. Surprisingly, dexamethasone treatment attenuated neither mononuclear phagocyte accumulation nor ex vivo mononuclear phagocytic activity.

The consistent effectiveness of antileukocyte therapies observed in this model may relate in part to the importance for outcome of delayed deterioration of motor function. In this model, rabbits exhibit an initial paraplegia that is resolved by 6 hours after ischemia and is followed by a secondary deterioration during the next 20 hours. This deterioration is either accompanied or produced by an inflammatory process because inflammatory markers such as cicosanoids, edema, and leukocyte infiltration increase concurrently. That small increases in the number of surviving motor units in the cord can result in easily detectable differences in motor function may also be important to demonstrating benefits of treatment. Species differences may also be important. All of these highly successful studies in spinal cord ischemia were conducted in rabbits, which exhibit a robust inflammatory response to various stimulants (i.e., the local Shwartzman reaction) relative to that in other species such as rats.

Clinical Studies

No specific intervention to modify leukocyte involvement in clinical stroke or cerebral ischemia has been investigated. Corticosteroid treatment, although shown to attenuate leukocyte involvement in most classical experimental models of acute inflammation, has, as described, failed to attenuate leukocyte involvement in models of CNS ischemia. Results of corticosteroid treatment in clinical stroke and in cardiac arrest and resuscitation have suggested no beneficial effect. Other factors, such as harmful effects of corticosteroid treatment on the brain, may also operate in this setting. Numerous drugs used in novel therapeutic approaches to cerebral ischemia have an important impact on the inflammatory process, and their effects on leukocyte function must be assessed. For example, recent success with the antiplatelet agent ticlopidine in clinical stroke may involve more than just inhibitory effects on platelet function. Ciuflietti et al recently demonstrated that ticlopidine significantly improved leukocyte filterability and attenuated activation of leukocytes from peripheral blood samples of patients treated for 21 days after stroke.

Impact of Stroke Risk Factors on Leukocyte Involvement

A more precise definition of the role of leukocytes in experimental thrombotic stroke will not be reached until rigorous short- and long-term outcome studies are performed in cerebral ischemia models similar to clinical thrombotic stroke, particularly models that incorporate stroke risk factors. For example, the marked PMNL infiltration after MCA occlusion observed by Barone et al likely depends on the use of SHR. Although one could argue that increased leukocyte accumulation in the presence of hypertension is an epiphenomenon of a larger and more reproducible infarct volume in SHR, much evidence suggests that such stroke risk factors could specifically heighten leukocyte involvement.

Production of inflammatory cytokines (tumor necrosis factor-α) in response to intravenous or intracisternal endotoxin administration is markedly increased in SHR compared with normotensive Wistar-Kyoto control rats. SHR exhibit PMNL and lymphocyte counts approximately double those of appropriate Wistar-Kyoto control rats, and PMNL in SHR exhibit a higher resting level of activation as assessed by nitroblue tetrazolium staining. In addition, aged SHR have a PMNL count about fivefold higher than normotensive age-matched controls. The increase in leukotrienes observed in the brain after ischemia and reperfusion is mediated largely (approximately 70%) by leukocytes during posts ischemic reperfusion, and leukotriene receptor antagonists reduced posts ischemic edema after carotid artery occlusion in SHR. The dramatic impact of atherosclerosis on the vasoconstrictor response to activated leukocytes has been demonstrated both in vitro and in vivo by Heistad's group, and a modified local Shwartzman reaction produces a stroke like lesion in a disproportionate number of rats only if an underlying stroke risk factor (diabetes, hypertension, or advancing age) is present. Although advancing age is
an important stroke risk factor, most experimental animals subjected to focal ischemia by vascular occlusion are relatively young, with ages corresponding to adolescence or young adulthood.

Indeed, although the incorporation of multiple stroke risk factors into experimental cerebral ischemia models is difficult and tedious, it may be important for future research. A stroke model in which animals with several underlying risk factors and chronic vascular disease are subjected to a subtle “insult” resulting in a cerebral thrombotic infarct would perhaps be the most clinically relevant model. The role of leukocytes could be appropriately defined, and other mechanistic features important to the pathogenesis of stroke could also be addressed. In addition, therapeutic approaches could be appropriately tested in a true stroke setting—acute brain and microcirculatory failure superimposed on chronic vascular disease. Such a model system would also allow for investigations of preventive strategies.

**Beneficial Effects of Polymorphonuclear Leukocytes and Monocytes in Stroke**

Any therapeutic approach designed to suppress immune function, even in a transient, specific, and reversible manner, must be scrutinized as to potential side effects. An increased incidence of nosocomial infection is the most obvious risk and has been reported by numerous investigators. In addition, cell-mediated immunity is depressed during the first week after stroke. Reductions in both lymphocyte number and function are observed. Similarly, even greater risk of infection was observed when therapies with even subtle effects on immune function (such as hypothermia) were used in the treatment of cerebral ischemia. Other, less obvious effects of immune modulation could also be influential. For example, inhibition of postischemic leukocyte accumulation and function might create disturbances in the complex interactions between the immune response and the regenerative response in the CNS. Recent work suggests that IL-1 (a monocyte/macrophage product) is an important inflammatory mediator produced after traumatic and ischemic brain injury. Although detrimental effects on blood–brain barrier permeability may result from IL-1 elaboration, IL-1 is also a signal for the local elaboration of the homeostatic and regenerative molecule nerve growth factor. Inhibition of monocyte accumulation could, thus, have a detrimental impact on reparative processes. A similar relation between the immune response to CNS injury and neovascularization has been proposed. These potential side effects of immune modulation must be addressed whenever promising results are obtained. Long-term outcome studies are essential to evaluate the potential effects of altering these interactions between leukocytes and regenerative processes.

**Summary**

Remarkable model specificity for the role of PMNL and monocytes/macrophages in cerebral ischemia is shown in the work reviewed. An important role has been suggested in models of cerebral air embolism and thromboembolism and in experimental spinal cord ischemia. Prior leukocyte removal improved intraischemic CBF in models of incomplete global brain ischemia. Leukocyte depletion or inhibition of leukocyte adhesion, however, did not improve outcome after either cardiac arrest or focal cerebral ischemia produced by vascular occlusion in an otherwise normal experimental animal. Although the role of leukocytes in experimental ischemia has begun to be addressed, their role in experimental stroke remains to be examined. Current research suggests that underlying stroke risk factors, including atherosclerosis, hypertension, diabetes, and advancing age, may be critical determinants of leukocyte contribution to stroke. Despite this information, few experimental models of cerebral ischemia actually mimic the clinical stroke setting of an acute thrombotic event superimposed on underlying chronic vascular disease, and no study has examined the role of leukocytes in such a model. Recent and continuing advances in our ability to selectively modify leukocyte adhesion and function suggest that future studies should seek to clarify the role of leukocytes in cerebral ischemia and stroke and to gauge the prophylactic and therapeutic potential of antileukocyte agents.

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