

Brief Reviews

Cytokines and Cell Adhesion Molecules in Cerebral Ischemia

Experimental Bases and Therapeutic Perspectives

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Abstract—The possibility of reopening an occluded cerebral artery by means of thrombolysis has renewed interest in a number of the several mechanisms that are active during acute cerebral ischemia. Over recent years, it has become apparent that leukocytes play a central role not only during the healing stage of brain infarction but also during the early phases of cerebral ischemia, when it is postulated that these cells produce harmful effects, particularly in the presence of reperfusion. This review is based on the critical analysis of more than 150 publications dealing with the role of leukocytes and some inflammatory mediators (cytokines, chemokines, and adhesion molecules) in cerebral ischemia. Animal studies indicate that leukocyte involvement is promoted by a variety of inflammatory molecules produced immediately after the onset of cerebral ischemia. Considerable experimental evidence suggests that these mediators play a key role in the progression from ischemia to irreversible injury (ie, cellular death and necrosis). However, the precise role of each molecule alone remains to be further elucidated as well as in relation to the complex network existing among different mediators. Progress in our understanding of the inflammatory mechanisms operating in cerebral ischemia has enabled the testing of new compounds with promising results in animals; in contrast, one recent controlled trial of an anti-leukocyte molecule in acute stroke patients showed negative results. This discrepancy may derive in part from our incomplete understanding of the complexity of the inflammatory mechanisms involved in cerebral ischemia. Our analysis suggests that until sufficient knowledge of the underlying disease mechanisms is acquired, more care should be taken when testing new and potentially efficacious drugs in stroke patients.

The scenario of acute ischemic stroke therapy is rapidly evolving, and thrombolysis has recently been approved for use in stroke patients by USA authorities. However, therapeutic recanalization of an occluded cerebral artery still appears to be a potentially risky option that can be applied only in the case of selected patients.^{1,2} The main limitation of cerebral thrombolysis is the narrow, 3-hour therapeutic “window” during which the thrombolytic agent has to be administered to be effective³; beyond this time limit, its effectiveness is neutralized by the high risk of cerebral hemorrhage.⁴

The rationale for the use of a number of other pharmacological treatments for acute stroke currently under study in both animals and humans is based on recent advances in the pathophysiology of brain ischemia, one of the most rapidly expanding and promising areas of which is the role of inflammation in stroke. This role involves (1) the molecules that form part of the inflammatory response and participate in the pathophysiological cascade after an ischemic insult and (2) the inflammatory or infectious processes that may predispose or precipitate atherothrombotic stroke.^{5–8} In this review, we reexamine some aspects of inflammation in acute stroke by focusing on the role of leukocytes and leukocyte-related

molecules, with the aim of providing the background necessary to understand the rationale of newly emerging therapeutic approaches. Our review is limited to experimental and human data concerning some of the inflammatory mechanisms active during the acute phase of cerebral ischemia.

Histological Inflammatory Aspects in Acute Cerebral Ischemia

An inflammatory reaction is a common response of the brain parenchyma to various forms of insult. It is histologically characterized by the infiltration of leukocytes, which in the case of cerebral ischemia are mainly polymorphonuclear leukocytes and monocytes/macrophages.⁹ Human data are scarce: an increased number of leukocytes has been documented in the cerebrospinal fluid of acute stroke patients 2 to 3 days after stroke¹⁰; a high number of radiolabeled polymorphonuclear leukocytes have been shown in slightly perfused brain areas as early as 6 to 12 hours after the ictus¹¹; and one autopsy study described intense leukocyte infiltration of the brain parenchyma 2 to 3 days after the stroke.¹²

However, the inflammatory response of ischemic brain parenchyma has been more thoroughly explored in animal

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Selected Abbreviations and Acronyms

CINC	= cytokine-induced neutrophil chemoattractant
ICAM	= intercellular adhesion molecule
IFN	= interferon
IL	= interleukin
MCA	= middle cerebral artery
MCP	= monocyte chemoattractant protein
MIP	= macrophage inflammatory protein
TGF	= transforming growth factor
TNF	= tumor necrosis factor

models. An increased number of polymorphonuclear leukocytes in the microvessels or ischemic brain parenchyma have been found in various species.^{13–21} Until recently, it was assumed that this inflammatory reaction was delayed, secondary to tissue necrosis, and was intended to remove tissue debris from the infarcted area. In rats with permanent occlusion of one MCA, leukocytes (particularly polymorphonuclear cells) can be recognized in the microvessels as early as 30 minutes after occlusion.²² In the same model, necrosis is detected only 72 to 96 hours after MCA occlusion.^{23,24} This study and others suggest that at least the early arrival of leukocytes in the brain parenchyma is independent of the presence of necrosis, and this idea leads to the hypothesis that these cells play a role in the progression from ischemia to brain infarction.

There are a number of mechanisms by which leukocytes may produce deleterious effects on ischemic parenchyma. It has been proposed that leukocytes obstruct the microvessels and contribute toward the so-called “no-reflow” phenomenon¹⁶; ie, the lack of complete recovery of cerebral blood flow in the ischemic area after reperfusion.²⁵ This plugging of microvessels under ischemic conditions may be the result of an interaction with endothelial cells, mediated by adhesion molecules and/or the loss of cell deformability due to actin polymerization and pseudopod projections.^{26,27} As reviewed by Härtl et al,²⁸ other detrimental effects of leukocytes during ischemia may be due to: (1) the release of vasoconstrictive mediators, such as superoxidase anions, thromboxane A₂, endothelin-1, and prostaglandin H₂²⁹; (2) an alteration in cerebral artery vasoreactivity; and (3) the release of cytotoxic enzymes, free oxygen radicals, NO, and products of the phospholipid cascade. The release of proteolytic enzymes such as elastase might damage endothelial cell membranes and the basal lamina, alter the blood-brain barrier, and contribute to the formation of postischemic edema. In addition, loss of the integrity of the endothelial cell–basal lamina lining might facilitate the escape of red blood cells and the hemorrhagic transformation of a brain infarct.^{30,31}

The view that leukocytes may cause additional damage to potentially still-viable tissue during acute cerebral ischemia is indirectly supported by data showing a smaller infarct volume in neutropenic animals than in normal controls.^{32–35} Furthermore, a significant correlation has been found in humans between the degree of leukocyte accumulation and infarct volume as assessed by CT.¹¹ The arrival of leukocytes in the ischemic area is a multistep process.³⁶ They first marginate in the venules, then adhere to endothelial cells, and finally

migrate into the brain parenchyma. During each of these three steps, their functions are regulated by the inflammation-related molecules that are produced soon after the onset of cerebral ischemia.

Cytokines and Cerebral Ischemia

The signals mediating the entry of leukocytes into the ischemic area have not yet been completely elucidated, but a number of clues suggest that cytokines play a key role in this process. Cytokines are low-molecular-weight glycoproteins that act as intercellular messengers and are produced by macrophages, monocytes, lymphocytes, endothelial cells, fibroblasts, platelets, and many other cell types. To produce cytokines, all of these cells need to be activated.³⁷ Cytokines act at very low concentrations on specific target-cell receptors, whose expression is modulated by the cytokines themselves. Binding of cytokines to receptors activates intracellular second-messenger systems (yet to be identified) and subsequently, protein kinases and phosphatases. These proteins cause gene regulation through the activity of transcription factors.³⁷ Cytokines are considered to be among the principal mediators of immunologic and inflammatory responses. In vitro, cytokines can be produced by astrocytes, neurons, and endothelial and microglia cells,^{38–40} and there are observations suggesting that this also holds true in humans affected by brain ischemia.⁴¹

Cytokines may have various functions during cerebral ischemia: on the one hand, they can attract leukocytes and stimulate the synthesis of adhesion molecules in leukocytes, endothelial cells, and other cells, thus promoting the inflammatory response of damaged cerebral tissue; on the other, they can facilitate thrombogenesis by increasing the levels of plasminogen-activating inhibitor-1, tissue factor, and platelet-activating factor^{42,43} and by inhibiting tissue plasminogen activator and protein S.⁴⁴ Examination of this last aspect is beyond the scope of the present review.

IL-1

IL-1 exists in two separate forms (α and β), which have only one-third sequence homology. IL-1 is overexpressed during brain ischemia, as documented by the induction of IL-1 β mRNA synthesis in rats with permanent MCA occlusion,^{45,46} transient global forebrain ischemia,^{47–50} or a ligated carotid artery associated with hypoxia.⁵¹ Attempts to detect IL-1 in the peripheral blood of stroke patients have so far been unsuccessful, conceivably because of its low plasma levels.⁵²

Evidence that IL-1 may play a deleterious role in cerebral ischemia derives from the observation that rats with a transient MCA occlusion have a larger brain infarction when recombinant human IL-1 β is injected into the lateral ventricle immediately after reperfusion.⁵³ Similar results have been obtained in rats with a permanent MCA occlusion.⁵⁴ The intraventricular injection of recombinant human IL-1 β also enhances the formation of brain edema and increases both the number of neutrophils in ischemic areas and neutrophil–endothelial cell adhesion.⁵³ The complex functions of IL-1 are mediated by specific cell-surface receptors and regulated by the IL-1 receptor antagonist.⁵⁵ Two main receptors for IL-1 have been identified; type I is present in many cell types and

binds IL-1 α and IL-1 β with similar affinity.⁵⁶ Type II is found on the surface of B cells, neutrophils, and macrophages and shows higher affinity for IL-1 β .⁵⁶ Types I and II are regulated differently in brain ischemia and may thus play separate roles. In spontaneously hypertensive rats, the mRNA for the type I IL-1 receptor is relatively highly expressed in the normal cortex, with a marked increase 5 days after cerebral ischemia.⁵⁵ Type II mRNA has low basal expression and a peak 12 hours after the onset of ischemia.⁵⁵ The possible mechanisms of intracellular signal transduction for IL-1 on peripheral immune cells include effects on cAMP, protein kinase C, and protein phosphorylation⁵⁶; these effects remain to be proved in brain ischemia. The IL-1–receptor interaction is quickly followed by the induction of immediate-early genes such as *c-jun* and *c-fos*.⁵⁷

Studies showing a reduction in infarct size after the administration of IL-1 antagonists or inhibitors provide further evidence of the importance of IL-1 in cerebral ischemia.^{53,58–64} The possible harmful mechanisms induced or activated by IL-1 include fever, increased heart rate and arterial blood pressure, enhancement of *N*-methyl-D-aspartate-mediated injury, proliferation of microglia, release of arachidonic acid, and stimulation of NO synthesis.⁶⁵ At present, the most widely recognized functions of IL-1 appear to be the induction of endothelial cell adhesion molecule expression and the promotion of neutrophil tissue infiltration.

TNF- α

TNF- α gene upregulation has been demonstrated in transient⁶⁶ and prolonged^{51,67} cerebral ischemia by the increased synthesis of TNF- α mRNA in the parenchyma. TNF- α plasma levels have also been found to be higher in acute stroke patients than in healthy control subjects.⁶⁸

The effects of TNF- α are mediated by specific receptors, the most well known of which are two proteins called p75 and p55 (according to their molecular weight).⁶⁹ The binding of TNF- α to its receptor is followed by the activation of a variety of proteins, such as protein kinase C, tyrosine kinase, mitogen-activated protein kinase, phospholipase A₂, and phosphatidylcholine-specific phospholipase C.⁷⁰ Among these, the mitogen-activated protein kinases are the most extensively studied, and they are divided into three main families: (1) the extracellular signal-regulated kinases, which are activated by growth factors; (2) the c-Jun NH₂-terminal kinases; and (3) the p38 kinases. The last two groups are activated by proinflammatory cytokines.^{71,72} The second step in the TNF- α signal transduction pathway, as for other cytokines, is intranuclear, with the activation of several transcription factors. One of these, nuclear factor- κ B, translocates from the cytoplasm to the nucleus, where it activates the promoter of the genes for adhesion molecules and other cytokines.⁷³

Like IL-1, TNF- α induces the expression of adhesion molecules by glial and endothelial cells,⁷⁴ thus favoring neutrophil adherence and accumulation in microvessels.⁶⁷ TNF- α is also involved in blood-brain barrier alterations, a proadhesive-procoagulant transformation of endothelial cell surfaces, and glial cell activation.⁷⁴ However, its role in cerebral ischemia remains to be established. Barone et al⁷⁵

showed that administration of TNF- α exacerbated the ischemic injury provoked by MCA occlusion in spontaneously hypertensive rats, although TNF- α toxicity was not related to direct neuronal damage, and also demonstrated that anti-TNF- α antibodies have a neuroprotective effect.⁷⁵ Similarly, the inhibition of TNF- α in mice with permanent MCA occlusion leads to a smaller infarct volume.⁷⁶ However, not all studies agree about the deleterious role of TNF- α in brain ischemia. When TNF- α is administered to mice 48 hours before MCA occlusion, it induces a protective effect.⁷⁷ Furthermore, the fact that mice lacking TNF- α receptors show a larger infarct area after MCA occlusion than do normal controls⁷⁸ suggests that TNF- α may be beneficial in the poststroke recovery phase. Finally, the specificity of TNF- α expression after cerebral ischemia has been challenged by preliminary human data showing that TNF- α is also expressed in the contralateral, nonischemic hemisphere.⁷⁹

IL-6

IL-6 plays a central role in host defense as well as in acute and chronic inflammatory activities^{80,81} and is expressed in response to various forms of brain injury.^{81–85} In the rat, IL-6 mRNA is overexpressed 3 hours after permanent MCA occlusion and reaches a peak at 12 hours; its expression remains high for at least 24 hours.⁸⁶

Higher IL-6 levels have been detected in the peripheral blood of patients with acute cerebral ischemia than in control subjects.^{52,87–89} Increased plasma and cerebrospinal fluid IL-6 levels are correlated with a larger infarct size on CT and magnetic resonance imaging scans^{87,88} and with a poorer clinical outcome,⁵² although it is not clear whether this simply reflects an overproduction of IL-6 by larger lesions or a directly damaging effect of IL-6 itself. In addition, it has not yet been completely elucidated whether IL-6 exerts anti-inflammatory or proinflammatory effects or both. Its anti-inflammatory effect depends on the inhibition of IL-1 and TNF- α production via a negative-feedback mechanism and stimulation of the production of their circulating antagonists: soluble TNF- α receptor and IL-1 receptor antagonist.^{80,90} Part of the anti-inflammatory function of IL-6 can be attributed to its ability to induce the release of corticotropin and cortisol^{91,92} and to promote the expression of acute-phase proteins^{93,94} that have antiproteinase and oxygen-scavenger functions.⁸¹ On the other hand, it has also been shown that IL-6 induces phospholipase A₂ gene expression and as a consequence, stimulates the production of leukotrienes, prostaglandins, and platelet activating factor,⁹⁵ all of which are involved in ischemic brain damage.⁹⁶

TGF- β

TGF- β is a cytokine that is known to regulate and stimulate cell proliferation and differentiation and to play a central role in tissue repair mechanisms.⁹⁷ In rats exposed to 10 minutes of global cerebral ischemia, increased expression of TGF- β mRNA is seen after 6 hours throughout the brain; this expression increases further at day 2 and subsides thereafter.⁹⁸

One recent human autopsy study has found TGF- β mRNA overexpression in ischemic tissue in comparison with samples taken from the contralateral, nonischemic side.⁹⁹ The

highest expression of TGF- β mRNA was detected in the tissue surrounding the infarction (the penumbra zone).⁹⁹ A so-far-unexplained finding is that serum TGF- β levels are lower in acute stroke patients than in control subjects, perhaps owing to an accumulation of this cytokine in the ischemic tissue.⁸⁹

Preliminary results indicate that TGF- β may play a protective role in brain ischemia.^{100–104} Some of the proposed reasons for this beneficial effect include (1) reduced neutrophil adherence to endothelial cells¹⁰⁵; (2) suppression of the release of potentially harmful oxygen- and nitrogen-derived products by macrophages^{106,107}; (3) the promotion of angiogenesis in the penumbra area⁹⁹; and (4) a reduction in the expression and efficacy of other cytokines, such as TNF- α ,^{108,109} possibly by blocking p38 kinase and the consequent inhibition of the TNF- α transduction mechanism.¹¹⁰

IFN- γ

IFN- γ is a protein produced by activated CD4+ and CD8+ T cells and, to a lesser degree, by natural killer cells.¹¹¹ IFN- γ is not produced by central nervous system cells, and its release at this level is possible only when a breakdown of the blood-brain barrier allows infiltration of lymphocytes into the brain parenchyma. Although the role of IFN- γ (and of all the IFNs) in brain ischemia has not been specifically addressed, blood-brain barrier damage and leukocyte penetration are among the first consequences of ischemic cerebral insults. Among many different functions, IFN- γ induces the expression of a variety of cytokines by stimulating p38 kinase¹¹⁰ and of class II major histocompatibility complex. Major histocompatibility complex is essential for macrophages to recognize antigen, and thus, IFN- γ could play a crucial role in the development of brain necrosis after an ischemic insult. Another possible role of IFN- γ in cerebral ischemia is the production of NO, with a consequent cytotoxic effect on brain cells; in vitro, IFN- γ stimulates the production of interferon regulatory factor-1, which induces NO synthase mRNA expression.¹¹²

Cell Adhesion Molecules in Cerebral Ischemia

Cell-Cell Adhesion

The adhesion of leukocytes to the endothelial surface and their subsequent migration from the microvessels into the brain parenchyma is mediated by a variety of molecules located on the surface of both leukocytes and endothelial cells. Adhesion molecules are conventionally divided into four main families, each of which has a different function: integrins, the immunoglobulin superfamily, cadherins, and selectins.¹¹³ Under normal conditions, there is little or no cell-surface expression of adhesion molecules¹¹⁴; their expression is induced by various inflammatory processes, such as cerebral ischemia, with the upregulation mediated by cytokines. Among the most studied adhesion molecules are ICAM-1 (immunoglobulin superfamily), which is located on the endothelial surface, and its leukocyte counterpart, integrin CD11/CD18; many others are currently under investigation. A number of animal studies have documented the upregulation of endothelial-leukocyte adhesion molecule 1 (also called E-selectin) in the microvessels,^{115–117} ICAM-1,^{118–122}

and P-selectin¹¹⁸ after focal cerebral ischemia in rodents and in nonhuman primates.

The possible relevance of adhesion molecules to the pathogenesis of ischemic brain damage has recently been corroborated by two studies demonstrating that, in comparison with normal controls, mice belonging to an ICAM-1-deficient strain show a marked reduction in cerebral infarction size after transient MCA occlusion.^{35,123} As reviewed by Feuerstein et al,⁷⁴ cytokines can also induce the in vitro expression of adhesion molecules in astrocytes, oligodendrocytes, and microglia. It is postulated that the presence of adhesion molecules on the surface of glial cells may facilitate the postischemic migration of leukocytes through the brain parenchyma.⁷⁴

One observation with potential clinical relevance is that the expression of adhesion molecules caused by administration of IL-1, TNF- α , and IFN- γ is higher in endothelial cell cultures from spontaneously hypertensive rats than in those derived from normotensive rats.¹²⁴ This finding suggests that ischemic injuries of comparable severity may lead to more severe consequences in hypertensive individuals.

Upregulation of adhesion molecules has been documented in human stroke patients. In a preliminary study, leukocytes from patients suffering an ischemic stroke or transient ischemic attack showed increased CD11a expression within 72 hours of the onset of symptoms in comparison with control subjects matched for age and risk factors.¹²⁵ Sobel et al¹²⁶ detected increased ICAM-1 expression on the surface of vessels from cerebral cortical infarcts in four patients. Soluble isoforms of adhesion molecules, which are believed to be shed from the surfaces of activated cells, can be quantified in serum: in comparison with normal control subjects, patients with acute stroke showed an initial increase in serum endothelial-leukocyte adhesion molecule-1 levels (up to 24 hours after stroke) and persistently increased levels of vascular cell adhesion molecule-1 (up to 5 days).¹²⁷ This temporal pattern is in line with the current view that selectins, such as endothelial-leukocyte adhesion molecule-1, mediate the initial low-affinity interaction between leukocytes and endothelial cells and promote the margination and rolling of leukocytes in the blood stream; subsequently, it is vascular cell adhesion molecule-1 (immunoglobulin superfamily) that causes the tight leukocyte-endothelial attachment and transendothelial migration.

When evaluating the levels of adhesion molecules in patients with cerebrovascular diseases, consideration should be given to the possible confounding effect of risk factors for atherosclerosis, a condition involving chronic inflammatory endothelial activation.¹²⁸ In acute ischemic stroke patients, serum ICAM-1 levels have been found to be lower than¹²⁹ or the same as¹²⁷ those of asymptomatic control subjects matched for age, sex, and a number of vascular risk factors. It can also be hypothesized that because of the long period (up to 72 hours) for enrolling patients, in one of these two studies,¹²⁹ the theoretical initial peak serum level of adhesion molecules could no longer be detected after they had bound to leukocytes and endothelial cells.

Cell-Matrix Adhesion

Another group of adhesion molecules is involved in cell-matrix interaction. Integrins, the most widely studied adhesion molecules of this group, are heterodimeric membrane glycoproteins formed by the combination of an α - and a β -subunit with an intracellular and an extracellular domain.¹¹³ At the basal membrane level, integrins connect endothelial cells to extracellular components, such as laminin and collagen. In the brain, endothelial cells, astrocytes, and the basal membrane contribute to form the blood-brain barrier, and their interconnection is mediated by integrins. Damage to these molecules may therefore lead to severe damage of the blood-brain barrier. Wagner et al¹³⁰ have demonstrated that integrin $\alpha_6\beta_4$, that under physiological conditions mediates the interaction between astrocytes and extracellular matrix, is rapidly damaged during focal cerebral ischemia/reperfusion. Other integrins play an important role in inflammatory neoangiogenesis, wound repair, and ontogenesis and may therefore be important for tissue repair after an ischemic insult. Among these, integrin $\alpha_v\beta_3$, a receptor for molecules sharing the common arginine-glycine-aspartic acid (RGD) sequence (eg, vitronectin, fibrinogen, fibronectin, and laminin), is expressed in basal ganglia microvessels in a nonhuman primate model of ischemia/reperfusion as early as 2 hours after MCA occlusion.¹³¹ The binding of ligand to integrins modifies the conformation of their intracellular domain so as to interfere with the cytoskeleton alignment, modify cellular structure, and confer on the cell the ability to migrate. Through these mechanisms, $\alpha_v\beta_3$ might play a role during the first stages of vascular reorganization after cerebral ischemia.

Chemokines in Cerebral Ischemia

As IL-1 β and TNF- α play a major role in promoting adhesion between endothelial cells and leukocytes, they are poor attractants for polymorphonuclear leukocytes and monocytes; other chemoattractant cytokines (the so-called chemokines) are believed to be specifically involved in guiding leukocytes through the parenchyma and toward the ischemic area.

Chemokines are low-molecular-weight molecules characterized by the presence, as a common structural pattern, of four cysteine residues and are divided into two main families (C-X-C and C-C chemokines) according to the presence or absence of an amino acid between the residues of the two most amino-proximal cysteines.³⁶ This structural distinction is reflected in vitro by a peculiar effect on different cell types: C-X-C chemokines tend to attract neutrophils, whereas C-C chemokines preferentially act on monocytes/macrophages. Each chemokine family binds to specific receptors formed by seven transmembrane domains that activate G proteins and subsequently an intracellular kinase cascade.¹³² Chemokine production and secretion are stimulated by a number of compounds, such as bacterial lipopolysaccharide, IL-1 α and -1 β , and TNF- α .³⁶

Few chemokines have so far been studied in some detail in experimental cerebral ischemia. Significant expression of CINC mRNA has been detected in the ischemic areas of spontaneously hypertensive rats 6 hours after permanent MCA occlusion¹³³; this expression peaks at 12 hours and

rapidly decreases at 24 hours. CINC has been shown to be homologous to three "gro" human proteins and "KC" in the mouse, and all of them are molecules acting predominantly as neutrophil chemoattractants.¹³⁴ In a model of rat transient focal ischemia, the first detectable level of CINC in the brain was observed 3 hours after reperfusion and preceded leukocyte infiltration.¹³⁵ In the sera of the same animals, a high concentration of CINC was found 60 minutes after MCA occlusion, with the peak concentration being reached 3 hours after reperfusion; a reduction in levels was observed between 6 and 48 hours.¹³⁵

The temporal expression of MCP-1 in brain ischemia follows that of CINC. High levels of MCP-1 mRNA have been found in the brains of rats with transient and permanent MCA occlusion at 6 hours, with the highest expression occurring between 12 hours and 2 days^{136,137}; increased MCP-1 expression was still detectable 5 days after permanent MCA occlusion.¹³⁶ The observations that in vitro IL-1 β and TNF- α induce MCP-1 gene expression¹³⁸ and that in vivo MCP-1 mRNA temporal expression follows that of IL-1 β and TNF- α mRNA^{45,67} suggest that IL-1 β and TNF- α induce MCP-1 expression in cerebral ischemia. According to Gourmalia et al,¹³⁹ 6 hours to 2 days after MCA occlusion, MCP-1 mRNA is present in rat astrocytes surrounding the ischemic tissue. After 4 days, MCP-1 mRNA is found in macrophages and microglial cells in the infarcted tissue.

The mRNA of another member of the C-C chemokine family, MIP-1 α , has been found to be overexpressed in cerebral ischemic areas.¹³⁷ This chemokine attracts monocytes and macrophages and modulates their activity in tissue undergoing an inflammatory process. Kim et al¹³⁷ found that the temporal expression of MIP-1 α parallels that of MCP-1 and that the distribution of MIP-1 α -positive cells was similar to that of activated astrocytes. MIP-1 α infused into the subarachnoid space of rabbits treated with Gram-negative bacteria initially attracts neutrophils and subsequently monocytes.¹⁴⁰ If this sequence also holds true in cerebral ischemia, this molecule could be in part responsible for the switch from a leukocyte- to a macrophage-based inflammatory response. The temporal profile of CINC, MCP-1, and MIP-1 α expression is in line with that of leukocyte accumulation in the ischemic brain parenchyma of nonhuman primates. In these models, polymorphonuclear cells are detected 24 to 72 hours after the onset of ischemia, followed at 7 to 16 days by monocyte and macrophage infiltration.^{9,13}

In vitro, smooth muscle cells stimulated by IFN and IL-1 β or TNF- α produce IFN-inducible protein-10, which is a chemoattractant for macrophages and activated T lymphocytes.¹⁴¹ Inducible protein-10 mRNA expression has been demonstrated in the rat after endothelial damage by balloon angioplasty.¹⁴¹ Because endothelial damage occurs during brain ischemia, it seems possible that this molecule could also play a role in this latter pathological condition.

The number of discovered chemokines is continuously growing, and so far, more than 20 members of this cytokine family have been identified. It is likely that in the future, the activity of other chemokines may be found to be increased after cerebral ischemia and their function elucidated.

Experimental Anti-Leukocyte Interventions

On the basis of the progress made in our understanding of inflammatory-like mechanisms in cerebral ischemia, an increasing number of molecules are currently being investigated in animals for their possible effectiveness in human acute stroke.

One group of studies has focused on therapeutic strategies related to the role of proinflammatory cytokines. The intraventricular and systemic administration of IL-1 receptor antagonist into rats reduces the volume of infarction caused by MCA occlusion^{54,59,62,64} or the ligation of one carotid artery associated with hypoxia.⁶¹ This reduction in infarct size is accompanied by a decreased number of leukocytes in the ischemic hemisphere and improved neurological function.⁶² Similar results have been achieved by means of the topical application of anti-IL-1 β neutralizing antibody,⁵³ zinc protoporphyrin,^{53,60,63} and fragments of lipocortin-1,⁵⁸ all of which act as IL-1 inhibitors. TGF- β administration has led to less severe histological damage in a rabbit model of embolic stroke¹⁰⁰ as well as in rats subjected to permanent MCA occlusion,¹⁰¹ transient global ischemia,¹⁰⁴ and hypoxia-ischemia cerebral insult.¹⁰³

A second group of studies has concentrated on molecules that are capable of blocking the adhesion between endothelial cells and leukocytes. The administration of anti-CD11b/CD18 monoclonal antibodies at various times after reperfusion reduces infarct volume in rats with MCA occlusion^{142,143} in a dose-dependent manner,¹⁴⁴ although single administration of the same drug 1 hour after permanent MCA occlusion led to less striking effects.¹⁴⁵ Treatment with antibodies against ICAM-1 or integrin CD11b/CD18 is also capable of reducing the number of apoptotic cells in the ischemic brain area.¹⁴⁶

The use of anti-ICAM-1 antibodies reduces infarct volume and the number of tissue polymorphonuclear leukocytes in rats with transient MCA occlusion,^{147,148} but the treatment is ineffective when administered to animals with permanent occlusion.¹⁴⁹ The same dichotomy between transient and permanent ischemia has been shown in one model of spinal cord ischemia.¹⁵⁰ These observations suggest that drugs blocking leukocyte-endothelial adhesion are effective only in the presence of reperfusion.

Because monoclonal antibodies are immunogenic and might have collateral effects in stroke patients, new nonimmunogenic molecules are now being tested in animal models with encouraging results.^{151,152} These are peptides or glycoproteins, which are produced by bacteria and which structurally or functionally mimic cell adhesion molecules and inhibit leukocyte function.

Conclusions

Leukocytes and Inflammatory Mediators in Cerebral Ischemia

The studies reviewed in this article emphasize the possible role of cytokines and cell adhesion molecules in the development of cerebral damage caused by an ischemic insult. Early overexpression of these inflammatory mediators promotes the recruitment of leukocytes in the ischemic area and represents a key mechanism for the evolution of tissue

damage. Leukocytes are in fact believed to have a deleterious effect in brain ischemia. In the microvessels, they first adhere to endothelial cells, interfering with local cerebral blood flow and enhancing the ischemic insult. Subsequently, leukocytes proceed through the endothelial lining and accumulate in the ischemic area, where they release a number of toxic molecules that contribute to the development of an infarction.

Relevance of Animal Models

Most of the evidence comes from animal studies; only preliminary observations relating to humans are available. It is plausible that our knowledge of the role of inflammation in human stroke will considerably expand in the future, in terms of both new molecules and a more precise definition of their action, but it is likely that animal models will continue to represent a fundamental source of information. The possibility that leukocyte alterations in experimental models may be determined by the surgical procedures used to induce the ischemic insult or by anesthesia has been refuted in a nonhuman primate model.¹⁵³ Moreover, the use of sham-operated animals (ie, animals in which all of the phases of the surgical procedure except artery occlusion are reproduced) makes it possible to control for the influence of surgery and anesthesia. Some of the substantial advantages of experimental models are the following: (1) The possibility of knowing the exact time of onset of ischemia means that the temporal pattern of the expression of the molecules under study can be investigated; (2) Access to brain tissue for pathological observation is simple; (3) New drugs can be tested. Nevertheless, it should be borne in mind that most of the conclusions are based on rodent experiments because data from nonhuman primate models (which are probably of greater relevance to human disease) are still scarce.

Therapeutic Perspectives

Acute stroke therapy is probably destined to change over the next few years. One of the crucial turning points will be definition of the time interval during which the administration of drugs is likely to be efficacious (therapeutic window), which is not easy because of individual patient variability. Most of the trials carried out so far suggest a very narrow (3 hours) therapeutic window, which in the case of thrombolytic agents, is due more to the increased occurrence of hemorrhagic transformation than a lack of efficacy beyond this limit. Animal and human studies have shown that the evolution of a brain infarct progresses over a longer period of time,^{23,24,154,155} and so the reopening of an artery could also be advantageous at a later time.

New drugs counteracting cytokine-mediated damage or opposing leukocyte-endothelial cell adhesion are already being investigated in animals with encouraging results. One perspective for the use of drugs capable of interfering with inflammation-related mechanisms is the possibility of widening the therapeutic window; in a rabbit model of embolic stroke, administration of anti-ICAM-1 antibodies has been shown to extend the time during which the thrombolytic agent was effective in dissolving blood clots.¹⁵⁶ The timing of arrival of many stroke patients to the emergency department¹⁵⁷ seems compatible with potential drug interventions

designed to interfere with inflammatory mediators and the natural evolution of the ischemic lesion. The inflammatory response of the cerebral parenchyma, with the above-discussed harmful implications, develops over days after the onset of ischemia in the animal,²⁰ and this is probably also true in humans.¹² Therefore, these therapeutic strategies could theoretically be undertaken far beyond the strict time limit of thrombolysis with some beneficial effects.

Possible future pharmacological treatments could be based on the inhibition of proinflammatory mediators (perhaps IL-1 and TNF- α) or enhancement of the effect of others (IL-6 and TGF- β). Some cytokines and chemokines, such as IFN- γ and MIP-1, seem to play a role in the passage from the first phase of the inflammatory response, characterized by the presence of polymorphonuclear cells, to the second, with monocyte and macrophage invasion and the development of frank necrosis. Blocking these molecules could prevent part of the brain parenchyma from becoming necrotic. Interfering with adhesion between leukocytes and endothelial cells represents a second therapeutic option. On the other hand, attempts could be made to potentiate the role of integrins that promote cell remodeling and neovascularization. Other targets for a possible pharmacological approach are the specific transduction pathway signals (eg, intracytoplasmic kinases) that are critical for cytokine production and their effects.

Caveats and Limitations

An understanding of the role of leukocytes and the mediators of inflammation in cerebral ischemia may have a very great impact on therapy, but a few notes of caution should be outlined. Although it currently seems that some cytokines, such as IL-1, have a detrimental effect and that others, such as TGF- β and IL-6, might be protective in some way, the precise effect of each mediator is still not completely known. Moreover, some data exist challenging the view that leukocytes are active in favoring the progression from cerebral ischemia to necrosis.¹⁵⁸⁻¹⁶⁰

The expectations aroused by animal studies have been somewhat dampened by the results of the first human trial using an anti-ICAM-1 murine monoclonal antibody (Enlimomab, Boehringer Ingelheim). In this double-blind, placebo-controlled trial, 625 patients with acute ischemic stroke were randomized within the first 6 hours of symptom onset to receive either anti-ICAM-1 antibodies (160 mg IV the first day, followed by 40 mg/d for 2 to 5 days after onset) or placebo.¹⁶¹ The 90-day outcome as measured by the modified Rankin Scale showed an improvement in 33% of the patients on placebo versus 27% of those receiving active treatment. Side effects (mainly fever, infections, and pneumonia) were recorded in 44% of the Enlimomab and 30% of the placebo patients; serious adverse effects (stroke progression, brain edema, and hemorrhagic transformation of the infarction) were observed in 22% of the Enlimomab versus 16% of the placebo patients; both of these differences were statistically significant. Moreover, the occurrence of cases of aseptic meningitis in the Enlimomab group underlines the limitations due to the immunogenic effect of this drug.

Is There Still a Role for Anti-Leukocytes Interventions in Human Stroke?

However, before setting aside anti-ICAM-1 antibodies as another ineffective drug in acute ischemic stroke, the preliminary results of the Enlimomab study should be critically considered. First of all, anti-leukocyte interventions appear to be particularly effective against reperfusion injury. Anti-ICAM-1 antibodies have been tested and proved efficacious in models of transient^{146-148,150} but not permanent¹⁴⁹ focal ischemia, presumably the condition of most of the patients enrolled in the Enlimomab trial. As shown by Bowes et al,¹⁵⁶ the use of anti-ICAM-1 might be beneficial only in combination with thrombolysis. Second, the time and route of drug administration could be crucial: in none of the reviewed animal studies was the first dose of anti-ICAM-1 antibodies delayed beyond 1 hour after reperfusion, and systemic administration could lead to a reduction in host defenses and a predisposition to infections. Finally, given the increased frequency of fever and aseptic meningitis among the treated patients, the immunogenic role of anti-ICAM-1 antibodies needs to be weighed very carefully.

In the light of the above considerations, among others, a multitherapeutic approach for ischemic stroke can be foreseen.¹⁶² The testing of new anti-leukocyte drugs in humans probably requires further elucidation of the pathophysiological role that these cells play during cerebral ischemia. At present, it would be dangerous to simply dismiss those drugs that may prove to be efficacious in different target populations.

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