Journal of Neuropathology and Experimental Neurology

Vol. 62, No. 2
February, 2003
pp. 127–136

Inflammatory Mechanisms after Ischemia and Stroke

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Abstract. Inflammation has been implicated as a secondary injury mechanism following ischemia and stroke. A variety of experimental models, including thromboembolic stroke, focal and global ischemia, have been used to evaluate the importance of inflammation. The vasculature endothelium promotes inflammation through the upregulation of adhesion molecules such as ICAM, E-selectin, and P-selectin that bind to circulating leukocytes and facilitate their migration into the CNS. Once in the CNS, the production of cytotoxic molecules may facilitate cell death. The macrophage and microglial response to injury may either be beneficial by scavenging necrotic debris or detrimental by facilitating cell death in neurons that would otherwise recover. While many studies have tested these hypotheses, the importance of inflammation in these models is inconclusive. This review summarizes data regarding the role of the vasculature, leukocytes, blood-brain barrier, macrophages, and microglia after experimental and clinical stroke.

Key Words: Adhesion molecules; Blood-brain barrier; Inflammation; Ischemia; Microglia; Stroke.

INTRODUCTION

Infection and trauma were the leading causes of death throughout evolutionary history until the advent of antibiotic therapy. The inflammatory response evolved to combat infection and promote tissue repair. Immune responses needed to be rapid and toxic to infectious organisms while causing minimal damage to the individual. The difficulty in maintaining this balance is illustrated by the ever-growing list of diseases that are influenced or caused by an aberrant immune response. While the normal brain is considered an immune-privileged organ, implying that the blood-brain barrier (BBB) subdues the inflammatory response and impedes infiltration of hematogenous inflammatory cells, the diseased brain is not so fortunate. Loss of the BBB and expression of proinflammatory molecules within the cerebral vasculature promote inflammation. There is currently a debate over whether inflammation is a beneficial, detrimental, or irrelevant consequence of clinical stroke. A growing body of evidence from animal models suggests that aspects of the inflammatory process impact CNS pathophysiology and repair. This review will focus on experimental evidence for current theories on inflammatory involvement and will discuss the relevance to human disease.

HUMAN DISEASE AND STROKE MODELS

Human stroke is a heterogeneous group of conditions, and the cerebrovascular inflammatory response differs depending on stroke etiology. Therapeutic strategies may be condition-specific, accounting for the failure of many clinical trials to find therapeutic benefit. For example, human stroke can be broadly categorized as hemorrhagic or ischemic, but ischemic stroke may have varying degrees of hemorrhagic transformation or BBB permeability. Hemorrhage leads to a more robust inflammatory response than pure ischemic stroke. Different types of ischemic stroke also have distinct inflammatory features. Ischemia due to global reductions in blood flow has different consequences than focal, embolic stroke. In addition, the composition of emboli and the location (arterial or venular) of occlusion may alter pathophysiology. The presence of reperfusion following occlusion plays a substantial role in the pathophysiology and potential efficacy of therapies in experimental models (1). These conditions are critical to consider when evaluating different mechanisms of disease and choosing animal models to study aspects of human disease.

Similarly, the inflammatory response differs depending on the animal model (2, 3). Disease models typically reduce the number of variables in order to improve an investigator's ability to reach a conclusion. The disadvantage in doing so is that eliminating variables changes the pathophysiology and conclusions may not apply to the clinical disease. Animal models of global cerebral ischemia model ischemia of cardiac arrest by reducing blood flow throughout the brain. Focal ischemia models use a variety of techniques to occlude cerebral vessels. The injury produced by ischemia models is highly reproducible and is an effective means to study ischemia. However, patients are most likely to experience embolic stroke, which contain atherosclerotic deposits, activated platelets, and clotting proteins that complicate the pathophysiology. To mimic the human disease more closely, models of embolic stroke were developed to study the combined effects of intravascular clots and emboli in addition to ischemia.

Embolic stroke in rats has been studied using either photochemically induced nonocclusive common carotid

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This work was supported by NIH Grants NS27127-11 and the Lois Pope Life Fellowship Program.

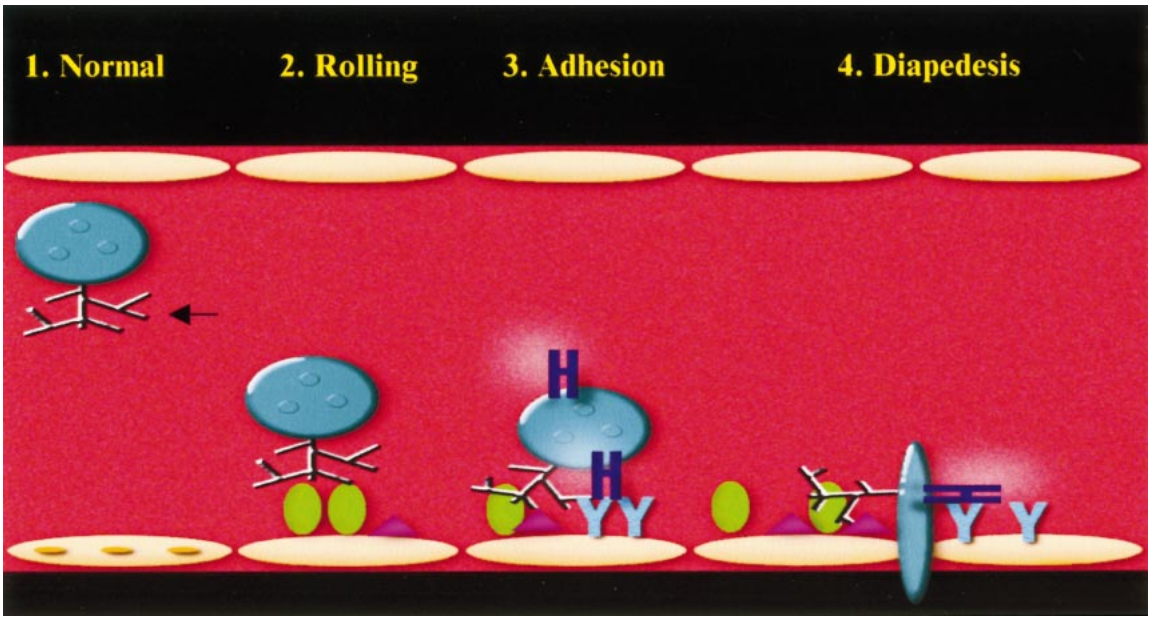

Fig. 1. Adhesion molecules promote inflammatory cell influx. 1. The vascular inflammatory response begins by expressing E (purple triangle) and P (green oval) selectin on the endothelial surface. 2. Sialyl Lewis X (marked by the arrow) expressing leukocytes bind to selectins causing the cells to roll along the surface. 3. Endothelium expressed ICAM (blue ''Y'') binds to β_2 integrins such as CD11a, b, and c (blue ''H'') on the leukocyte surface and facilitates firm adhesion to the vessel wall. 4. Upon stimulation with other inflammatory molecules, the leukocyte squeezes through the wall (diapedesis) and into the adjacent tissue.

artery thrombosis (CCAT) to produce platelet emboli or direct injection of fragmented clots (4–6). Emboli travel from the common carotid artery or from a catheter introduced intra-arterially to the cerebral vasculature. Some emboli lodge in cerebral vessels leading to focal areas of infarction. BBB permeability, endothelial damage, vascular dysregulation, inflammatory cell diapedesis, and significant numbers of intraparenchymal inflammatory cells have been observed in rat brain following embolic stroke (6–8). These studies led investigators to hypothesize that embolic stroke results in a primary vascular injury that may promote inflammation. There also is evidence that stasis following middle cerebral artery occlusion (MCAO) leads to intravascular thrombosis, which may be an important component of ischemic models as well (9–11).

VASCULAR INFLAMMATION AND STROKE

Activated platelets interact with the endothelium and facilitate its conversion from an anti-inflammatory, antithrombotic state to a pro-inflammatory, pro-thrombotic state. These changes are mediated through altered expression of released and cell surface signaling molecules (12). This phenomenon was demonstrated by exposing human activated or inactivated platelets to porcine endothelial cells. Only activated platelets enhanced tissue factor activity (pro-thrombotic), upregulated E-selectin (pro-inflammatory), and secreted endothelin-1 (vasoconstrictive). Platelet-released interleukin-1 (IL-1) and other cytokines may contribute to endothelium conversion and enhance inflammation (13).

Adhesion Molecules

Cell adhesion molecules permit interactions between endothelial cells, platelets, leukocytes, and lymphocytes. The classes of adhesion molecules most commonly attributed to inflammatory processes include the integrins, selectins, and members of the immunoglobulin superfamily (IgCAM). Integrins are transmembrane, heterodimeric molecules composed of an α and β subunit. They bind to extracellular matrix components as well as other adhesion molecules. Selectins are calcium-dependent, transmembrane glycoproteins that bind to carbohydrates. L-selectin (CD62-L) and E-selectin (CD62-E) are found on leukocytes and endothelial cells respectively, while P-selectin (CD62-P) is found on both platelets and endothelial cells. IgCAMs are transmembrane proteins with an immunoglobulin-like domain that are found on many cells and bind to other adhesion molecules and extracellular matrix components.

Following endothelial damage or ischemia, altered adhesion molecule expression promotes cellular interactions that are critical for inflammatory and thrombotic processes (12) (Fig. 1). Von Willebrand Factor (vWF) and P-selectin are released from Weibel-Palade bodies and expressed on the endothelial surface. Leukocytes express the oligosaccharide antigen sialyl Lewis-X (SLe^x), which

binds to endothelial-expressed P- and E-selectin and enables leukocytes to roll along the endothelium. Once slowed by binding to selectins, contact between leukocyte β_2 (CD18) integrins and endothelial ICAM-1 (a type of IgCAM) mediate leukocyte adhesion to and emigration through the vascular wall (14). Monocytes expressing the $\alpha_4\beta_1$ integrin undergo a similar process by binding to vascular cell adhesion molecule (VCAM). P-selectin and the integrin GPIIb/IIIa expressed on the platelet surface bind to leukocytes and facilitate the inflammatory process by forming an endothelium-platelet-leukocyte bridge (12).

Experimental Models

Increased endothelial adhesion molecule expression has been observed following experimental stroke in animal models (Fig. 2A, B) (3, 14–16). P- and E-selectins are upregulated in a similar time course, following 2 hours of transient MCAO, thrombotic, or embolic stroke in rats (17). P-selectin expression peaked at 6 hours and gradually declined to near baseline after 96 hours. E-selectin also increased by 2 hours, and peaked between 4 and 12 hours, depending on the model. E-selectin gradually declined and returned to baseline after 72 hours (17). An increase in P-selectin also was observed following permanent middle cerebral artery occlusion (pMCAO) in rats, peaking between 2 and 8 hours and returning to baseline by 1 day (16). These early changes reflect the role of selectins in the quick recruitment of inflammatory cells.

Upregulation of ICAM-1 also was observed following 2 hours of transient MCAO in rats (Fig. 2C, D). The number of ICAM-1 positive vessels increased gradually by 2 hours, peaked at 46 hours, and returned to near baseline after 96 hours (18). ICAM-1 mRNA increased gradually after tMCAO, peaked between 10 and 24 hours, and remained elevated for at least 166 hours throughout the ischemic hemisphere (18, 19). Rodent models are the most commonly used for a variety of technical reasons, but their relevance to human conditions is questionable. Baboon models of tMCAO also demonstrated increases in both P-selectin and ICAM-1 during ischemia and up to 24 hours after reperfusion. Longer time points were not examined in the baboons so the temporal profile cannot be completely compared to rodent studies. However, these data do provide evidence that the adhesion molecule response is well conserved (15).

To test the hypothesis that these vascular changes and subsequent infiltration of inflammatory cells affect stroke outcome, interventions targeting adhesion molecules have been tested in experimental stroke models. Treating rats at the start of reperfusion with the SLe^x analogue CY-1503 reduced infarct volume by 42% and the number of myeloperoxidase-positive cells within the infarct by 60% after 2 hours tMCAO (20). Using the same model, pretreatment but not post-treatment with the P-selectin antibody RMP-1 also decreased infarct volume by 70% and hemorrhagic area by 90%, compared to vehicle (21). Adhesion molecules may worsen outcome by promoting inflammatory cell movement into the CNS or by allowing them to accumulate in vessels, thereby reducing cerebral blood flow (CBF) upon reperfusion. The latter hypothesis may be responsible for the phenomenon whereby CBF remains low (or has a rebound reduction) after reperfusion. The roles of P-selectin in stroke and in reducing CBF were tested using a mouse model of 45 min tMCAO. When P-selectin null (−/−) mutants were compared to wild type (+/+), significant decreases in polymorphonuclear leukocytes (PMNs) and infarct volume as well as an increase in blood flow following reperfusion were observed. In addition, a P-selectin antibody was given to wild type mice either before or immediately following ischemia. Significant decreases in infarct volume were observed in both pretreated and post-treated animals and an improvement in blood flow was observed in the pretreatment group (22).

Similar results have been observed using antibodies against ICAM-1. Rats were infused with the anti-ICAM-1 antibody 1A29 at 1 hour and 22 hours of reperfusion following 2 hours tMCAO or 2 hours and 24 hours of ischemia during pMCAO. A significant decrease in infarct areas was observed in treated tMCAO rats but not in treated pMCAO rats compared to vehicle controls (1). ICAM-1 null (−/−) mice subjected to permanent or transient MCAO exhibited reduced infarct volumes compared to wild type (+/+). Interestingly, the decrease in infarct volume was improved using the antibody RB6-8C5 to deplete granulocytes, suggesting a potential benefit of combination therapy. While there was no difference between null and wild type mice in cortical microperfusion 15 min after reperfusion, null mice had a small but significant improvement in a semiquantitative evaluation of perfused microvessels (23). Reducing ICAM expression and subsequent neutrophil migration may be one benefit of therapeutic hypothermia (24). Hypothermia is neuroprotective in CNS injury and is believed to work through a variety of mechanisms, including attenuating inflammation (25). Such studies provide a good argument that pro-inflammatory changes in the vasculature have a detrimental effect on stroke outcome.

Clinical Studies

To evaluate whether pro-inflammatory molecules play a role in clinical stroke as they do in experimental models, investigators examined adhesion molecule expression in living stroke patients and in postmortem specimens. While adhesion molecules function on the cell surface, some are shed into the blood, and their plasma concentration can be measured using the enzyme-linked immunosorbent assay (ELISA). Plasma values of adhesion

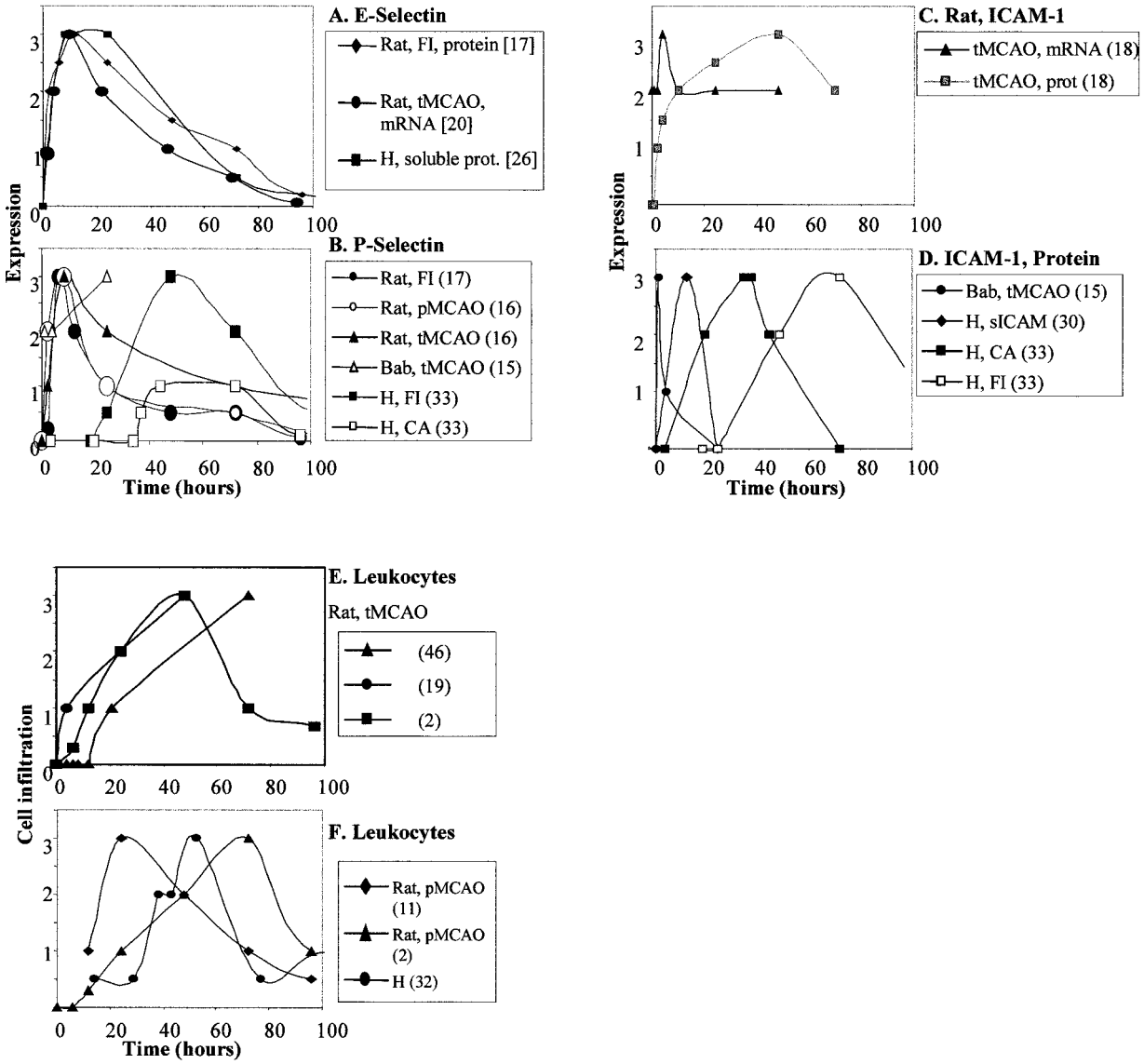

Fig. 2. Temporal profile of adhesion molecule expression (**A–D**) and leukocyte influx (**E–F**) in various stroke models. Data from different studies were examined and ranked independent of magnitude as follows: peak value = 3, high values = 2, and slightly elevated values = 1. Human studies usually included 1 patient per time point. **A:** E-selectin mRNA and protein peaked within the first 30 hours and slowly declined. **B:** P-selectin also increased early in rat and baboon (Bab) stroke models but has been reported at later time points in humans (H). **C:** ICAM-1 mRNA increased early in rats, followed by protein, which remained elevated up to 70 hours after injury. **D:** In baboon models, ICAM-1 protein expression peaked very early and quickly declined. When vessels were examined for membrane-bound ICAM-1 protein expression after cardiac arrest (CA) or focal ischemia (FI), protein expression peaked after 24 hours. Human soluble ICAM (sICAM) and ICAM-1 in baboon models peaked much earlier. **E:** Leukocyte influx after rat tMCAO began within the first day after injury but peaked between days 2 and 3. **F:** Two reports of leukocytes after rat pMCAO yielded very different results, reporting peaks either 1 or 3 days after stroke. Human values for mixed stroke types peaked between 2 and 3 days. Abbreviations: FI, focal ischemia; p (permanent) middle cerebral artery occlusion, pMCAO; t (transient) middle cerebral artery occlusion, tMCAO; CA, cardiac arrest; H, human; Bab, baboon.

molecules in stroke patients were measured and compared to those of control patients with vascular risk factors but no history of stroke. Patients with vascular risk factors had significant plasma elevations of ICAM, decreases of L-selectin, and no changes in VCAM or E-selectin compared to individuals without risk factors (26). Stroke patients had increased E-selectin between 4 hours and 1day after their stroke, and VCAM was elevated between 4 hours and 5days compared to normal controls (26). Other studies comparing stroke patients to normal controls found that soluble ICAM either increased after 1 day (27), decreased (28), or was unchanged between 2 days and 1 week (29). Soluble E-selectin also was found to have either no change in less than 1 day (27), an

increase between 2 days and 1 week (29), or decreased after 5 days compared to control (30). Apparent discrepancies in these data may have a variety of explanations including variation in the model, assays or specimen collection. Plasma levels can change because of alterations in expression and release or their binding to nonsoluble substrate such as inflammatory cells. Inflammatory cells increased expression of CD11a in both TIA and stroke patients (31). Increased CD18 was only observed in TIA patients (31). Both molecules bind to adhesion molecules, opening the possibility that decreases in soluble adhesion molecules may be due to increased binding to leukocytes, which makes interpretation of these data difficult and confusing.

By staining vessels from patients who died at different times following stroke or cerebral ischemia, a temporal profile of ICAM, P-selectin, and E-selectin expression can be constructed. Lindsberg et al reported more robust and greater numbers of ICAM stained vessels in patients who died of focal stroke between 14 hours and 6 days (32). Time points were usually based on 1 patient. Love and Barber studied 11 focal stroke and 11 cardiac arrest patients and usually had 1 patient per time point with a maximum of 3. They observed weak ICAM staining 2 to 3 days after atherothrombotic stroke and 19 to 48 hours after cardiac arrest. P-selectin was observed in patients between 1 and 7 days following atherothrombotic stroke. Staining was strong in 3 patients at 2 and 3 days. Three cardiac arrest patients had weak P-selectin staining between 37 and 48 hours following cardiac arrest. Staining was not observed at other time points (33). Future studies need to duplicate and add to these results with the hope of providing better clinical evidence of adhesion molecule expression. If adhesion molecules are upregulated, they may be useful therapeutic targets and temporal profile information may be used to guide clinical trial design. A recent trial of the murine ICAM-1 antibody Enlimomab found that treatment worsened outcome as measured by neurological score and mortality (34). A follow up study using the murine anti-rat ICAM-1 antibody in rats also found an increase in infarct volume and no efficacy. While neutrophil adhesion was reduced, immune activation in response to the foreign protein probably accounted for the experimental and clinical results (35).

BLOOD BRAIN BARRIER PERMEABILTY AND MATRIX METALLOPROTEINASES

BBB permeability may be enhanced and sometimes initiated by inflammatory processes. It may occur acutely (immediate), early (min-hours), or delayed (days) following injury (36). Acute changes were observed immediately following thromboembolic stroke (7, 37) and are likely due to direct mechanical damage to the vasculature. Many brain regions exhibited transient BBB disruption, but infarcts remained leaky for at least 7 days (7). Once injury has occurred, inflammatory molecules such as bradykinin, histamine, platelet-activating factor, cytokines, and matrix metalloproteinases (MMPs) are released from a variety of different cells. These factors may be responsible for some of the delayed permeability changes observed in stroke models (36). Once the BBB degrades, inflammatory cells can move more easily into damaged areas (6). These cells also release inflammatory molecules that further alter BBB permeability.

MMPs are enzymes that break down components of the extracellular matrix (ECM). MMPs are released as a zymogen and are activated by cleavage into their active form. They have been widely studied in cancer biology as potential mediators of metastasis and angiogenesis. Their involvement in angiogenesis suggests potential beneficial roles in tissues exposed to ischemia. New vessel growth may improve collateral flow and protect brain regions against future ischemic episodes. In the short term, however, BBB permeability and ECM degradation promotes inflammation and edema.

MMP-9 and MMP-2 have been studied in stroke models and in the plasma of patients. MMPs are studied using the traditional techniques of immunohistochemistry and Western blotting as well as gel zymography to measure activity. In some models of focal ischemia, MMP-9 activity increases early (15–48 hours), followed by MMP-2 (5 days) (38, 39). The activity of MMPs was associated with BBB permeability (38) as well as with neutrophil and macrophage accumulation (39). Demonstrating the potential role of MMPs in focal ischemia, an MMP-9 blocking antibody administered 1 hour before MCAO reduced infarct size at 24 hours (39). Plasma concentrations of MMP-9 were observed in stroke patients between 12 and 48 hours following stroke. A positive correlation was noted between MMP level, infarct volume and final NIHSS score. MMP-2 also was examined in this study, but no association between stroke severity and expression was noted. The authors suggest that MMP-2 may correlate more with neovascularization after injury, while MMP-9 may be primarily involved with edema (40).

LEUKOCYTES

Pathophysiology

Leukocytes may contribute to stroke damage by reoccluding vessels after reperfusion or by entering infarcted tissue and exacerbating cell death through cytotoxic interactions. To establish a pathological role for leukocytes, they must be found at the lesion site while the infarct is getting worse and there should be an association between the numbers of leukocytes and infarct severity such that leukocyte depletion results in reduced cell death or infarct volume and vice versa (41). A thorough review by Emerich et al (41) concluded that no study has successfully demonstrated that leukocytes satisfy any criterion for causing cell death or increased infarction due, in

part, to a lack of sufficient controls. However, many investigators have found suggestive evidence that leukocytes contribute to infarction.

Leukocyte Contributions to the CBF Reduction

Evidence that leukocytes may be responsible for reducing CBF in experimental models consists of observations that 1) pro-inflammatory adhesion molecules are upregulated on the vascular surface and on leukocytes (discussed in the previous section); 2) leukocytes more readily adhere to the vasculature following stroke; and 3) inhibiting leukocyte adherence improves CBF. In vivo microscopy through an open cranial window was used to observe fluorescently labeled leukocytes aggregating in and adhering to vessels following 2 hours of transient MCAO in rats (42). At 15, 30, and 60 min after reperfusion, significant increases in leukocyte adhesion to postcapillary venules were observed. There also was a significant but not as robust increase in adhesion within capillaries and arterioles after 30 min of reperfusion. Blood-cell velocities were decreased during reperfusion, associating CBF changes with leukocyte accumulation (42).

Evidence of leukocyte adhesion after transient MCAO in the baboon suggests that these mechanisms may be applicable to patients. To identify occluded vessels, animals were perfused at high pressures and electron micrographs of leukocyte-endothelial contact were examined for the presence of adhesions. After 1 hour of reperfusion, individual PMNs and aggregates were observed occluding capillaries and larger vessels. Platelet and fibrin plugs were seen often on one or both sides of leukocyte ''occlusions,'' lending more support to the hypothesis that these cells were actually occluding vessels for some length of time. Roughly 30% to 40% of microvessels did not reperfuse relative to the contralateral side (10). Treatment with antibodies to integrin β_2 (CD18) prior to reperfusion improved CBF in vessels with diameters between 7.5 and 50 μm. Neurological score was evaluated in baboons with and without the antibody but no difference was noted. However, the observation period in this study was only 1 hour following reperfusion, and benefits in neurological score may be delayed (9). Rabbits pretreated with anti-neutrophil antibodies before embolic stroke had significantly increased regional CBF and reduced infarct volume, suggesting that leukocyte adhesion or influx may increase pathology (4).

Leukocyte Influx

If PMNs were to enter the brain or spinal cord, the production of bacteriocidal products, including proteases, phospholipases, nitric oxide (NO), hydrogen peroxide, and superoxide (O_2^-), could worsen pathology by damaging tissue that survived the initial ischemic insult. Histological analysis of rat brains after permanent MCAO demonstrated that leukocytes do migrate into the infarcted region. PMN influx peaked 24 hours after ischemia when 0.5 to 3.5 PMNs per $\times$1,000 microscopic field were observed. The number of monocyte/macrophages increased gradually to about 13 per field at 168 hours (11).

PMNs are observed within the brain parenchyma but it is unclear whether they are present in numbers sufficient to be detrimental and whether they are producing cytotoxic substances (Fig. 2E, F). To address this question, infarct volume and measurements of cytotoxic substances were compared in animals with and without neutrophil depletion. Matsuo et al used the formation of ascorbyl radical to assess the production of free radicals within the ischemic hemisphere of rats after transient MCAO. They found that neutrophil depletion by anti-neutrophil antibody administration prevented the surge of oxygen radicals (43), decreased myeloperoxidase activity within the infarct region (the enzyme that converts hydrogen peroxide to hypochlorous acid) and reduced water content and infarct size (44). The CD11b/CD18 ligand known as neutrophil inhibitory factor (NIF) was studied in rat models of permanent and transient MCAO. Improvements in infarct volume and neurological outcome were observed when NIF was administered before and up to 2 hours after reperfusion (45). It is difficult to distinguish rheologic from parenchymal effects of neutrophil depletion. This common complication has been pointed out by a number of authors (41, 43) and would require additional studies to definitively associate PMN influx with aggravated pathology. However, not all studies found evidence that PMNs are involved in worsening pathology. Using models of partially reperfused focal ischemia with neutrophil depletion, Hayward et al (46) found that very few PMNs migrated into the lesion and did so after infarct volume was established. They also found no benefit to neutrophil depletion in their model (Fig. 2E).

Human Studies

Investigators have examined blood, CSF, and ex vivo tissue samples for signs of a leukocytic response in stroke patients. To test the hypothesis that generalized inflammation influences the development of stroke, the prevalence of prior infection was measured in stroke patients compared to neurological patient controls and was approximately 3-fold higher in patients the week prior to a stroke (47). Other observations also suggest detrimental roles for generalized inflammation. Increased white blood cell count was a weak but significant predictor of fatal brain swelling with an odds ratio of 1.08 (48). A positive linear correlation was observed between infarct size and peripheral blood PMN count (49). Leukocytes from stroke patients express adhesion molecules that manifest

as greater tendencies to aggregate and adhere to substrates such as fibronectin and laminin. Seventy-one percent of patients with a major stroke (>7.5 on the Canadian Neurological Scale) had leukocytes with an abnormally elevated tendency to aggregate compared to only 19% of those with a minor stroke. Both major and minor stroke groups had increased platelet and leukocyte aggregation over matched controls (28, 50).

Leukocyte infiltration was studied in living stroke patients using single-photon emission computed tomography and leukocytes labeled with technetium-99m hexamethylpropyleneamine oxime. The asymmetry index between ipsilateral and contralateral sides was measured up to 11 weeks after acute stroke and in chronic stroke patients. Significant differences were noted between the chronic and acute stroke patients within the first week although asymmetry tended to persist for up to 5 weeks (51). These data do not distinguish between leukocytes within the vasculature or parenchyma but do suggest that leukocytes accumulate after stroke in living patients. CSF analysis from stroke patients provided early evidence that leukocytes cross the BBB and that different types of stroke have their own vulnerabilities to inflammatory responses. Patients were divided into those having infarcts with or without collaterals, embolic infarcts (assumed to have a hemorrhagic component), or lobar hematoma. PMNs were observed in infarcts with collaterals, embolic and lobar hematomas but not in infarcts without collaterals. Numbers of PMNs increased between 1 and 2 days, peaked at 3 days, and returned to baseline by 2 weeks. The peak increase for embolic and pale infarcts was about 5 cells per ml while lobar hematomas peaked at 1,000 cells/ml. Comparisons of leukocyte numbers in CSF or infarcted tissue from patients that died during the study suggested that CSF cytology reflected intraparenchymal leukocyte accumulation. In addition, histopathology verified that hemorrhagic infarcts and lobar hematoma contained greater numbers of PMNs than the other stroke types (52). It is apparent from human data that leukocytes are active following stroke, accumulate in the brain, and migrate into the parenchyma. Whether these cells are present in sufficient numbers to cause clinically relevant pathology remains to be determined.

MICROGLIA

Pathophysiology

The intrinsic neuroinflammatory response is mediated by microglia and perivascular cells. Perivascular cells reside around blood vessels and constitutively express markers identifying them as resident macrophages. Microglia are generally considered derived from the monocytic lineage. They reside in a ''resting'' but vigilant state throughout the parenchyma where they monitor the extracellular milieu for changes signifying injury. In the resting state, microglia express few surface molecules identifying them as potential phagocytes. Initial activation involves morphological changes and upregulation of the surface molecules isolectin-B4, CR3, and microglial response factor-1 (MRF-1) (53). In this partially active state, microglia proliferate and migrate to sites of injury but are not phagocytic and do not produce cytotoxic molecules. In the presence of dying neurons, microglia become true phagocytes, expressing the same markers as peripheral macrophages, including MHC class I, MHC class II, and leukocyte common antigen. In this state they are virtually indistinguishable from peripheral macrophages (54).

Whether the microglial response to injury is beneficial or detrimental is a topic of debate. Microglia produce toxic molecules such as NO, oxygen radicals, arachidonic acid derivatives, and cytokines. They are able to kill neurons in vitro, however, their transition to cytotoxic phagocytes is closely regulated and the opportunity to kill normal neurons in vivo may not arise. In areas of severe damage, microglia can phagocytose debris and attack microorganisms that may infect the damaged area. In addition, microglia produce neurotrophins in vitro (53), as well as TGF-β1 and plasminogen, which are involved with tissue repair and neurite outgrowth (54).

Global Ischemia

Microglia become activated within minutes following transient global ischemia and proliferate in the cortex and striatum. This early proliferation is independent of injury severity. 5-bromo-2′-deoxyuridine-5′-monophosphate (BrdU) intercalates into the DNA of dividing cells and serves as a marker of cell proliferation. In one study, BrdU was administered before sublethal (2.5 min) ischemia to evaluate the potential role of dividing cells in ischemic preconditioning of the CA1 hippocampus. Microglia (labeled with G4-isolectin) were observed in their activated state, undergoing cell division after sublethal ischemia but were found in the cortex and striatum, not in the hippocampus (55). During lethal global ischemia, microglia activate throughout the brain and accumulate in the damaged hippocampus as phagocytic cells. Activation decreases in surrounding areas but peaks within the damaged hippocampus between 4 and 6 days (3).

Focal Ischemia

Microglia within the ischemic core are likely killed during the ischemia. By 1 hour of reperfusion following transient MCAO, amoeboid cells (probably activated microglia) were observed in areas of selective neuronal ''shrinking'' and not in areas of neuronal loss (56). Round and amoeboid cells were first observed in the ischemic core between 2 and 4 hours but were notably increased 10 hours and continued to increase between 70 and 166

hours after reperfusion (56). These probably represent migrating microglia from the penumbra. To distinguish between the contribution of microglia and peripheral macrophages to neuroinflammation, dichloromethylene diphosphonate containing liposomes were used to deplete peripheral macrophages before photothrombotic cerebral infarction. Using ED1 to mark activated microglia and macrophages, the numbers of ED1$^+$ cells were no different between macrophage depleted and control animals after 3 days of reperfusion. Macrophage-depleted animals had significantly reduced numbers of ED1$^+$ cells after 6 days of reperfusion (57). Activated microglia seem to migrate into damaged regions early on, followed by macrophages at later time points. Photothrombotic infarction is a permanent model of vascular thrombosis so macrophages may infiltrate earlier with reperfusion.

Experiments that prevent microglial activation have attempted to determine whether these cells are beneficial or detrimental in experimental stroke models. Minocycline has been tested as a neuroprotective agent and attenuates microglial activation. Minocycline treatment before and after global ischemia yielded greater than 7-fold improvement in the number of surviving neurons (58). Treatment with minocycline reduced infarct volume following embolic stroke (5) and focal ischemia (59). Therapeutic hypothermia decreased injury and microglial activation following transient forebrain ischemia (60) but not after embolic stroke (5). Discrepancies in studies using hypothermia may be due to differences in the rewarming phase. Gradual rewarming may result in reduced pathology when abrupt rewarming may yield no improvement (61). However, it is not clear that the neuroprotective effects of minocycline or hypothermia are due to reduced microgliosis or effects on neurons themselves.

Human Studies

Human studies have begun to address the location and temporal profile of microglia and macrophage accumulation. [^{11}C]PK1195 (1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide) is a ligand for the peripheral benzodiazepine binding site that is predominately found on activated microglia and macrophages. Using positron emission tomography and T1-weighted magnetic resonance imaging (MRI), Gerhard et al measured increased [^{11}C]PK1195 binding in the infarcted hemisphere of stroke patients (62). Regions of increased binding extended beyond the lesioned area identified by T1-weighted MRI and was observed between 5 and 53 days after injury. This technique may be useful to further elucidate the progression of microgliosis and macrophage accumulation in a variety of neurological diseases. More classical techniques to study inflammation ex vivo have also been reported. Tomimoto et al (63) used immunohistochemistry to rank expression of cytokines and glial markers as none, trace, moderate, or marked in the brains of control, Alzheimer disease, and infarcted patients. Infarcted brains were observed to have marked and moderate increases more often than control and Alzheimer diseased brains. Most lesions with a known duration from injury were between 12 and 40 days. The longest interval between injury and death in this study was 15 months, and marked expression of glial markers was still reported in this patient (63). An immunohistochemistry study by Schwab et al (64) used interleukin-16, morphology, and double labeling to identify inflammatory cells in control and infarcted brains. Intravascular neutrophils were observed between 1 and 2 days after infarction and at later time points were observed infiltrating the parenchyma. Between days 3 and 4, IL-16$^+$ cells were identified as either perivascular lymphocytes or parenchymal monocytes. They remained elevated weeks and months after infarction. IL-16$^+$ cells were observed in infarcted and surrounding areas but areas remote from the infarct did not stain above control levels (64). These studies all support the conclusion that microglial activation and/or macrophage accumulation occurs early after injury and they remain activated for weeks and perhaps months after injury. Additionally, these studies showed evidence of active cells in the infarct core as well as peri-lesional areas. What remains to be determined is whether therapies targeted to these phenomena improve neurological outcome.

There are some reasonable, theoretical arguments and experimental evidence to support a beneficial role of microglia and macrophages following brain injury. If the infarct core is not cleaned up, infection becomes more likely, lipid peroxidation may produce free radicals, and other cytotoxic molecules from dying cells may damage adjacent tissue. Rapalino et al demonstrated the beneficial effects of macrophages in a rat model of spinal cord transection (65). They injected autologous macrophages preexposed to peripheral nerve segments into the transected spinal cord and observed a partial recovery of motor function and nerve fibers transcending the injury site in the treated animals, suggesting that macrophages participate in recovery processes (65).

Summary

There is substantial evidence that inflammation occurs following clinical and experimental stroke and is composed of vascular, hematogenous, and intrinsic responses. The upregulation of adhesion molecules on the vascular surface supports both inflammatory cell adhesion and influx, although the clinical importance of these processes is not resolved. While inflammatory cells may potentially cause harm by producing cytotoxic molecules, microglia and macrophages may be beneficial through their roles in repair and scavenging necrotic debris. Finally, neuroprotective strategies such as hypothermia attenuate the

inflammatory response, but whether reduced inflammation is cause or effect needs to be determined. A better understanding of the time course and consequences of neuroinflammation may aide in therapeutically promoting beneficial and reducing harmful aspects of neuroinflammation.

ACKNOWLEDGMENT

The authors thank Jenny Altman for generously donating the artwork for Figure 1.

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