Intracortical Hemorrhage Injury in Rats: Relationship Between Blood Fractions and Brain Cell Death

Mengzhou Xue, MD; Marc R. Del Bigio, MD, PhD, FRCPC

Background and Purpose—Intracerebral hemorrhage is associated with stroke and head trauma. The purposes of this study were to investigate the effect of intracortical injections of autologous whole blood and blood components on inflammatory cell infiltration and brain cell death and to determine if nonhemorrhagic lesions differ in these respects.

Methods—Eighty-seven adult rats were subjected to intracortical injections of autologous whole blood or allogeneic plasma, erythrocytes, leukocytes, “activated” leukocytes, and serum. Injections of saline or mineral oil were controls. Blood injections were compared with cortical freeze injury and pial devascularization. Rats were perfusion-fixed 48 hours after injection or lesioning. Eosinophilic neurons, TUNEL-positive cells, brain damage area, infiltrating neutrophils, and CD8a-immunoreactive lymphocytes were quantified.

Results—Damage area, dying cells, and inflammatory infiltrate were significantly greater after autologous whole blood, leukocyte, and “activated” leukocyte injections than injection of other fractions.

Conclusions—These results suggest that extravasated whole blood causes a greater degree of cortical cell death and inflammation than ischemic lesions of similar size. Leukocytes “activated” by systemic illness might exacerbate the injury. Secondary hemorrhagic phenomena suggest that the harmful effect is directed toward both brain cells and the vasculature. Further studies are required to delineate the mechanism(s). (Stroke. 2000;31:1721-1727.)

Key Words: hematoma ■ inflammation ■ in situ nick-end labeling ■ lymphocytes ■ neutrophils

Intracortical hemorrhage can be a consequence of bleeding into an ischemic infarct, rupture of vascular malformation, amyloid angiopathy, or trauma. Although hematomas in the cortex are amenable to surgical therapy, they remain a significant management problem perhaps because the blood itself has adverse effects beyond its space-occupying effect. Several animal models of intracerebral hemorrhage have been developed, and experiments indicate that space-occupying effect, brain edema, ischemia, and neurotoxicity might all be involved in the brain damage that follows hemorrhage. Ischemia may be induced by direct mechanical compression from the hematoma and/or vasoconstrictor substances in blood. Neutrophilic inflammation is considerable in the vicinity of cerebral hematoma. Neutrophils release a variety of cytokines, such as tumor necrosis factor-α, interleukin-6, and interferon-γ (IFN-γ), which might play an important role in ischemic and traumatic brain damage. Work from one group has suggested that thrombin and erythrocyte degradation products are responsible for edema production. An understanding of evolution of brain injury after intracortical hemorrhage is important to determine the strategy of treatment. Therefore, the purpose of this study was to investigate the early effects of autologous whole blood and compare its effect with that of separated blood components after intracortical injection. We hypothesized that cellular components of blood would cause greater cortical cell death and inflammation than soluble blood proteins. We have chosen a survival period of 48 hours because we have observed that inflammation and the frequency of dying cells both peak 48 to 72 hours after intracerebral injections of autologous blood and after pial devascularization (unpublished data). To test the secondary hypothesis that hemorrhagic brain injury causes more inflammation than nonhemorrhagic injury, we compared the damage caused by blood product injections with that caused by cortical freeze injury and pial devascularization.

Materials and Methods

Animal Preparation

All experimental procedures were done in accordance with guidelines of the Canadian Council on Animal Care. The local experimental ethics committee approved protocols. Eighty-seven young adult male Sprague-Dawley rats weighing between 175 and 250 g were used. Ten groups of 5 to 8 rats each (see Table 1) were used for injections of blood fractions, saline, or mineral oil. Because the blood components, which were derived from donor rats and prepared fresh, had limited stability ex vivo, randomization of the experiment was only partial with saline and mineral oil controls randomly included among batched recipient animals. Cortical freezing and pial...
devascularization experiments were done later; therefore, these animals were not randomized among the blood fraction recipients.

### Intracortical Hemorrhage Model
For cortical injection, each rat was anesthetized with pentobarbital (50 mg/kg IP) and placed in a stereotactic frame. Under aseptic conditions, a midline scalp incision was made, a hole was drilled in the skull (3 mm lateral to midline and 0.02 mm anterior to coronal suture), a 100-μL syringe was secured to the frame, and a 25-gauge needle was introduced into the deep cerebral cortex 2.5 mm below the surface of the skull. Pilot experiments wherein blood was injected to these coordinates showed that there was minimal extension into white matter or the subarachnoid compartment after short survival. Each rat received a 50-μL injection of either normal saline, plasma, serum, erythrocytes, leukocytes, “activated” leukocytes, autologous whole blood, or mineral oil over a period of 5 minutes. Mineral oil was chosen as additional inert control because it has greater viscosity than saline and was expected to better mimic the minimal space-occupying effect of the infused blood. After infusion, the needle was left in place for 3 minutes and then removed slowly. The bone hole was sealed with bone wax, the scalp wound was sutured, and the animal was placed in a clean cage with free access to food and water. Invasive physiological monitoring was not used because it significantly increased the anesthesia time.

### Whole Blood and Blood Component Separation
Autologous whole blood was obtained from anesthetized rats by placing the tail end in 40°C water for 30 seconds followed by cutting the tail tip. Freely dripping whole blood (50 mL) was collected in a sterile syringe, which was then affixed to the stereotactic frame and immediately injected into the cerebral cortex. To obtain autologous blood components, 2 donor rats were anesthetized with pentobarbital and 5 mL blood was removed from the heart. Fresh blood was put in a syringe, which was then affixed to the stereotactic frame and 5 mL blood was injected into the deep cerebral cortex 2.5 mm below the surface of the skull. Pilot experiments wherein blood was injected to these coordinates showed that there was minimal extension into white matter or the subarachnoid compartment after short survival. Each rat received a 50-μL injection of either normal saline, plasma, serum, erythrocytes, leukocytes, “activated” leukocytes, autologous whole blood, or mineral oil over a period of 5 minutes. Mineral oil was chosen as additional inert control because it has greater viscosity than saline and was expected to better mimic the minimal space-occupying effect of the infused blood. After infusion, the needle was left in place for 3 minutes and then removed slowly. The bone hole was sealed with bone wax, the scalp wound was sutured, and the animal was placed in a clean cage with free access to food and water. Invasive physiological monitoring was not used because it significantly increased the anesthesia time.

### Freeze and Pial Devascularization Lesions
We observed that injections of autologous whole blood were frequently associated with lesions much larger than the area directly infomed with blood. To test the secondary hypothesis that hemorrhagic brain injury causes more inflammation than nonhemorrhagic injury, we attempted to create relatively nonhemorrhagic lesions of similar size in a similar dorsal cortical location. For induction of cortical freeze lesion, rats were anesthetized with pentobarbital and placed in a stereotactic frame. The scalp was incised along the midline. A copper rod (end diameter 2 mm) cooled to −170°C in liquid nitrogen was applied to the skull surface for 4 minutes (3 mm lateral to midline, 0.02 mm anterior to coronal suture). The scalp wound was sutured, and the animal was placed in a clean cage with free access to food and water. To study the early histological changes, rats were killed 4, 8, and 24 hours (n=2 each) after freezing in addition to the 48-hour survival period used for comparison with blood injections.

For pial devascularization, rats were anesthetized and placed in a stereotactic frame. The scalp was incised along the midline. A rectangle 3 mm wide and 5 mm long was delineated in the right hemisillk, the rostral margin 1 mm anterior to the bregma, and the medial margin 1 mm lateral to the midline. The bone was removed carefully by enlarging a drill hole with rongeers. Cortical blood vessels were interrupted by gently pulling the pia/arachnoid away with a needle (23 gauge) bent at the tip. Hemostasis was achieved by gentle tamponade with cotton-tipped applicators. The bone hole was covered with surrounding soft tissue, the scalp wound was sutured, and the animal was placed in a clean cage with free access to food and water. Rats were killed 4, 8, 24 (n=2 each), and 48 hours (n=4) after pial devascularization.

### Histological Examination
Forty-eight hours after intracortical injection or lesioning, rats were anesthetized and perfused through the heart with 300 mL of 5% paraformaldehyde in 0.1 mol/L PBS. The brain was removed and stored in the same fixative for up to 10 days. Fixed brains were cut coronally ~2 mm on either side of the needle entry site, which was identifiable on the brain surface. Brain slices were dehydrated and embedded in paraffin. Sections (6 μm) were cut, and each 30th section from the rostral to the caudal portion of the residual hematoma cavity was stained with hematoxylin and eosin stain. At the level of the needle site with maximal brain damage area, a variety of histological and immunohistochemical stains were performed.

Chloracate esterase staining was used to assess neutrophils. Sections were dewaxed and hydrated, washed with distilled water, and incubated for 60 minutes in 4% sodium nitrite, 4% new fuchsin, and 1% esterase substrate solution (naphthol AS-D chloracate dissolved in N,N dimethyl formamide) in phosphate buffer at pH 7.4. Slides were washed with distilled water, counterstained with Mayer’s hematoxylin for 3 minutes, dehydrated, cleared, and mounted. Neutrophilic granulocyte cytoplasm was stained bright red. Immunohistochemical localization of CD8a was performed. Sections

### Table 1. Cortical Injury and Inflammation After Injection of Whole Blood or Blood Fractions

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Total Damage Area, mm²</th>
<th>Eosinophil Neurons/0.25 mm²</th>
<th>TUNEL-Positive Cells/0.25 mm²</th>
<th>Neutrophils/0.25 mm²</th>
<th>CD8a-Positive Cells/0.25 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=7)</td>
<td>0.1±0.1</td>
<td>5.1±3.0</td>
<td>1.3±1.1</td>
<td>3.4±1.6</td>
<td>2.4±3.8</td>
</tr>
<tr>
<td>Whole blood (n=8)</td>
<td>2.8±2.5*</td>
<td>69.5±40.2*</td>
<td>25.3±23.3*</td>
<td>50.6±45.9*</td>
<td>36.5±10.3*</td>
</tr>
<tr>
<td>Erythrocytes (n=8)</td>
<td>0.3±0.1</td>
<td>6.5±6.7</td>
<td>4.4±3.3</td>
<td>5.0±1.9</td>
<td>12.6±8.1</td>
</tr>
<tr>
<td>Leukocytes (n=8)</td>
<td>0.3±0.2</td>
<td>41.1±20.0*</td>
<td>74.8±40.9*</td>
<td>50.5±12.3*</td>
<td>20.0±15.1*</td>
</tr>
<tr>
<td>“Activated” leukocytes (n=6)</td>
<td>5.9±2.9*</td>
<td>37.5±4.7*</td>
<td>55.4±1.8*</td>
<td>26.8±5.1*</td>
<td>18.5±7.6*</td>
</tr>
<tr>
<td>Plasma (n=8)</td>
<td>0.2±0.1</td>
<td>4.9±3.0</td>
<td>5.6±2.3</td>
<td>3.3±1.6</td>
<td>4.5±2.6</td>
</tr>
<tr>
<td>Serum (n=6)</td>
<td>0.2±0.2</td>
<td>8.8±4.2</td>
<td>4.5±5.3</td>
<td>3.8±2.8</td>
<td>7.3±8.4</td>
</tr>
<tr>
<td>Mineral oil (n=5)</td>
<td>0.2±0.1</td>
<td>6.6±4.3</td>
<td>3.2±3.1</td>
<td>3.6±3.4</td>
<td>3.2±2.3</td>
</tr>
</tbody>
</table>

All data are expressed as mean±SD. Cell counts are expressed as the number of cells per 250×250-μm areas (ie, 0.25 mm²).

*P<0.004 vs saline, plasma, serum, erythrocyte, and mineral oil groups (ANOVA with Bonferroni-Dunn intergroup comparisons).
were dewaxed and hydrated, washed with distilled water, quenched with 0.3% H₂O₂, blocked with 10% normal serum, and incubated with anti-rat CD8a monoclonal antibody (clone G28 diluted 1:400; PharMingen International) at 4°C overnight. Slides were then washed with Triton PBS, incubated in biotinylated goat anti-mouse IgG (1:300) 1 hour at room temperature, washed, incubated with peroxidase-HRP (1:400) for 30 minutes at room temperature, colored with diaminobenzidine-H₂O₂ solution, washed, and coverslipped. Control sections were processed with omission of the primary antibody. TUNEL (terminal deoxynucleotidyl transferase [TdT]-mediated deoxyuridine triphosphate [dUTP]-biotin nick end labeling) was used to identify dying cells. Paraffin-embedded sections were dewaxed and hydrated, then incubated in 20 μL/ml proteinase K for 15 minutes. TUNEL was accomplished with the use of an Apoptag in situ kit (Intergen). After immersion in equilibration buffer for 10 minutes, sections were incubated with TdT and dUTP-digoxigenin in a humidified chamber at 37°C for 1 hour and then incubated in the stop/wash buffer at 37°C for 30 minutes. Sections were washed with PBS before incubation in anti-digoxigenin-peroxidase solution (1:500 in PBS) for 30 minutes at room temperature and colored with diaminobenzidine-H₂O₂ solution. Sections were counterstained with methyl green. Negative control sections were treated similarly but incubated in the absence of TdT enzyme, dUTP-digoxigenin, or anti-digoxigenin antibody. TUNEL-positive nuclei with chromatin condensation and fragmented nuclei were considered as probable apoptotic cells. TUNEL-positive cells with diffuse light-brown labeling of nucleus and cytoplasm were considered as probable necrotic cells.

**Determination of Damaged Brain Area**

The area of brain damage on the hematoxylin and eosin–stained coronal slice at the level of the needle injection site was defined by the presence of blood collections, tissue rarefaction caused by edema, and dying cells around the injection sites. Computerized planimetry was used to measure the damage area on “camera lucida” drawings of the brain section.

**Cell Counts**

Although the observer was technically blinded to the nature of the injection, because of some obvious differences (for example, between whole-blood and saline injections), blinding in an absolute sense was not possible. With the use of an ocular graticule and ×250 ocular magnification (objective magnification ×20), eosinophilic neurons, neutrophils, CD8a immunoreactive cells, and TUNEL-positive dying cells were counted in 4 randomly selected fields (each area 250×250 μm) adjacent to but not including the needle insertion/injection site, which was defined by the presence of erythrocytes in all cases (see Figure 1). Areas with large blood vessels were avoided. In brains with large areas of damage after autologous blood injection, similar counts were made at the edge of the lesion, distant from the injection site. Cortical freeze and pial devascularization lesions were assessed in 4 randomly selected areas at the periphery of the lesions.

**Data Analysis**

All data are expressed as mean±SD. Data were analyzed with StatView 5 software (SAS Institute Inc). ANOVA with the Bonferroni-Dunn test was used for intergroup comparisons. Fisher’s r to z test was used for correlations.

**Results**

All rats tolerated the surgical procedures well, and there were no surgical deaths. Residual hematomas in the cerebral cortex around the needle site were apparent at 48 hours. A small quantity of blood was in the adjacent white matter of 7 rats. Some rats in the whole-blood and in both leukocyte injection groups exhibited additional blood in the subarachnoid space (4 of 8 whole blood, 2 of 8 leukocyte, and 3 of 6 “activated” leukocyte recipients). This extension was associated with areas of hemorrhagic and edematous brain damage far beyond (up to 2 mm) the site of injection. Because such extension was never seen after injection of saline, mineral oil, plasma, or serum, and because pilot experiments showed that 50 μL of blood remained confined to the cortex in the immediate term, this suggests that a secondary hemorrhagic phenomenon and not a simple surgical complication had occurred.

Microscopically, whole-blood injections appeared as single or multiple contiguous collections of blood cells in a column surrounding the needle tract. Leukocyte injections appeared as smaller collections of intact neutrophils and fragmented leukocytes with nuclear debris. Small collections of blood cells around the needle insertion site were seen all in other groups. In the vicinity of all lesions, neutrophils were seen adherent to blood vessel walls or passing out of capillaries and venules into the neuropil. Eosinophilic neurons, TUNEL-positive cells with nuclear labeling presumed to indicate apoptosis, and TUNEL-positive cells with cytoplasmic labeling presumed to indicate necrosis were detected in cortical damage areas. Cells that exhibited membrane immunoreactivity for CD8a were small, with minimal cytoplasm and round nuclei. On the basis of the morphology of the cells and the known specificity of the antibody, we believe these to be natural killer (NK) cells and/or activated cytotoxic T-lymphocytes. Neutrophils were also noted in the subarachnoid compartment adjacent to the needle entry sites and at sites of subarachnoid hemorrhage.

Quantitative analysis showed that the total area of cortical damage was significantly larger after injection of 50 μL of whole blood or “activated” leukocytes than other blood fractions (Table 1), particularly when there was associated subarachnoid hemorrhage. At the microscopic level, eosinophilic neurons, TUNEL-positive dying cells, neutrophils, and CD8a-immunoreactive lymphocytes adjacent to the injection site were significantly more abundant after whole-blood, leukocyte, and “activated” leukocyte injections than the other groups (Table 1). For the population as a whole, the quantities of dying cells and inflammatory cells were all intercorrelated (r=0.556 to 0.795, P<0.0001). The total damage area correlated poorly with TUNEL-positive cells and eosinophilic neurons per unit area in microscopic fields (r=0.412 and 0.544 respectively, P<0.001) and not with the inflammation. Comparison of microscopic changes between the core and...
edge of hemorrhagic lesions that had enlarged beyond the site of whole-blood injection showed that the quantity of dying cells and inflammatory cells was approximately half at the periphery.

To test the secondary hypothesis that hemorrhagic brain lesions are associated with more inflammation than nonhemorrhagic lesions, we compared the lesions that had enlarged after whole-blood injections with freeze lesions and pial devascularizing lesions in the dorsal cortex (Figure 2 and Table 2). Our goal was to create lesions of similar size in the same location, but we did not entirely succeed in this respect. Pial devascularization lesions exhibited a mixture of edematous and hemorrhagic brain at 8 hours, as previously documented. By 48 hours, the core was necrotic, and damage extended laterally to the margins of the cranectomy and deep to the white matter. Freeze lesions were pale on gross inspection, and only microscopic petechiae were noted 8 hours after freezing. By 48 hours, the majority of cells in the central region were necrotic, with no basophilic staining of the nuclei. The surrounding viable brain was rarefied as the result of edema. The core of all large lesions exhibited advanced necrosis with only vague cell outlines apparent, absence of chromatin staining, minimal inflammation, and only very rare TUNEL-positive cells. The margins of these lesions were edematous and exhibited both dying cells and inflammation. At the microscopic level, lesions associated with blood injections exhibited more inflammation and cell death than the partially hemorrhagic devascularizing lesion and the relatively nonhemorrhagic freeze lesions (Table 2).

**Discussion**

Intracerebral hemorrhage causes brain damage through multiple mechanisms. Direct tissue destruction by the hemorrhagic event with dissection of blood along tissue planes occurs immediately. This is followed by development of edema and possibly ischemic damage caused by raised intracranial pressure and distortion or vasospasm in the microvasculature. Delayed damage could result through a variety of mechanisms including local ischemia, release of toxins by blood breakdown products, thrombin release, or leukocyte infiltration. This study demonstrates that injection of a small quantity of whole blood into the cerebral cortex of rats is associated with cell death and inflammatory cell infiltration at 48 hours. In a prior experiment, we have shown that this time represents the peak for both processes. The tissue distortion was minimal, and therefore mechanisms other than ischemia due to distortion are presumed to play a role. Plasma or serum alone and concentrated erythrocytes had negligible effect. Concentrated leukocytes had a mild adverse effect, whereas “activated” leukocytes had a strong adverse effect. Two issues must be discussed. First, what is the nature of the early response to the released blood products? Second, what is the reason that some blood components are more harmful than others? One could argue that the experiment should have been conducted by injecting 50 μL whole blood, ≈20 μL erythrocytes, ≈25 μL plasma, and ≈2 μL leukocytes to account for the true relative volumes of these blood fractions. We were, however, concerned that this would be confounded by different volumes of dispersion. Had this been done, the effect of erythrocytes and plasma, which were not significantly different than saline control, would have been even less. The effect of the leukocytes might be exaggerated in the experimental protocol that was used.
After injection of the blood, we observed cell death, inflammatory cell infiltration, and in some cases distant hemorrhagic/ischemic damage. In the immediate vicinity of the injection site, neuronal death was characterized by DNA damage and cytoplasmic hypereosinophilia. Although it is likely that the 2 features represent cells at different stages of death or those dying by different mechanisms (ie, apoptosis or necrosis), our data do not add anything to explain the mechanism of delayed neuronal cell death, a subject of heated debate.15–37 Reactive neuronal expression of heat shock protein (HSP72) and/or loss of MAP2 immunoreactivity indicative of proteolysis and impending death has been documented within 5 hours after intracerebral hemorrhage in humans. Increased astroglial expression of glial fibrillary acidic protein (HSP72) and/or loss of MAP2 immunoreactivity indicative of proteolysis and impending death has been documented within 5 hours after intracerebral hemorrhage in humans. Increased astroglial expression of glial fibrillary acidic protein and metallothionein occurs within 18 to 48 hours.38

Inflammation is an obvious response to the injections. Although one might argue that the neutrophils had been injected and had not entered by diapedesis, we attempted to count cells only beyond the margin of the primary injection site. Substantial margination of leukocytes along the blood vessel lumen supports the idea that they subsequently enter the brain tissue. Chemotaxis of these neutrophils and lymphocytes, and later of monocytes, is mediated by α- and β-chemokines and complement.39,40 In rat brain injury experiments, intense neutrophilic infiltrate has been previously documented around collagenase-induced hematomas,5,13 contusions,41,42 and ischemic sites43 beginning at 6 to 12 hours and peaking at 48 to 72 hours. In human brains, neutrophil infiltration is apparent 5 to 72 hours after hemorrhage or contusion.1,38,44–46 The present experimental comparison of hemorrhagic and nonhemorrhagic lesions suggests that something in, or a process initiated by, extravasated blood promotes chemotaxis. Neutrophils can release potentially harmful factors such as oxygen radicals57 or cytokines including tumor necrosis factor-α, interleukin-6, and IFN-γ, which seem to play a role in brain damage.15,16 Neutrophils can also exacerbate brain injury by obstructing microvessels, which then causes local ischemia.48 Many experimental studies have documented infiltration of NK cells into injured brain.39,49,50 One study of brain contusions in rats showed that NK and T-cell infiltration was more prevalent than neutrophilic infiltrate.51 We also observed a significant number of CD8a-immunoreactive cells, which could be either NK cells and/or activated cytotoxic T-lymphocytes51 that could be directly injurious or indirectly so through release of IFN-γ.

We observed that the injection of whole blood or the leukocyte fraction caused injury, whereas injection of erythrocytes, plasma, and serum had minimal effects. However, it is conceivable that specific blood/plasma fractions exert damage at different times. Other investigators showed that cerebral edema develops only 3 days after erythrocyte injection, and they suggested that hemoglobin released from lysed erythrocytes is toxic to brain.11 The toxic effect of hemoglobin on neurons has been demonstrated in vitro.52 In this study, neither plasma nor serum caused more damage than saline injection. Thrombin, which is a component of plasma, has been shown to cause brain edema and seizures after intracerebral injection.8,33,53 There are several possible explanations for the discrepancy. The thrombin effect might be mediated only by very large doses and is therefore relatively less important in vivo. Thrombin might for some reason be inactivated during processing of the plasma. Finally, the “pharmacodynamics” might be altered because a blood clot allows slow focal release whereas plasma injection would diffuse rapidly leading to lower regional concentrations.

The leukocyte fraction, and in particular “activated” leukocytes, caused greater injury than other blood fractions. When one interprets the intensity of the leukocyte-mediated response, one must keep in mind that leukocytes occupy <1% of the volume of whole blood. Therefore, the leukocyte injection groups received a dose equivalent to 5 mL of whole blood.54 Regardless, “activated” cells appear to be more harmful, perhaps through production of more of the deleterious mediators mentioned above. This might help to explain the observation that fever during the first 3 days after intracerebral hemorrhage is an independent predictor of poor prognosis in patients.55 To further study the role of leukocytes in this model, one could inject microwave-killed cells whose proteins are inactivated, lysed cells, and supernatant from cultured cells in resting or active states. This would help to determine whether the noxious agents are actively or passively released. It is also important to recognize that activated platelets, which we did not study directly, are included in the leukocyte fraction. Platelets can release serotonin and platelet-derived growth factor, which are capable of increasing vascular permeability and causing vasoconstriction.56
Finally, we must try to explain why injection of some blood fractions was associated with enlargement of the lesions well beyond the limits of the injected substance. Several studies suggest that mechanism other than mass effect are involved in the contribution of blood to perihematoma edema formation, because blood produces larger lesions than would be expected from its space occupying effects alone.\(^57\) This might be explained by a secondary effect of the injected substances on the vasculature through agents that promote vasospasm and/or increased vascular permeability.\(^58\) Whole blood has greater adverse effects on cerebral blood flow than hemoglobin or albumin when injected into the subarachnoid space, presumably because of vasoconstrictive agents.\(^59\) It is because of these larger lesions that we also used extravascular whole blood, presumably because of vasoconstrictive and coagulative necrosis,\(^60\) and pial devascularization, which has been promoted as a model of ischemia.\(^61\) Our goal was to create large nonhemorrhagic lesions that could be compared with the lesions caused by blood injections. The freeze lesion was reasonably successful in this regard, having caused only minimal hemorrhage but considerable release of plasma.\(^62\) At the margin it was associated with considerably fewer dying cells and neutrophils than the whole blood injection. The devascularization injury was associated with deep hemorrhage that probably was a consequence of deep vein avulsion. This mixed hemorrhagic/ischemic lesion was associated with more dying and inflammatory cells than the freeze lesion but fewer than the whole-blood injection.

In conclusion, extravascular whole blood, and perhaps the leukocyte fraction in particular, appears to play an important role in cortical damage. The magnitude of delayed cell death and inflammation is greater than that after nonhemorrhagic injury. Although coexistent, we cannot state that inflammation is necessarily a cause of neural cell injury. The precise molecular and chemical mechanisms remain to be determined but probably are multiple and include secondary ischemia, inflammatory cell products, and iron-mediated effects.

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**Editorial Comment**

It has become obvious in recent years that the space-occupying effect of intracerebral hematomas is not nearly as important as other factors in determining how much damage is done to the brain. In view of the disappointing results of (even early) surgical treatment of spontaneous intracerebral hematomas, other avenues must be sought to improve the outcome for these unfortunate patients. The authors have made a valuable contribution in this article, showing a major role for leukocyte activation and inflammation in the pathophysiology of these lesions. Obviously, the next step would be to assess experimental hematomas in animals with leukopenia or impaired leukocyte function. It is true that in ischemic stroke this avenue has not yet led to clinically significant results in patients, but hopefully the pathophysiology of hemorrhagic stroke is less multifactorial than that of ischemic stroke.

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