Blood Cell-Derived RANTES Mediates Cerebral Microvascular Dysfunction, Inflammation, and Tissue Injury After Focal Ischemia–Reperfusion

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Background and Purpose—Although chemokines have been implicated in cardiovascular diseases, few studies have addressed the role of these inflammatory mediators in ischemic stroke. This study tested the hypothesis that RANTES (CCL5; regulated on activation, normal T-cell expressed and secreted) mediates the cerebral microvascular dysfunction, inflammation, and tissue injury induced by brain ischemia and reperfusion.

Methods—After 60-minute middle cerebral artery occlusion and reperfusion, the adhesion of leukocytes and platelets in cerebral venules, infarct volume, and blood–brain barrier permeability were measured in wild-type mice (WT), RANTES-deficient mice (RANTES−/−), WT mice transplanted with RANTES−/− bone marrow (RANTES−/− WT), and control bone marrow chimeras (WT>WT). The concentration of RANTES and several cytokines was also measured by enzyme-linked immunosorbent assay and a cytometric bead array.

Results—The enhanced leukocyte and platelet adhesion, increased blood–brain barrier permeability, and tissue infarction elicited in WT and WT>WT mice after middle cerebral artery occlusion and reperfusion were significantly blunted in RANTES−/− mice. Similar attenuation of the middle cerebral artery occlusion and reperfusion-induced responses were noted in RANTES−/− WT chimeras. Although RANTES deficiency did not alter the changes in tissue cytokine levels elicited by middle cerebral artery occlusion and reperfusion, plasma concentrations interleukin-6, interleukin-10, and interleukin-12 were all reduced.

Conclusions—These findings implicate blood cell-derived RANTES in the microvascular, inflammatory, and tissue injury responses of the brain to ischemia and reperfusion. (Stroke. 2008;39:000-000.)

Key Words: cerebral infarct  ■  chemokines  ■  cytokines  ■  platelets  ■  RANTES

There is a growing body of evidence that implicates inflammatory cells and mediators in pathogenesis of ischemic stroke. A variety of cytokines and chemokines are produced by postischemic brain tissue and these mediators are thought to attract inflammatory cells to the site of injury through mechanisms that involve activation of endothelial cells and other circulating cells (eg, platelets). Several cytokines, including tumor necrosis factor-α and interleukin (IL)-1β, have been shown to contribute to inflammatory cell recruitment, blood–brain barrier dysfunction, and tissue injury in animal models of ischemic stroke.1 Chemokines, and their receptors, have also been implicated in pathogenesis of ischemic stroke. Patients with a stroke exhibit elevated plasma levels of the chemokines monocyte chemoattractant protein-1 (MCP-1) and IL-8.2,3 Studies in animal models have also revealed the same chemokines (eg, MCP-1) contribute to the leukocyte recruitment, brain infarction, and edema elicited by cerebral ischemia–reperfusion (I/R).4–6 Although a role for other chemokines in stroke pathogenesis has been inferred based on the elevated plasma levels detected after a stroke, their potential contribution to this disease process has not been directly assessed.

RANTES (CCL5; regulated on activation, normal T-cell expressed and secreted), a member of the CC-chemokine family, promotes the directed migration of leukocytes into damaged or inflamed tissue. Aggregates of RANTES that form on cell surfaces have been shown to act as powerful activators of leukocytes. Although the chemotactic properties of RANTES are mediated through engagement with its receptor (CCR5), the leukocyte activation effect of the chemokine reflects an oligomerization-dependent interaction with cell surface glycosaminoglycans.7,8 RANTES is produced by a variety of cells, including T-lymphocytes, platelets, endothelial cells, smooth muscle cells, and glial cells.

Received December 20, 2007; final revision received February 1, 2008; accepted February 6, 2008.
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© 2008 American Heart Association, Inc.
Stroke is available at http://stroke.ahajournals.org DOI: 10.1161/STROKEAHA.107.513150
Although all of these cells likely contribute to the pathogenesis of ischemic stroke, it remains unclear whether RANTES produced by some or all of these cells mediates the inflammation, microvascular dysfunction, and tissue injury induced by cerebral I/R.

The overall objective of this study was to assess the contribution of RANTES to brain inflammation and injury in an animal model of ischemic stroke. The recruitment of leukocytes and platelets, blood–brain barrier permeability, infarct size, and blood and tissue concentrations of different cytokines and chemokines were monitored and compared between wild-type and RANTES-deficient mice. To assess the contribution of blood cells versus parenchymal cells to the RANTES-mediated injury responses, bone marrow chimera mice were created. Our findings strongly implicate this chemokine in the pathogenesis of experimental ischemic stroke and suggest that the RANTES that mediates the cerebral inflammation, blood–brain barrier dysfunction, and tissue infarction is largely derived from circulating blood cells.

Methods
Animal Preparation
Male C57BL/6J mice (wild type [WT]) were obtained (n=20 for sham operation, n=31 for I/R) from Jackson Laboratories (Bar Harbor, Maine). RANTES knockout mice (RANTES−/−) on a C57BL/6J background were originally generated by Danoff and coworkers.9 A breeding pair of RANTES−/− was obtained from Dr Sally Sarawar in the Torrey Pines Institute for Molecular Studies, San Diego, Calif. A breeding colony was established in the animal resource facility of the Louisiana State University Health Sciences Center, Shreveport (n=28). The experimental procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee and were in compliance with the guidelines of the National Institutes of Health.

Creating Bone Marrow Chimera Mice
Bone marrow (BM) cells, collected from the femurs and tibias of donor mice (WT and RANTES−/−), were injected (2×10^6 BM cells) through the femoral vein into recipient mice (congenic WT with the same phenotype as C57BL/6J mice; B6.SJL-PtprcaPep3b/BoyJ), after total-body irradiation sufficient to eliminate the recipient blood cells. The BM chimeras were kept in autoclaved cages with 0.2% neomycin added to drinking water for the first 2 weeks. Six to 8 weeks later, reconstitution of BM cells was verified by flow cytometry by testing for the percent blood leukocytes positive for CD45.1 (recipient isoform of CD45) versus CD45.2 (donor isoform of CD45). Successful BM reconstitution was set as ≥90% of the CD45.2-positive population in total leukocytes as previously described.10 Two BM chimeras were produced: 1) WT recipients receiving BM from RANTES−/− donor mice (RANTES−/−; n=24); and 2) WT recipients receiving BM from WT donor mice (WT; n=18, as positive controls).

Middle Cerebral Artery Occlusion and Reperfusion
Mice were anesthetized with 100 mg/kg intraperitoneal ketamine and 10 mg/kg xylazine. Transient (60 minutes) focal cerebral ischemia was induced by left middle cerebral artery occlusion using the intraluminal filament method. A 7-0 silicone-coated nylon monofilament (Doccol Corporation) was advanced through the external carotid artery. In the sham group, the arteries were visualized but not disturbed. After 60 minutes, the fiber was removed. Middle cerebral artery occlusion reperfusion (MCAO/R) was verified using a laser Doppler flowmeter to monitor cerebral blood flow during ischemia and reperfusion. Core temperature was kept at 36°C. Percentage survival was calculated for mice allowed to reperfuse for 24 hours.

Intravital Videomicroscopy
During these experiments, blood pressure was monitored through a femoral artery and blood gas was analyzed at the end. The procedures used to monitor blood cell–vessel wall interactions in murine cerebral venules are described elsewhere in detail.11 Briefly, at 4 hours after reperfusion, a cranietomy (1 mm posterior, 4 mm lateral from the bregma) was made under controlled ventilation. The cranietomy was soaked with artificial cerebrospinal fluid, and a glass coverslip was placed over it. An upright fluorescent microscope with a 3CCD video camera system was used to observe randomly selected 100-μm segments of pial venules (25 to 50 μm diameter). A total of 100×10^6 platelets from donor mice were labeled ex vivo with carboxyfluorescein diacetate succinimidyl ester.12 These green fluorescent platelets were administered to recipient mice followed by continuous infusion of 0.02% Rhodamine 6G, which labeled leukocytes red. Adherent leukocytes and platelets were defined as cells bound to venules for ≥30 and 2 seconds, respectively. Cell adhesion data were expressed as number of cells per square millimeter of venular surface, calculated from venular diameter and length, assuming cylindrical geometry.

Infarction Volume
After 24 hours of reperfusion, 1-mm-thick coronal sections of the brain were immersed in 0.05% 2,3,5-triphenyltetrazolium chloride solution for 30 minutes. The total areas of each brain section and the infarcted region were quantified with the software program, NIH image. Infarct volume was corrected for edema as previously described.13

Blood–Brain Barrier Dysfunction
A 2% solution of Evans blue (Sigma-Aldrich) was injected (4 mL/kg) intravenously immediately after MCAO/R or after sham operation. Twenty-four hours later, the blood was obtained for plasma collection and the brain was sampled after transcardial perfusion with phosphate-buffered saline (PBS; 100 mm Hg, 5 minutes). The cerebral hemispheres and the plasma were homogenized, sonicated, and then centrifuged. The supernatant was diluted with ethanol and the concentrations of Evans blue in brain tissue and plasma were measured using a fluorescence spectrophotometer (FLUOstar Optima; BMG LABTECH, Inc). Blood–brain barrier (BBB) permeability was normalized by dividing tissue Evans blue concentration (μg/g brain weight) by the plasma concentration (μg/mL).

RANTES in Plasma and Brain Tissue
Twenty-four hours after reperfusion, the blood was sampled with citrate to prevent platelet activation, and plasma was obtained. Thereafter, the mice were transcardially perfused with PBS (without heparin) for 5 minutes. The brain samples were homogenized and sonicated in PBS containing protease inhibitors (protease inhibitor cocktail; Sigma-Aldrich) followed by centrifugation for collection of the supernatant. RANTES levels in brain tissue supernatant and plasma were measured using an enzyme-linked immunosorbent assay kit (Quantikine for mouse RANTES; R&D systems). In addition to the groups outlined here, a group of recombinant activating gene-1-deficient mice (Rag-1−/−; B6.129S7-Rag-1<tm1Mom>/J), which are deficient in both T- and B-lymphocytes, was added for this parameter (n=6). RANTES is produced by T-cells but not B-cells. Therefore, Rag1−/− mice were used to address whether T-cells could be a source of RANTES after MCAO/R.

Cytokines in Plasma and Brain Tissue
Twenty-four hours after reperfusion, the blood was collected into heparin-coated syringe, and plasma was obtained. After a 5-minute transcardial perfusion of PBS, the brain hemispheres were homogenized, sonicated, and centrifuged in PBS containing protease inhib-
Results

Physiological Parameters
The Table shows the values of body weight, mean arterial blood pressure, blood pH, PaCO₂, O₂ saturation, and survival rate. There was no significant difference between groups for any parameter except survival rate, which was significantly lower in WT-I/R mice when compared with WT-sham and RANTES−/−-I/R groups.

Blood Cell–Vessel Wall Interactions and Blood Cell–Blood Cell Interactions
Figure 1 summarizes the blood cell–vessel wall interactions induced in cerebral venules after sham surgery (WT-sham) and at 4 hours of reperfusion after a 60-minute ischemic challenge in WT-I/R, RANTES−/−, WT>WT, and RANTES>WT mice. RANTES−/− and RANTES>WT mice exposed to MCAO/R exhibited significant reductions in the number of adherent leukocytes (Figure 1A) and platelets (Figure 1B) compared with WT-I/R and WT>WT-I/R mice. The magnitude of the reductions in adherent leukocytes and platelets were very similar for the RANTES−/− and RANTES>WT mice.

Infarct Volume
The brain infarct volumes in WT, RANTES−/−, WT>WT, and RANTES>WT mice subjected to 60 minutes of middle cerebral artery occlusion and 24 hours of reperfusion are presented in Figure 2A. The infarct volumes in RANTES−/− (17.0±1.9%) and RANTES>WT (18.0±0.6%) were significantly smaller than in WT (29.7±2.0%) and WT>WT (30.0±2.6%) mice. RANTES−/− and RANTES>WT mice showed nearly identical reductions in infarct volume compared with control (WT and WT>WT) mice.

Blood–Brain Barrier Dysfunction
Figure 2B summarizes the changes in BBB permeability to Evans blue induced by 60 minutes ischemia and 24 hours reperfusion in the different experimental groups. BBB permeability in the left (injured) hemisphere of WT mice (0.066±0.007) and WT>WT mice (0.060±0.006) after I/R was significantly elevated compared with sham-WT mice (0.005±0.001), RANTES−/− mice (0.022±0.005) and RANTES>WT chimeras (0.023±0.009) exhibited marked reductions in BBB permeability compared with WT-I/R and WT>WT-I/R groups.

RANTES Levels in Brain Tissue and Plasma
The objective of this series of experiments was to determine if RANTES levels in postischemic brain tissue (Figure 3A) and plasma (Figure 3B) are elevated after MCAO/R and whether blood cells are the major source of the RANTES accumulation elicited by I/R. To address the possibility that T-cells, a well-known source of RANTES, contributing to the I/R-induced chemokine accumulation, Rag-1 knockout mice (Rag-1−/−), were included in the analysis. Transcardial PBS perfusion of the brain under physiological pressure was applied to minimize the contribution of trapped blood to the tissue RANTES estimates without eliminating RANTES bound to the vascular endothelial surface. The tissue RANTES measurements reveal a significant accumulation of the chemokine after I/R, which did not differ statistically among WT, Rag-1−/−, and RANTES>WT groups. However, RANTES was not detected in brain tissue after I/R in RANTES−/− mice, suggesting that the tissue levels are largely derived from parenchymal cells rather than circulating blood cells. However, estimates of RANTES concentration in plasma indicates that much of the chemokine appearing in this compartment is derived from circulating blood cells, because RANTES>WT mice exhibited a plasma concentration that was 42% of WT and Rag-1−/− mice. The comparable values for RANTES in Rag-1−/− and WT mice suggest that lymphocytes are an unlikely source of the chemokine in plasma. RANTES−/− mice exhibited an 82% reduction in plasma RANTES compared with WT mice.

| Table. Values for Different Physiological Variables in Sham-Operated WT Mice (WT-sham) and WT Mice (WT-I/R), RANTES Knockout Mice (RANTES−/−), WT Mice Transplanted With WT BM Cells (WT>WT), and WT Mice Transplanted With RANTES−/− BM cells (RANTES>WT) Subjected to I/R |
|---------------------------------|-----------------|----------------|-----------------|-----------------|-----------------|
|                                | WT-Sham         | WT-I/R         | RANTES−/−       | WT>WT           | RANTES>WT       |
| Body weight, g                 | 23.7±0.4        | 23.4±0.5       | 25.9±0.6        | 24.7±0.6        | 23.5±0.5        |
| Mean arterial blood pressure, mm Hg | 79.4±3.2       | 82.1±1.5       | 82.1±6.4        | 77.4±3.5        | 79.0±4.6        |
| pH                             | 7.30±0.05       | 7.35±0.03      | 7.29±0.06       | 7.29±0.02       | 7.33±0.03       |
| PaCO₂, mm Hg                   | 38.0±7.2        | 42.2±3.8       | 41.3±6.9        | 41.9±1.2        | 43.0±3.9        |
| O₂ saturation, %               | 99.3±0.1        | 99.4±0.1       | 99.3±0.3        | 99.2±0.2        | 99.4±0.1        |
| Survival rate, %               | 100             | 78.7*          | 96.8†           | 88.9            | 89.7            |

Data are expressed as mean±SE. *P<0.05 versus WT-sham. †P<0.05 versus WT-I/R by χ² analysis.
Plasma Cytokine Levels

Figure 4 summarizes the changes in plasma concentrations of different cytokines (IL-12, tumor necrosis factor-α, interferon-γ, MCP-1, IL-10, IL-6) in WT-sham, WT-I/R, and RANTES−/− mice. MCAO/R elicited significant increases in plasma IL-10 (108.1±21.4 pg/mL) and IL-6 (365.1±100.4 pg/mL) concentration in WT mice, and these changes were significantly blunted (24.6±19.3 and 83.7±21.5 pg/mL, respectively) in the RANTES−/− mice subjected to I/R. Although plasma IL-12 concentration was unaffected by MCAO/R in WT mice, RANTES−/− mice exhibited a small but significant reduction (63%) compared with WT-sham mice.

Brain Cytokine Levels

The changes in brain tissue cytokine concentrations after MCAO/R are summarized in Figure 5. MCAO/R elicited significant reductions in IL-12 and IL-10 in the left (injured) hemisphere of WT mice, whereas corresponding increases in tumor necrosis factor-α, MCP-1, and IL-6 were detected.
Figure 2. Infarct volume (A) and BBB permeability to Evans blue (B) induced by I/R in WT mice, RANTES knockout mice (RANTES−/−), WT mice transplanted with WT bone marrow cells (WT>WT), and WT mice transplanted with RANTES−/− bone marrow cells (RANTES> WT). The tables beneath the panels show the predicted ability of vascular endothelium and blood cells to produce RANTES (*P<0.05 versus WT-I/R, †P<0.05 versus WT>WT, ‡P<0.05 versus WT-sham, §P<0.05 versus contralateral hemisphere in the same group).
Figure 3. Brain tissue (A) and plasma (B) concentrations of RANTES after I/R in WT mice, Rag-1 knockout mice (Rag-1−/−: lymphocyte-deficient mice), WT mice transplanted with RANTES−/− bone marrow cells (RANTES−/WT), and RANTES knockout mice (RANTES−/−). The table beneath each panel lists the potential sources of RANTES in each group for either brain tissue or plasma (*P<0.05 versus WT-sham, †P<0.05 versus WT-I/R, ‡P<0.05 versus Rag-1−/−, §P<0.05 versus contralateral hemisphere in the same group).
However, these responses were unaffected by RANTES deficiency.

**Discussion**

Relatively little attention has been devoted to the contribution of chemokines to the pathogenesis of ischemic stroke. Although recent work has implicated CCL2 (MCP-1) and CCR2 in cerebral I/R injury in mice, a role for other members of the CC-chemokine family such as RANTES (CCL5) in stroke has not been previously addressed. RANTES has been implicated in variety of pathological conditions, including atherosclerosis, asthma, AIDS, cancer, transplantation, and inflammatory bowel disease. Like other chemokines, RANTES mediates chemotaxis or cell migration through G-protein coupled receptors at nanomolar concentrations, and aggregates of RANTES can induce cell activation (prolifer-
Figure 5. Brain tissue concentrations of IL-12, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), MCP-1, IL-10, and IL-6 after I/R in WT mice and RANTES knockout mice (RANTES−/−; *P<0.05 versus WT-sham, †P<0.05 versus contralateral hemisphere in the same group).
ation, apoptosis, cytokine release) through a G-protein coupled receptor-independent pathway at micromolar concentrations. Inasmuch as inflammatory cell recruitment into the postischemic brain is considered an important pathophysiological component of this disease process, RANTES appears to be a worthy candidate mediator of the inflammatory responses to ischemic stroke.

The results of our study provide 2 major lines of evidence that implicate RANTES in the inflammation, microvascular dysfunction, and tissue injury elicited in a murine model of focal cerebral ischemia. First, we demonstrate that brain tissue, not plasma, levels of RANTES are significantly elevated in WT mice subjected to focal cerebral I/R. Second, significant protection against the I/R-induced inflammatory and tissue injury responses was noted in RANTES−/− mice as well as in RANTES>WT chimeras.

The results of this study suggest that RANTES plays a major role in the recruitment of both leukocytes and platelets into the cerebral microvasculature after I/R. This effect on leukocyte recruitment is consistent with the well-documented effects of RANTES on leukocyte migration. Indeed, RANTES has been implicated in the trafficking and retention of leukocytes in atherosclerotic plaques, in the leukocyte adhesion in cerebral venules of mice with experimental autoimmune encephalitis as well as in monocyte arrest on monolayers of cultured endothelial cells. It has been previously shown that RANTES liberated from α-granules of activated platelets or platelet microparticles binds to glycosaminoglycans on the endothelial cell surface where it promotes leukocyte adhesion. The avidity of RANTES for glycosaminoglycans would ensure that blood cell-derived RANTES is concentrated on the endothelial cell surface in the inflamed or damaged tissue. Although platelets are known to express CCR1, 3, and 4, all of which can interact with RANTES, the results of a previous in vitro study indicate that RANTES per se does not promote the activation or aggregation of platelets or the adhesion of platelets to endothelium. Thus, the effect of RANTES on I/R-induced platelet adhesion observed in our study may reflect the binding of platelets to leukocytes rather than direct binding to vascular endothelium as supported by our finding that 89% of I/R-induced platelet adhesion in WT-I/R mice could be attributed to platelet interactions with leukocytes rather than with the vascular wall. Hence, a reduction of leukocyte adhesion due to RANTES deficiency would be expected to elicit a comparable reduction in platelet adhesion.

Our observation that the BBB dysfunction induced by cerebral I/R was greatly attenuated in RANTES−/− mice suggests that this chemokine either directly or indirectly increases BBB permeability. Studies in the lung have failed to demonstrate a role for RANTES in the increased vascular permeability induced by I/R. There is evidence, however, that RANTES can promote the migration of leukocytes across monolayers of brain endothelial cells. This observation, coupled with reports linking leukocyte transmigration to BBB permeability, suggests that the directed movement of leukocytes across the BBB may contribute to the increased BBB permeability observed in our study. However, it remains unclear whether such trafficking of leukocytes does occur to a significant extent at 24-hour reperfusion and whether this could account for the large increment in BBB permeability. Another possible explanation is that RANTES activates adherent leukocytes, which in turn release mediators that disrupt the BBB.

A variety of cells are known to produce RANTES, including T-lymphocytes, platelets, endothelial cells, smooth muscle cells, and glial cells, and so on. To address whether the source of RANTES that mediates the I/R-induced inflammation, microvascular dysfunction, and tissue injury is circulating blood cells or cells lining the vasculature or glial cells, we used bone marrow chimeric mice. Our findings indicate that RANTES>WT chimeras exhibit attenuated blood cell adhesion, BBB permeability, and tissue infarction responses similar to those observed in RANTES−/− mice. This suggests that a circulating blood cell is the likely source of RANTES that mediates the I/R-induced cerebral responses. The persistent elevation of brain tissue RANTES in RANTES>WT chimeras (Figure 3A) suggests that nonblood cells (endothelial cells, vascular smooth muscle cells, and/or glial cells) are likely to account for the majority of the I/R-induced elevation of brain tissue RANTES. Of the total RANTES detected in brain tissue of WT-I/R, it may be estimated from Figure 3A that approximately 40% is derived from blood cells, whereas 60% is derived from nonblood cells. Nonetheless, RANTES>WT chimeras clearly exhibited protective responses comparable to RANTES−/− mice, suggesting that deposition of blood cell-derived RANTES onto the endothelial cell surface is of equal or greater importance to RANTES generated from parenchymal cells in mediating the inflammatory and injury responses to I/R. Although parenchymal cell-derived RANTES can move to the endothelial surface by transcytosis, the liberation of RANTES from blood cells is a much faster response and is more effective for the initiation of the pathogenesis.

The BM chimeras developed from RANTES−/− mice provide useful insights into the potential contribution of blood versus vascular/extravascular cells to the RANTES-mediated responses. However, there is some evidence in the literature suggesting that transplanted BM cells may gain access to the extravascular compartment when chimerization is achieved. For example, it has been shown that, 3 months after BM transplantation, a significant number (over 10 000 cells/brain) of transplanted cells are detected in the brain, but most of the cells are distributed in the areas of the brain that lack a BBB, like the leptomeninges and circumventricular organs. These cells preserved their hematopoietic identity (macrophage) and were able to replace the resident population of leptomeningeal macrophages. The transdifferentiation of donor cells into neurons was rarely seen except in the cerebellum. In another study, it was shown that donor-derived cells start to appear at perivascular/leptomeningeal sites as early as 2 weeks after BM transplantation. These reports suggest that we cannot exclude the possibility that our RANTES>WT chimeras may have included donor-origin (RANTES−/−) macrophages in circumventricular organs and perivascular regions, which could have offered a more protective against I/R injury.
Our RANTES measurements in Rag-1<sup>−/−</sup> mice suggest that lymphocytes are an unlikely source of the chemokine. However, this does not exclude the possibility that T-lymphocytes, which have been implicated in the pathogenesis of ischemic stroke,<sup>30</sup> could influence the release of RANTES from other cells. Platelets are a more likely source, because they are known to release large quantities of the chemokine from α-granules when activated.<sup>31</sup> Although we made an effort to directly address the role of platelets in the RANTES-mediated brain responses to I/R by rendering mice thrombocytopenic using antiplatelet serum (rabbit antismouse thrombocyte antiserum; Accurate Chemicals), the animals (n = 7) could not tolerate the I/R protocol due to excessive brain hemorrhage after the reperfusion. The absence of an elevated RANTES concentration in plasma after I/R (Figure 4B) is consistent with clinical data showing that serum RANTES concentration does not differ between patients with stroke and normal subjects,<sup>32</sup> but this may reflect the efficient binding of locally released RANTES to glycosaminoglycans on cerebrovascular endothelial cells. Indeed, RANTES is reported to have a higher affinity to heparin than other chemokines (RANTES > MCP-1 > IL-8 > MIP1α).<sup>27</sup>

The mechanisms underlying the inflammatory and microvascular alterations mediated by RANTES in cerebral I/R remain unclear. Because the MCAO/R model is associated with a significant increase in tissue and plasma levels of cytokines, we addressed whether the protection observed in RANTES<sup>−/−</sup> mice is associated with a blunted cytokine response. Our findings revealed that the enhanced cytokine levels elicited by cerebral I/R largely do not differ between WT and RANTES<sup>−/−</sup> mice, especially in brain tissue, in which increases in tumor necrosis factor-α, MCP-1, IL-6, and reductions in IL-12 were detected in the injured hemisphere of both WT and RANTES<sup>−/−</sup> mice. However, significant reductions in plasma IL-6 and IL-10 concentrations were noted in RANTES<sup>−/−</sup> compared with WT-I/R mice.

Although it is well recognized that cerebral I/R is associated with increases in both plasma and tissue IL-6 concentrations, the pathophysiological importance of these changes remains controversial with some reports proposing IL-6 as a mediator of injury,<sup>33,34</sup> and others suggesting that the cytokine is protective against ischemic stroke.<sup>35,36</sup> IL-10, on the other hand, is more universally considered to be an anti-inflammatory cytokine with neuroprotective effects.<sup>37</sup> RANTES is a potent inducer of IL-10 production by peripheral mononuclear cells;<sup>38</sup> therefore, RANTES deficiency might be expected to lower plasma IL-10 levels as noted in our study. Nonetheless, it remains unclear whether the changes noted in plasma cytokines in RANTES<sup>−/−</sup> mice account for the protection afforded in this mutant.

In conclusion, our findings implicate RANTES as a mediator of I/R-induced BBB disruption, tissue injury, and the inflammatory and prothrombogenic phenotype assumed by the cerebral microvasculature after focal I/R. Blood cells, probably platelets, are the likely source of RANTES that mediates these responses. The protective effect of RANTES deficiency may be linked to changes in the plasma concentrations of certain cytokines, namely IL-6, IL-10, and IL-12. RANTES-directed interventions may prove useful in the treatment of ischemic stroke.

**Acknowledgments**

We thank Dr. Sally R. Sarawar from the Torrey Pines Institute for Molecular Studies, San Diego, Calif, for providing a breeding pair of RANTES<sup>−/−</sup> mice.

**Source of Funding**

This work was supported by a grant from the National Heart Lung and Blood Institute (HL26441).

Disclosures

None.

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*Stroke*. published online July 17, 2008;
*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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