CD18-Mediated Neutrophil Recruitment Contributes to the Pathogenesis of Reperfused but Not Nonreperfused Stroke

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Background and Purpose—Neutrophil (PMN) recruitment mediated by increased expression of intercellular adhesion molecule-1 expression (ICAM-1, CD54) in the cerebral microvasculature contributes to the pathogenesis of tissue injury in stroke. However, studies using blocking antibodies against the common β2-integrin subunit on the PMN, the counterligand for ICAM-1 (CD18), have demonstrated equivocal efficacy. The current study tested the hypothesis that mice deficient in CD18 would be protected in the setting of reperfused but not nonreperfused stroke.

Methods—Two groups of mice were studied, those whose PMNs could express CD18 (CD18+/+) and those mice hypomorphic for the CD-18 gene (CD18−/−). PMNs obtained from CD18−/− or CD18+/+ mice were fluorescently labeled and tested for binding to murine brain endothelial monolayers. Using a murine model of focal cerebral ischemia in which an occluding suture placed in the middle cerebral artery (MCA) is removed after 45 minutes (transient ischemia, reperfused stroke) or left in place (permanent ischemia, nonreperfused stroke), cerebral infarct volumes (% ipsilateral hemisphere by TTC staining), cerebral blood flow (CBF, % contralateral hemisphere by laser-Doppler flowmetry), and survival (%) were examined 24 hours after the initial ischemic event. Adoptive transfer studies used 111In-labeled PMNs (from either CD18+/+ or CD18−/− mice) to examine the relative accumulation of PMNs in the ischemic region.

Results—PMNs obtained from CD18−/− mice exhibit reduced adhesivity (compared with CD18+/+ PMNs) for both quiescent and cytokine-activated endothelial monolayers. CD18−/− mice (n=14) subjected to transient focal cerebral ischemia demonstrated a 53% decrease in infarct volumes versus CD18+/+ mice (n=26, P<0.05), improved penumbral CBF at 24 hours (1.8-fold, P=0.02), and a 3.7-fold decrease in mortality (P=0.02). However, when CD18−/− mice (n=12) were subjected to permanent focal cerebral ischemia, no differences were noted in infarct volume, mortality, or CBF versus similarly treated CD18+/+ mice (n=10). There was a greater accumulation of CD18+/+ PMNs in the ischemic zone of CD18+/+ animals than CD18−/− animals subjected to reperfused stroke (82% increase, P=0.02), although there was no difference between groups when subjected to permanent MCA occlusion.

Conclusions—Deficiency for the CD18 gene confers cerebral protection in a murine model of reperfused stroke, but this benefit does not extend to CD18-deficient animals subjected to permanent MCA occlusion. These data suggest that anti-PMN strategies should be targeted to reperfused stroke and may perhaps be used in conjunction with thrombolytic therapy that establishes reperfusion. (Stroke. 1999;30:1110-1117.)

Key Words: antigens, CD18 ■ endothelium ■ leukocytes ■ reperfusion ■ stroke, experimental ■ mice

When a patient suffers an ischemic stroke, there are relatively few established therapies that can be initiated immediately and have been proved to improve outcome. Initial studies of embolic stroke in rabbits showed that lysing thrombi could reduce neurological damage.1 Recent clinical trials have shown early reperfusion to be beneficial in terms of reducing long-term morbidity.2,3 Local intraarterial thrombolysis with recombinant prourokinase has shown initial promise in small, early trials if given within a 4-hour window,4,5 although, as with all thrombolytic agents, intracerebral hemorrhage remains a concern. Streptokinase increases mortality in ischemic stroke.6 Although timely reperfusion can rescue jeopardized brain tissue, there are theoretical risks attendant with reperfusion in addition to the recognized risk of hemorrhagic conversion. In the highly vulnerable reperfusion period, recruited neutrophils release a veritable firestorm of reactive oxygen intermediates, corrosive acids, and cytolytic enzymes.7 Recent evidence suggests that reperfusion may be deleterious in certain circumstances, leading to an increase in myocardial8 or cerebral9 infarct size.

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In one report of reversible unilateral middle cerebral artery/common carotid artery occlusion in the rat, up to 72% of the ischemic damage to the brain was a consequence of reperfusion injury. However, because of the recent recognition of the primacy of timely reperfusion in cerebral tissue salvage in the face of an ischemic event, it becomes imperative to understand mechanisms through which potential toxicities of the reperfusion milieu can be diminished.

There are recent data showing that P-selectin and ICAM-1, potent neutrophil adhesion receptors whose expression is increased on the surface of postischemic cerebral endothelial cells, participate in the pathogenesis of both neutrophil recruitment and cerebral tissue injury in reperfused stroke. However, there is limited data to indicate whether inhibiting P-selectin- or ICAM-1-mediated leukocyte recruitment may be beneficial in strokes that fail to reperfuse. Patients usually present after the safety window for thrombolytic intervention, and it is therefore important to understand whether mechanisms that protect in reperfused stroke might enable salvage of jeopardized penumbral tissue in strokes that do not reperfuse. In a recent clinical trial in which a blocking antibody to human ICAM-1 was administered within 6 hours after stroke, no therapeutic benefit was observed and the trial was aborted (Reference 12 and Stephen Polmar, oral communication). The reasons for this clinical failure are unclear, but one reason this trial may have failed to demonstrate a beneficial effect of anti-leukocyte adhesion therapy is that the majority of the patients did not reperfuse (Stephen Polmar, personal communication).

ICAM-1 mediates firm neutrophil arrest to activated endothelial cells by binding to $\beta_2$-integrins, heterodimeric adhesion receptor glycoproteins expressed on the neutrophil surface. CD18 is the common $\beta_2$-subunit located on the neutrophil surface (common to both LFA-1 [CD11a/CD18] and Mac-1 [CD11b/CD18]) and is responsible for ICAM-1-mediated leukocyte adhesion to endothelial cells. Its role in the pathogenesis of ischemic cerebral damage has not been clearly defined. In animal models of cerebral or spinal stroke, administration of blocking antibody to CD18 either improved outcome or had no effect on outcome. Based on the recent identification of the importance of ICAM-1 in the pathogenesis of reperfused stroke, we hypothesized that mice deficient in CD18 would be protected from cerebral ischemia; furthermore, given the negative data in the EnlimoMab trial, we hypothesized that the beneficial effects of CD18 deficiency would be most apparent in a model of reperfused (compared with nonreperfused) stroke. To test these hypotheses, deletion mutant mice (hypomorphic for CD18) were used to study the effects of focal cerebral ischemia with or without reperfusion.

Materials and Methods

Animals

Adult male C57BI/6J mice homozygous null for the Igkb2 mutation (C57BI/6J-Igkb2tm1Bay, derived from the tenth generation of backcrossing with C57BI/6J mice, and therefore comprised of 99.8% of the C57BI/6J genotype) and controls (C57BI/6J) were obtained from The Jackson Laboratory (Bar Harbor, Maine). The C57BI/6J-Igkb2tm1Bay mice are hypomorphic homozygotes for the CD18 allele; throughout this paper, these mice will be referred to as CD18 --/--; C57BI/6J mice will be designated CD18 +/+ . All mice were between 7 and 10 weeks of age and weighed between 23 and 32 grams at the time of surgery. The latter part of the C57BI/6J-Igkb2tm1 designation is one of nomenclature: Igkb2=integrin beta 2; tm1=targeted mutation; 1; Bay=Baylor, the institution where the mice were created.

Middle Cerebral Artery Occlusion

Mice were subjected to transient and permanent middle cerebral artery (MCA) occlusion through procedures that were approved by the university’s Animal Care and Use Committee and are similar to those which have been recently reported in detail. Briefly, mice were anesthetized with an intraperitoneal injection of 0.3 mL of a combination of ketamine (10 mg/mL) and xylazine (0.5 mg/mL). A rectal temperature probe (Yellow Springs Instruments) connected via thermocouple to an infrared heat source was used to maintain a core temperature of 36°C to 38°C during the perioperative period. With the animal in the supine position, a 1-cm midline incision was made and the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were identified and exposed. With the aid of an operating microscope (10–40× zoom, Leica), the CCA was isolated with 4–0 silk sutures, and proximal and distal control of blood flow was obtained by applying gentle traction to the silk sutures, effectively occluding the CCA. After cautery and transection of the ECA, an arteriotomy was made on the proximal stump, and a heat-blunted nylon suture was introduced into the proximal lumen. The suture tip was then advanced up the ICA to the origin of the right MCA, after which the arteriotomy site was cauterized, and traction on the CCA was released. Total carotid occlusion time was <2 minutes in all cases. Transcranial measurements of relative cerebral blood flow (CBF, as described below) were used to confirm occlusion of the MCA. Occlusion was considered to be technically adequate if $\geq$50% reduction in relative CBF was observed immediately after placement of the intraluminal occluding suture.

In animals undergoing permanent MCA occlusion, the intraluminal MCA suture was left in place, all skin incisions were closed with surgical staples, and the animal was placed in an incubator to maintain core temperature at a constant 37°C for 90 minutes during the animal’s recovery from surgery and anesthesia. Animals undergoing transient MCA occlusion were maintained at a core temperature of 37°C with the occluding catheter in place. After 45 minutes of ischemia, CBF was again assessed, the catheter was withdrawn, and the arteriotomy site was cauterized. Reestablishment of blood flow to the MCA distribution was ascertained by laser Doppler flowmetry. All incisions were subsequently closed with surgical staples; the animals were maintained at 37°C and allowed to recover from the effects of anesthesia in an incubator for 90 minutes. All animals were then returned to their respective cages and given free access to food and water.

Quantitation of Cerebral Infarct Volumes

On postoperative day 1 (24 hours postoperatively), the mice were anesthetized, relative CBF measurements were again evaluated, and the animals were sacrificed by rapid decapitation. One mm thick coronal brains sections were cut using a mouse brain matrix (Activation Systems, Inc), and sections were immersed in 2% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma Chemical Co) in 0.9% saline solution, as described. Serial sections were then photographed adjacent to a 1-cm reference bar, and areas of infarction were traced by a member of the team blinded to the experimental conditions. Through the use of computerized imaging software (NIH Image), total volume of infarcted tissue was expressed as a percentage of total right hemispheric volume. Although this method for calculating infarct volumes may overestimate infarct volumes because of swelling of the ipsilateral brain, especially during the early period (<24 hours) under study, all animals were treated identically and all infarct volumes calculated in the same manner.
Evaluation of CBF
CBF evaluations were performed using a 0.7-mm, 70-cm long straight laser Doppler flowmeter (Perimed, Inc.). After reflecting the skin overlaying the translucent calvarium in anesthetized animals, both cerebral convexities were visualized. By placing the probe over the right and left hemispheres perpendicular to the calvarial surface 2 mm posterior to the bregma, 6 mm lateral to the midline (designated “core”), and 3 mm lateral to the midline (designated “penumbra”), relative blood flow measurements were obtained. Measurements were then obtained using these rigid coordinates so that they would be objective and reproducible, but due to slight variations in infarct regions, they may not represent true core or penumbral regions as defined by other techniques. Measurements were obtained on all animals before MCA occlusion, immediately after introduction of the intraluminal suture, and immediately before sacrifice. In instances where animals were subjected to transient focal cerebral ischemia, additional measurements were obtained before removal of the occluding intraluminal suture and again immediately after MCA perfusion was reestablished. Data are expressed as the ratio of the Doppler signal intensity of the ischemic compared with that of the nonischemic hemisphere.

Neurological Examination
Twenty-four hours after MCA occlusion and reperfusion, before being given anesthesia, mice were examined for neurological deficit by use of a 4-tiered grading system:18 A score of 1 was given if the animal demonstrated normal spontaneous movements; a score of 2 was given if the animal was noted to be turning to the right (clockwise circles) when viewed from above; a score of 3 was given if the animal was observed to spin longitudinally (clockwise when viewed from the tail); and a score of 4 was given if the animal was crouched on all fours, unresponsive to noxious stimuli. This scoring system has been previously described in mice.18

Preparation and Administration of 111In-Oxine-Labeled Murine Neutrophils (PMNs)
Homogenous C57B1/6J-Igh2m1B8ay and wild type C57B1/6J mice were anesthetized, and 0.5 to 1.0 mL of blood was withdrawn from each mouse by percutaneous intracardiac puncture with a 22-gauge needle and transferred to a sterile test tube with 0.1 mL sodium citrate at room temperature. Blood was then diluted 1:1 with PBS, transferred to a 15-mL conical tube containing Ficoll-Hypaque (Pharmacia LKB Technology), and centrifuged at 1800 rpm for 20 minutes at room temperature. The buffy coat was then gently transferred to a second conical tube and centrifuged at 1400 rpm for 15 minutes at 4°C. The supernatant was aspirated, and red blood cells were subjected to hypotonic lysis; the remaining cells were then resuspended in PBS. The sample was centrifuged at 1200 rpm for 12 minutes at 4°C, the supernatant was decanted, and the hypotonic lysis step was repeated until the specimen was free of erythrocytes. The leukocytes were then resuspended in PBS to a count of 5 to 7.5×106 cells/mm³ and incubated at 37°C for 30 minutes with 100 μCi 111In-oxine (Amersham Medipysics). The neutrophils were then centrifuged at 1800 rpm for 5 minutes and washed 3 times with PBS at 37°C. The neutrophils were then resuspended to a final concentration of 1.0×106 cells/mL. Final counts were adjusted to the corresponding parametric volume (approximately 3×10⁶ cells/mL). Neutrophils were then administered to each mouse by percutaneous intracardiac injection. Cerebral blood flow (CBF) was measured 30 minutes after injection.

Data Analysis
CBF, infarct volumes and 111In-PMN deposition were compared using the Student’s t test for unpaired variables. Neurological deficit scores were compared using the Mann-Whitney U test. Survival analysis was tested using contingency analysis with the Chi square statistic. Values are expressed as the mean±SEM, with a P<0.05 considered statistically significant.

Results
In order to determine the specific role of CD18 in the pathogenesis of tissue injury in stroke, CD18+/− mice were used. Because there can be variations in cerebrovascular anatomy between strains of mice which can affect the severity of cerebral ischemic tissue damage,21 initial experiments were performed to ascertain that the CD18+/− mice did not have grossly detectable variations in cerebrovascular anatomy. For these experiments, an antemortem intravascular injection of India Ink was used to define the Circle of Willis and its principal branches. These experiments showed, from a gross morphologic perspective, that for both CD18+/+ and CD18+/− mice, there is a complete Circle of Willis with no evidence of aberrant communicating vessels between the
that primary neutrophils, obtained from CD18
2
monolayers; values shown are mean
nm. The results shown represent the data from 5 endothelial cell
in response to application of an excitation wavelength of 485
nm. After a series of repetitive washes, adherent
1
1
CD18
cells were detected based on the emission of 530 nm light
the endothelial cells. Figure 1.

Figure 1. CD18-dependent binding of neutrophils to quiescent
or activated endothelium. Murine brain endothelial cells21 were
grown to confluence on a 24-well plate and either left unstimu-
ated (Control) or exposed to recombiant murine IL-1b for 20
hours. Neutrophils were harvested from either CD18 −/− or
CD18 +/+ mice, fluorescently labeled, and coincubated with
the endothelial cells. Because CD18 is expressed on the neutrophil surface, in
counterligand ICAM-1, which is an integral
endothelial membrane protein, the next set of experiments
were performed to demonstrate that CD18 −/− neutrophils
do indeed exhibit diminished capacity to bind to both quies-
cent endothelium, as well as endothelium which has been
activated with IL-1, a known potent inducer of ICAM-1 on
the endothelial cell surface.24 These studies [Figure 1] show
that primary neutrophils, obtained from CD18 −/− mice,
have a diminished capacity to bind to murine brain endothel-
ial cells21 both under resting conditions and after endothelial
cells have been stimulated with IL-1.

To elucidate the role of CD18 in stroke, radiolabeled
PMNs were infused into mice immediately prior to stroke,
and their relative accumulation into the ischemic hemisphere
quantified. The MCA of CD18 +/+ and CD18 −/− mice
was transiently occluded (for 45 minutes) and then allowed
to reperfuse for the duration of the 24 hour observation period.
There was a significant accumulation of radiolabeled CD18
+/+ PMNs in the ipsilateral hemisphere of both CD18 +/+ and
CD18 −/− mice (ratio>1.0). However, accumulation
was greater in the CD18 +/+ mice [Figure 2, comparison A].
In contrast, when the MCA occluding suture was left in place
to create a permanent model of stroke, at the same 24 hour
time point, there was less overall PMN accumulation and no
significant difference in relative 111In-CD18 +/+ PMN
accumulation in the ipsilateral hemisphere between CD18 +/+ and
CD18 −/− mice [Figure 2, comparison B]. To assess the
effect of different ischemic conditions, mice were subjected
to either transient or permanent MCA occlusion (Figure 2,
comparisons C & D). These data show that transient MCA
occlusion is associated with greater ipsilateral PMN accumu-
lation than following permanent MCA occlusion. Taken
together, these data lead us to conclude that (1) CD18 −/−
mice demonstrate reduced accumulation of PMNs compared
with CD18 +/+ animals subjected to transient ischemia, and
(2) transient cerebral ischemia with its associated reperfu-
sion causes a greater accumulation of neutrophils in the isch-
emic hemisphere than under conditions of permanent MCA
occlusion.

These experiments, in which CD18-expressing PMNs ac-
umulated to a lesser degree in CD18 −/− than CD18 +/+ animals [Figure 2, comparison A], suggested to us that there
is a role for neutrophil-induced neutrophil recruitment; the
defect in CD18 expression on native neutrophils in CD18
−/− animals would not otherwise be expected to alter the
recruitment of CD18 +/+ neutrophils to the ischemic cere-
bral microvasculature, because the CD18 −/− animals are
not deficient for the CD18 counterligand, ICAM-1, or other
adhesion receptors. Initial experiments using labeled CD18
−/− PMNs showed reduced accumulation compared with
labeled CD18 +/+ PMNs [Figure 2, comparisons E & F],
confirming the relative importance of CD18 expression in
PMN recruitment in stroke (as was shown in comparison A).
mechanisms of neutrophil recruitment, we next performed adoptive transfer experiments using radiolabeled CD18−/− PMNs. Because these CD18-deficient PMNs provide the label (and therefore, are the only cells whose accumulation is tracked), the most likely explanation for their recruitment to ischemic foci is their adhesion via non–CD18-dependent mechanisms. When CD18−/− PMNs were infused just prior to transient MCA occlusion [Figure 2, open bars], the relative accumulation of neutrophils in the ipsilateral hemisphere exceeded unity in both CD18−/− and CD18+/+ mice, suggesting the participation of non–CD18-dependent adhesive mechanisms (eg, selectins) in the capture of neutrophils.

**Stroke Outcome**

The next series of experiments was designed to study the functional significance of CD18 expression in stroke. For these experiments, measurements were made of CBF as well as infarct volumes in both CD18+/+ and CD18−/− mice. Preoperative relative CBFs, measured as the ratio of ipsilateral to contralateral Doppler signals, was similar for both CD18−/− and CD18+/+ mice [Figure 3, “Preop”]. Both groups demonstrated reduction of blood flow which exceeded 50% at the time the suture was placed at the level of the MCA [Figure 3, “Occlusion”]. After 45 minutes of occlusion, the MCA occluding suture was withdrawn, the animal was turned prone, the Doppler probe was positioned, and relative CBF was recorded [Figure 3, “Reperfusion”]. Even at this relatively early time point, there was a tendency for relative CBF to be higher in the CD18−/− animals than the CD18+/+ controls. By the time of sacrifice at 24 hours, this difference became more pronounced and statistically significant in the penumbral region [Figure 3, “Sacrifice”].

To establish the overall pathophysiological significance of CD18 expression in stroke, cerebral infarct volumes were calculated. In transient cerebral ischemia, there was a marked (53%) reduction in cerebral infarct volumes in CD18−/− mice compared with CD18+/+ mice (P<0.05; Figure 4). This reduction in infarct volumes in the CD18−/− mice was accompanied by a reduction in mortality (3.7-fold reduction in mortality, P<0.02 versus CD18+/+ mice). In contrast, when mice were subjected to permanent cerebral ischemia, no differences were noted in either infarct volumes or mortality between the two groups [Figure 5]. When mice were examined for neurological deficit (prior to anesthesia and sacrifice) at the 24-hour time point, there was a trend toward reduced neurological deficit in the CD18 null mice for both transient and permanent middle cerebral artery occlusion [Figure 6].

**Discussion**

Safe therapeutic options for the treatment of evolving stroke are extremely limited. Although reports by the NINDS2 and ECASS3 suggest that establishing early reperfusion with thrombolytic agents may help reduce the neurologic morbidity and mortality of a thrombotic or embolic stroke, the window of opportunity for therapeutic administration is exceedingly narrow. There are clearly theoretical advantages to establishing reperfusion (ie, restoration of oxygen and nutrient delivery to ischemic cerebral tissue), yet there is also the possibility that reintroduction of blood and blood-borne cells (such as PMNs) can exacerbate damage due to ischemia per se.9 There are numerous mechanisms by which PMNs can...
PMNs rapidly upregulate surface CD18 expression,\(^{25,26}\) the lumen and by stiffening on activation. In fact, activated obstruction of flow, due to adhesion to microvascular endothelium and by stiffening on activation. In fact, activated PMNs rapidly upregulate surface CD18 expression,\(^{25,26}\) which can amplify their adhesive potential. Previous studies have confirmed both a detrimental role for neutrophils in the pathogenesis of cerebral tissue injury in stroke, as well as a contributory role for various leukocyte adhesion receptors, such as ICAM-1\(^{11}\) and P-selectin.\(^{10}\)

In the current work, we explored the pathogenic role of an important leukocyte counterligand to endothelial ICAM-1 (CD18) in stroke. CD18 is a member of the integrin superfamily of adhesion glycoproteins, a family which consists of a number of membrane spanning glycoproteins that promote cell-cell and cell-matrix interactions. Integrins are heterodimers consisting of 1 unique alpha subunit and 1 of 3 common beta subunits: \(\beta_1, \beta_2,\) and \(\beta_3.\) Within the \(\beta\) family, there exist 3 distinct heterodimers, with the common CD18 subunit shared by distinct subunits (CD11a, CD11b, and CD11c). The CD11b/CD18 heterodimer (Mac-1) avidly binds to ICAM-1 on the endothelial surface, as does CD11a/CD18 (LFA-1), although the latter also binds ICAM-2 and ICAM-3. Both bind fibrinogen, as does CD11c/CD18. Although mice that lack CD18 cannot show ICAM-1 dependent cellular adhesion mediated via either Mac-1 or LFA-1, there are additional adhesive effector mechanisms (such as P-selectin-mediated leukocyte adhesion\(^{10}\)) which may still be active in CD18-null mice. The studies presented here show that CD18 \(-/-\) mice exhibit diminished leukocyte recruitment and are significantly protected in the setting of reperfused stroke, indicating a potent pathophysiological role for CD18 in this setting. However, somewhat unexpectedly, in the setting of permanent focal cerebral ischemia, the lack of CD18 was not protective. These data suggest that a leukocyte antiadhesive strategy may work best when combined with a reperfusion therapy. In support of this hypothesis, antibody to ICAM-1 enhanced the ability of tPA to improve neurological outcome in a rabbit model of embolic stroke, and antibody to CD18 showed efficacy with tPA at doses that were by themselves ineffective.\(^{27}\)

The current studies also permitted us to examine the general proinflammatory role of CD18 in the postischemic brain. The topographic localization of CD18 on circulating leukocytes rather than as a fixed adhesion receptor on the vessel wall enabled us to perform adoptive transfer experiments, which demonstrated that neutrophil recruitment amplifies neutrophil recruitment. In the first of these experiments, we injected radiolabeled CD18 \(+/+\) PMNs into CD18 \(-/-\) or CD18 \(-/-\) mice subjected to transient focal cerebral ischemia under conditions identical to prior experimental protocols and compared these data to CD18 \(+/+\) PMNs. Because other than hypomorphism for CD18, the counterligands and other adhesion receptors are functionally intact in both types of recipient mice, one would expect no significant difference in the amount of accumulation of radiolabeled CD18 \(+/+\) PMNs between the 2 groups. However, this was not the case; there was a significantly diminished binding of these PMNs in CD18 \(-/-\) animals. As the preponderant population of native PMNs in each recipient presumably accumulates to a greater or lesser degree depending on the presence or absence of functional CD18, it is reasonable to speculate that reduced accumulation of the native PMN population in the CD18 \(-/-\) animals inhibited further recruitment of PMNs with fully competent adhesion receptors. Proof of reduced accumulation of CD18 \(-/-\) PMNs in the CD18 \(-/-\) mice comes from the adoptive transfer experiments in which CD18 \(-/-\) PMN deposition was tracked in CD18 \(-/-\) mice; these mice had the lowest PMN deposition of all groups of mice subjected to transient cerebral ischemia.

The current studies contribute to the growing evidence implicating a detrimental role for PMNs in stroke. In the mouse model of stroke, absolute reduction in the numbers of circulating PMNs before transient focal ischemia is by itself sufficient to improve stroke outcome.\(^{13}\) Although leukocyte recruitment occurs within minutes of reperfusion in a murine model of stroke, it continues for at least the ensuing 24 hours and is only partially blocked by the absence of the P-selectin gene,\(^{10}\) suggesting an active role for other mechanisms of leukocyte recruitment. In humans, CD11a and CD18 are both upregulated in the leukocytes of patients with ischemic stroke and transient ischemic attacks.\(^{28}\) However, it has been difficult to tease out the pathogenic role of CD18 in PMN recruitment in stroke using blocking antibodies. Administration of a blocking antibody to CD18 (clone designate R 3.3) demonstrated therapeutic efficacy effective in an ischemia-reperfusion model of spinal cord injury\(^{29}\) but not in a model of irreversible cerebral embolic stroke.\(^{16}\) In the latter study, administration of a monoclonal antibody to CD18 (clone designate MoAb 60.3) did not improve CBF or evoked potentials. By using mice with severe functional hypomorphism of the CD18 gene product, the current studies support our hypothesis that CD18 is indeed pathogenic in PMN recruitment and cerebral tissue damage in stroke. Taken together, these studies demonstrate that reperfusion repre-
sents an especially vulnerable period for the brain, providing the potential benefits of restoring nutritive blood flow to an ischemic region while simultaneously opening the flood gates for a massive influx of activated PMNs. These studies suggest that stroke outcomes may be improved by anti-leukocyte adhesive strategies that are specifically targeted to the reperfusion period.

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References


aberrations beyond the expected (for example, significant changes in cerebral microvessels, circulation, and tissue due to “lifelong” diminished encounter with neutrophils), the study points to the possibility that antiadhesion strategies aimed at disruption of the interaction of neutrophils with the endothelium could result in histological and functional benefits only in cases in which reperfusion of the ischemic zone has been established after a brief (45-minute) ischemic period. The clinical significance of this observation needs to be evaluated vis-à-vis the clinical reality as follows: (1) Is reperfusion of ischemic brain in the common stroke situation a prevalent phenomenon? (What percentage of the patients with MCA occlusion reperfuse?) This issue has significant literature background to suggest that, indeed, a significant number of patients reperfuse (30% to 70%).1-4 (2) Does reperfusion in stroke patients occur early enough and in a significant number of patients to afford therapeutic opportunity? In fact, the majority of patients who reperfuse are not within the early (few hours) time frame5; late reperfusion (12 to 24 hours to weeks) may not provide/result in medical benefits because (a) the erratic nature of the event (no predictors) and (b) the reperfusion may not be of “nurturing quality,” as the tissue perfused may be largely dysfunctional at that time. Furthermore, it is not clear whether agents that work in models of reperfusion demonstrate efficacy because their action needs the presence of blood flow or because of reperfusion injury which the agent abolishes. Thus, it is highly questionable whether therapeutic agents aimed to block the CD18 adhesion receptors will be useful therapy in the majority of stroke patient treated within few hours after the ictus; in fact, the recent failure to establish efficacy with enlimomab (the anti–ICAM-1 murine antibody) in stroke trials supports this suggestion (with the caveat that the latter agent’s liabilities and mode of delivery and not the mechanism of action are at fault). In any case, anti-CD18 agents may be useful as adjunct therapeutics to targeted and timed reperfusion induced by tPA (and other thrombolitics), either by systemic or local delivery, at a certain time frame that is as yet unspecified in humans.

From the preclinical research perspective, it is rather intriguing to speculate on reasons that CD18-deficient mice had no improved outcome after permanent ischemia. Current dogma supports a role for the penumbra region in the outcome of the ischemic damage. Because the penumbra is perfused, albeit at lower level, neutrophil accumulation and its putative consequence—exacerbation of damage—should have been noticed. Alas, this elegant study is a perfect example of how preclinical data may provide compelling evidence for a potential therapeutic utility of an available agents (anti-CD18—neutralizing antibodies), yet careful analysis of the clinical context must be exercised to identify the discrete opportunity, if any, of CD18 antagonists in stroke.

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References
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