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Role of T Lymphocytes and Interferon-γ in Ischemic Stroke

Gokhan Yilmaz, MD*; Thiruma V. Arumugam, PhD*; Karen Y. Stokes, PhD; D. Neil Granger, PhD

Background**—**Although lymphocyte recruitment and activation are associated with cerebral ischemia-reperfusion (I/R) injury, the contributions of specific lymphocyte subpopulations and lymphocyte-derived interferon-γ (IFN-γ) to stroke remain unknown. The objectives of this study were to define the contribution of specific populations of lymphocytes to the inflammatory and prothrombogenic responses elicited in the cerebral microvasculature by I/R and to investigate the role of T-cell–associated IFN-γ in the pathogenesis of ischemic stroke.

Methods and Results**—**Middle cerebral artery occlusion was induced for 1 hour (followed by 4 or 24 hours of reperfusion) in wild-type mice and mice deficient in lymphocytes (Rag1$^{-/-}$), CD4^{+} T cells, CD8^{+} T cells, B cells, or IFN-γ. Platelet and leukocyte adhesion was assessed in cortical venules with intravital video microscopy. Neurological deficit and infarct volume were determined 24 hours after reperfusion. Rag1$^{-/-}$, CD4^{+} T-cell$^{-/-}$, CD8^{+} T-cell$^{-/-}$, and IFN-$\gamma^{-/-}$ mice exhibited comparable significant reductions in I/R-induced leukocyte and platelet adhesion compared with wild-type mice exposed to I/R. Infarct volume was reduced and I/R-induced neurological deficit was improved in immunodeficient Rag1$^{-/-}$ mice. These protective responses were reversed in Rag1$^{-/-}$ mice reconstituted with either wild-type or, to a lesser extent, IFN-$\gamma^{-/-}$ splenocytes. B-cell–deficient mice failed to show improvement against ischemic stroke injury.

Conclusions**—**These findings indicate that CD4^{+} and CD8^{+} T lymphocytes, but not B lymphocytes, contribute to the inflammatory and thrombogenic responses, brain injury, and neurological deficit associated with experimental stroke. Although IFN-γ plays a pivotal role in stroke-induced inflammatory responses, T lymphocytes appear to be a minor source of this cytokine. (***Circulation*****. 2006;113:2105-2112.**)

Key Words: cerebral ischemia ■ leukocytes ■ lymphocytes ■ microcirculation ■ platelets

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Cerebrovascular diseases, including ischemic stroke, are associated with high mortality worldwide. The microvasculature of postischemic brain assumes an inflammatory phenotype that is manifested as endothelial activation and barrier dysfunction, enhanced generation of oxidants and inflammatory mediators, and the recruitment of adherent leukocytes and platelets.[1] The contention that inflammation is an early and rate-determining step in the microvascular dysfunction and tissue injury associated with cerebral ischemia-reperfusion (I/R) is supported by several reports that describe a reduction in brain edema and infarct size in animal models of stroke treated with antibodies that block leukocyte adhesion.[2,3] Although most attention has been devoted to the possible role of neutrophils in the tissue responses to cerebral I/R because this leukocyte subset accumulates early after reperfusion, there is evidence that T lymphocytes also accumulate in the postischemic brain within the first 24 hours after reperfusion.[4] However, a functional role for neutrophils in the development of stroke-associated injury remains controversial, and the contribution of specific lymphocyte subpopulations and their products to the pathogenesis of ischemic stroke has not been systematically addressed.

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In most organs, including the brain, neutrophils are the first leukocytes to accumulate and infiltrate the tissue on reperfusion, followed by monocytes and then T lymphocytes. Although data supporting a role for T cells, in particular CD8^{+} cytotoxic T lymphocytes,[4–6] in ischemic brain injury are both limited and indirect, a large body of evidence invokes a major role for lymphocytes in the pathogenesis of I/R injury in other vascular beds, including intestine, liver, and kidney.[7–11] Furthermore, it has become increasingly apparent that the role of T lymphocytes is not restricted to the late reperfusion phase; rather, these cells have been strongly implicated in the neutrophil infiltration that occurs shortly after reperfusion[10] by producing/releasing proinflammatory cytokines that induce the expression of endothelial cell adhesion molecules. Although T-cell–derived interferon-γ (IFN-γ) has been shown to contribute to the injury elicited by I/R in other organs[7] and IFN-γ mRNA is increased in rat brain tissue after permanent focal cerebral ischemia,[12] less is known about the contribution of this cytokine to cerebral I/R injury. B lym-

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From the Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, Shreveport.
*Drs Yilmaz and Arumugam contributed equally to this article.
Correspondence to D. Neil Granger, PhD, Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, 1501 Kings Hwy, Shreveport, LA 71130-3932. E-mail dgrang@lsuhsc.edu

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phocytes also have been implicated in I/R-induced tissue injury[13]; however, this response in the kidney appears to be independent of leukocyte recruitment. It remains unclear whether B cells contribute to I/R injury in other organs, including the brain.

In the present study, an established murine model of transient focal cerebral ischemia was used to address the following objectives: (1) to define the role of specific subpopulations of lymphocytes ($CD4^+$ T cells, $CD8^+$ T cells, B cells) and IFN-γ in the inflammatory and prothrombogenic responses elicited in the cerebral microvasculature by I/R, (2) to determine whether lymphocytes are an important source of IFN-γ, and (3) to define the contribution of these lymphocyte populations and IFN-γ to the tissue damage and neurological deficit associated with ischemic stroke.

Methods

Animals

All experiments were performed on male mice (21 to 25 g) with a C57Bl/6J background (Jackson Laboratories, Bar Harbor, Me). The experimental procedures were approved by the Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee. A sham group (wild-type C57Bl/6J [WT] exposed to sham operation; 4 hours, n=6; 24 hours, n=8) and the following 9 groups exposed to 1 hour of middle cerebral artery occlusion (MCAO) and 4 or 24 hours of I/R were studied: WT (4 hours, n=10; 24 hours, n=8), $CD8^+$ T-cell$^{-/-}$ (mice deficient in $CD8^+$ T lymphocytes) (4 hours, n=5; 24 hours, n=6), $CD4^+$ T-cell$^{-/-}$ (mice deficient in $CD4^+$ T lymphocytes) (4 hours, n=5; 24 hours, n=6), B-cell$^{-/-}$ (mice deficient in B lymphocytes) (4 hours, n=5; 24 hours, n=5), neutropenic (WT mice given 150 μg antineutrophil serum IP as previously described[14] [RB6–8C5, kindly provided by Stephen B. Pruett, Louisiana State University Health Sciences Center, Shreveport] 24 hours before I/R, which reduced circulating neutrophil counts by >98%) (24 hours, n=5), Rag1$^{-/-}$ (lymphocyte-deficient mice) (4 hours, n=7; 24 hours, n=7), IFN-$\gamma^{-/-}$ (IFN-γ–deficient mice) (4 hours, n=5; 24 hours, n=7), Rag1$^{-/-}$←WSC (Rag1$^{-/-}$ mice reconstituted with 50×10^6 whole splenocytes from WT mice intravenously 5 days before I/R as described previously[14]) (4 hours, n=5; 24 hours, n=5), and Rag1$^{-/-}$←IFN-$\gamma^{-/-}$SC (Rag1$^{-/-}$ mice reconstituted with whole splenocytes from IFN-$\gamma^{-/-}$ mice 5 days before I/R) (4 hours, n=5; 24 hours, n=6). Flow cytometric analysis confirmed that Rag1$^{-/-}$, $CD4^+$ T-cell$^{-/-}$, $CD8^+$ T-cell$^{-/-}$, and B-cell$^{-/-}$ mice were completely deficient in the relevant lymphocyte populations. Blood total leukocyte counts in Rag1$^{-/-}$ mice were decreased by ≈50%, which is consistent with complete depletion of the normal resting lymphocyte population in C57Bl/6 mice (data not shown). More experiments were performed for the neurological score and infarct data than stated above for intravital microscopy, and these numbers are reported on the relevant graphs.

Middle Cerebral Artery Occlusion

Mice were anesthetized by intraperitoneal injection of ketamine hydrochloride (50 mg/kg) and xylazine (2.5 mg/kg). Transient focal cerebral ischemia was induced by MCAO using the previously described intraluminal filament method.[15] Briefly, the blunted tip of a 6-0 nylon monofilament was advanced to the level of the carotid bifurcation via the internal carotid artery until light resistance was felt. The distance from the nylon thread tip to the internal carotid artery–pterygopalatine artery bifurcation was slightly >6 mm, and the distance to the bifurcation of the internal and external carotid arteries was slightly <9 mm. The monofilament was removed after 60 minutes of occlusion. In the sham group, these arteries were visualized but not disturbed.

Cerebral blood flow measurements were obtained from all groups (n=3 to 5) with the Vasamedics laser Doppler (Vasamedics Inc, St Paul, Minn) by placing the laser probe at the level of the dura, directly above the ischemic region. Cerebral blood flow measurements were obtained before and after MCAO to determine whether the reduction in cerebral blood flow caused by MCAO differed between the various mutant mouse models.

Intravital Fluorescence Microscopy

After a 4- or 24-hour reperfusion period, the mice were anesthetized by intraperitoneal injection of ketamine hydrochloride (50 mg/kg) and xylazine (2.5 mg/kg). The right jugular vein was cannulated for intravenous administration of the labeled platelets and rhodamine-6G. The femoral artery was cannulated to monitor mean arterial blood pressure and to sample arterial blood for blood gases (Omni-Modular System). All mice were tracheotomized and artificially ventilated with room air during observation by intravital microscopy.

The head of each mouse was fixed in a plastic frame in the sphinx position. The left parietal bone was exposed by a midline skin incision, followed by a craniectomy at 1 mm posterior from the bregma and 4 mm lateral from the midline. The dura mater was not cut because the fluorescently labeled blood cells were easily observed and intracranial pressure was well maintained in the absence of this procedure. Artificial cerebrospinal fluid was placed over the exposed brain tissue. The observation area represented the major infarcted region after MCAO. A 12-mm glass coverslip was placed over the craniectomy, and the space between the glass and dura mater was filled with artificial cerebrospinal fluid.

Platelets were isolated from a donor animal using a series of centrifugation steps and labeled ex vivo with carboxyfluorescein diacetate succinimudyl ester as described before.[16] We have previously shown that this platelet isolation procedure does not activate the platelets.[17] In each mouse, 100×10^6 platelets were infused over 5 minutes, yielding ≈10% of the total blood platelet count. The platelets were allowed to circulate for 5 minutes before images of the microcirculation were recorded. Once the platelet data were collected, endogenous leukocytes were labeled in vivo by infusing rhodamine-6G (100 μL; 0.02%) over 5 minutes and allowing it to circulate for 5 minutes before observation.

An upright Nikon microscope equipped with a SIT camera (C2400-08; Hamamatsu Photonics K.K., Shizuoka, Japan) and a mercury lamp was used to observe the cerebral microcirculation. Five randomly selected segments of cerebral venules were chosen for observation of platelet and leukocyte adhesion for 1 minute each in each mouse. Venules from the dura mater were avoided by confirming the origin of the observed cerebral venules and by evaluating adhesion in venules 30 to 60 μm in diameter (the diameter of dura venules is normally <20 μm) and at least 100 μm in length. Adherent platelets and leukocytes were defined as cells remaining stationary on the venular wall for ≥30 seconds and were expressed as the number of cells per 1 mm^2 venular surface, calculated from the diameter and length, assuming that the vessel was cylindrical.

Neurological Assessment

The functional consequences of cerebral I/R injury were evaluated at 24 hours of reperfusion by using a 5-point scale neurological deficit score (0=no deficit, 1=failure to extend right paw, 2=circling to the right, 3=falling to the right, 4=unable to walk spontaneously)[18] and assessed in a blinded fashion.

Detection and Quantification of Cerebral Infarction

At the end of a 24-hour reperfusion period, mice were killed with a lethal dose of pentobarbital (150 mg/kg IP). The brains were immediately removed, and 2-mm coronal sections were cut and then stained with 2% 2,3,5-triphenyltetrazolium chloride, as previously described.[19] The total areas of each brain section and the infarction were quantified on digitized images with a computerized image analysis program (NIH Image). Infarct volume was expressed as a percentage of ipsilateral hemisphere. In separate groups of WT and Rag1$^{-/-}$ mice, infarct volume also was determined at 72 hours of reperfusion.

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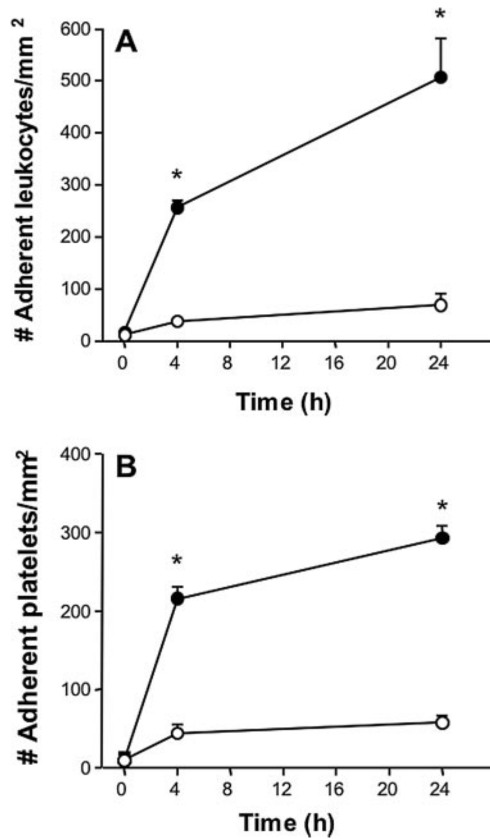

Figure 1. Changes in leukocyte (A) and platelet (B) adherence observed in pial venules after 60 minutes of ischemia followed by either 4 or 24 hours of reperfusion in WT mice (●). Sham-operated mice also are represented (○). *$P<0.05$ vs corresponding sham and control values.

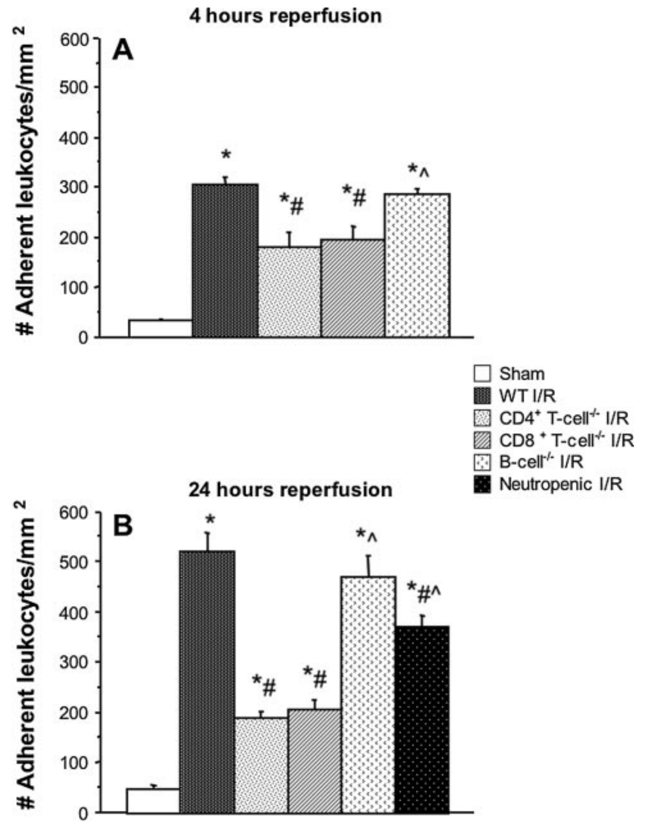

Figure 2. Adherent leukocytes in cerebral venules after MCAO followed by either 4 (A) or 24 (B) hours of reperfusion in WT mice and mice deficient in $CD4^+$ T cells, $CD8^+$ T cells, B cells, or neutrophils. *$P<0.05$ vs shams; #$P<0.01$ vs WT I/R; ^$P<0.05$ vs $CD4^+$ T-cell$^{-/-}$ and $CD8^+$ T-cell$^{-/-}$ groups.

Statistical Analysis

All experimental results are expressed as mean±SEM. Statistical comparisons were made with ANOVA, followed by Tukey-Kramer post hoc analysis. Statistical significance was assessed at $P<0.05$.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Blood Pressure, Gas Analysis, Cerebral Blood Flow, and Circulating Blood Cells

Blood pressure and gas analysis measurements revealed no statistically significant differences between any of the experimental groups. The cerebral blood flow measurements obtained immediately before and after MCAO showed an ≈90% reduction in blood flow to the MCAO infarct region, which did not differ between groups.

Blood Cell–Vessel Wall Interactions

Time-Dependent Blood Cell Adhesion

A negligible level of leukocyte and platelet adhesion was noted in cerebral venules of WT sham-operated mice (Figure 1). A sharp rise in the recruitment of both leukocytes (Figure 1A) and platelets (Figure 1B) occurred within 4 hours of reperfusion. By 24 hours of reperfusion, leukocyte adhesion was increased a further 70%, whereas adherent platelets were elevated by an additional 13% above the 4-hour value.

Roles of $CD8^+$ T Cells, $CD4^+$ T Cells, and B Cells

Deficiency of either $CD4^+$ T cells or $CD8^+$ T cells partially reduced MCAO-induced leukocyte (Figure 2) and platelet (Figure 3) recruitment at 4 hours of reperfusion and resulted in a more dramatic reduction in the adhesion responses at 24 hours of reperfusion. In contrast, mice lacking only B cells did not exhibit an attenuation of the MCAO-induced leukocyte (Figure 2) or platelet (Figure 3) adhesion responses at either 4 or 24 hours of reperfusion.

Role of Neutrophils

Neutropenic mice were analyzed only at 24 hours of reperfusion because we have previously reported a significant reduction in leukocyte adhesion in neutropenic mice undergoing MCAO and 4 hours of reperfusion.[2] Although these mice also demonstrated an attenuated leukocyte adhesion response at 24 hours of reperfusion (compared with the untreated MCAO group), the attenuating effect was much reduced and leukocyte adhesion remained significantly higher than levels detected in sham-operated animals (Figure 2B). I/R-induced platelet recruitment at 24 hours was unaffected in neutropenic WT mice compared with untreated WT mice (Figure 3B).

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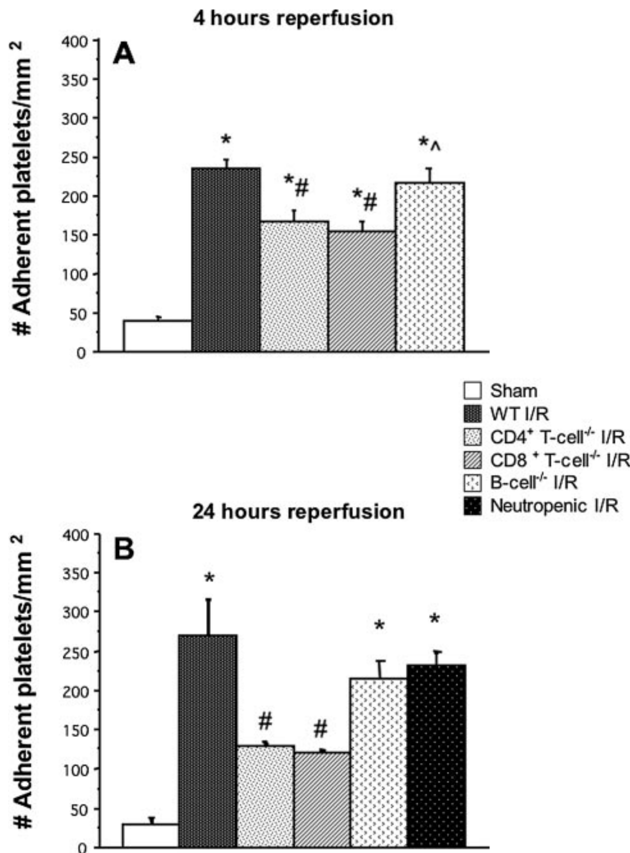

Figure 3. Adherent platelets in cerebral venules after MCAO followed by either 4 (A) or 24 (B) hours of reperfusion in WT mice and mice deficient in $CD4^+$ T cells, $CD8^+$ T cells, B cells, or neutrophils. *$P<0.001$ vs shams; #$P<0.05$ vs WT I/R; ^$P<0.05$ vs $CD8^+$ T $cell^{-/-}$.

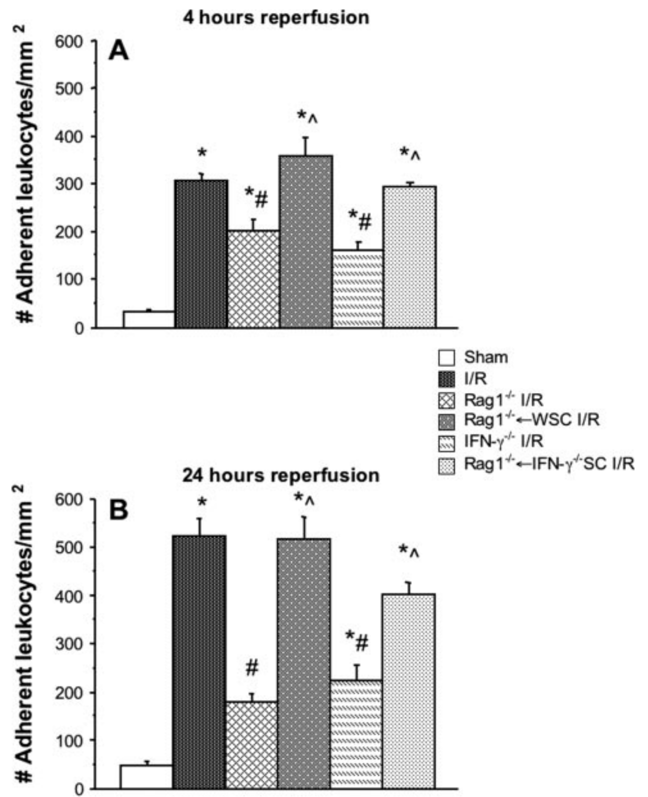

Figure 4. Adherent leukocytes in cerebral venules after MCAO followed by either 4 (A) or 24 (B) hours of reperfusion in WT mice and mice deficient in T and B lymphocytes ($Rag1^{-/-}$) or $IFN\text{-}\gamma^{-/-}$. Separate groups of $Rag1^{-/-}$ mice received whole splenocytes isolated from WT ($Rag1^{-/-}$←WSC) or $IFN\text{-}\gamma^{-/-}$ ($Rag1^{-/-}$←$IFN\text{-}\gamma^{-/-}$SC) animals 5 days before induction of stroke. *$P<0.01$ vs shams; #$P<0.001$ vs WT I/R; ^$P<0.05$ vs $Rag1^{-/-}$.

Role of Lymphocyte-Derived IFN-γ

Leukocyte (Figure 4A) and platelet (Figure 5A) adhesion responses at 4 hours of reperfusion were significantly reduced after MCAO in mice lacking both T and B lymphocytes ($Rag1^{-/-}$), although they remained significantly higher than in shams. Similar to mice deficient in either $CD4^+$ or $CD8^+$ T cells (Figure 2 and Figure 3), the protective effect of total lymphocyte deficiency was more pronounced at 24 hours of reperfusion, with a more dramatic reduction in leukocyte (Figure 4B) and platelet (Figure 5B) adhesion, although the latter did not reach statistical significance. Administration of whole splenocytes from WT mice restored the leukocyte and platelet adhesion responses in $Rag1^{-/-}$ mice ($Rag1^{-/-}$←WSC group) at both 4 and 24 hours of reperfusion to the magnitude found in WT mice. $IFN\text{-}\gamma^{-/-}$ mice showed a reduction in leukocyte (Figure 4) and platelet (Figure 5) adhesion similar to that observed in $Rag1^{-/-}$ mice at both 4 and 24 hours of reperfusion (compared with WT mice). $Rag1^{-/-}$ mice reconstituted with $IFN\text{-}\gamma^{-/-}$ splenocytes exhibited leukocyte and platelet adhesion responses at 4 hours of reperfusion that were similar to levels observed in WT mice. However, at 24 hours, the restoration of leukocyte adhesion was only partial, although leukocyte adhesion was significantly elevated compared with $Rag1^{-/-}$ mice. Platelet adhesion in the $Rag1^{-/-}$ ←$IFN\text{-}\gamma^{-/-}$SC group at 24 hours was comparable to that in WT and $Rag1^{-/-}$←WSC mice.

MCAO-Induced Cerebral I/R Injury

Roles of CD8+, CD4+ T cells, and B Cells

Figure 6 shows that $CD8^+$ T cells, and to a lesser extent $CD4^+$ T cells, are important in the development of postischemic cerebral infarct. Mice lacking these T-cell subtypes exhibited comparable reductions in neurological score, although this did not reach statistical significance. In contrast, B-cell deficiency did not afford any benefit against MCAO-induced infarct volume or neurological deficit (Figure 6).

Role of Neutrophils

Mice rendered neutropenic were not protected against the development of cerebral infarct after MCAO (Figure 6A). However, neutropenia conferred a 39% reduction in the neurological score of WT mice after MCAO, although this did not reach significance (Figure 6B).

Role of Lymphocyte-Derived IFN-γ

Cerebral infarct size and neurological deficit were significantly reduced in $Rag1^{-/-}$ mice (Figure 7). The infarct size remained significantly lower in $Rag1^{-/-}$ mice subjected to 60 minutes of ischemia and 72 hours of reperfusion (11.8±2.55) compared with WT mice (27.6±4.90) subjected to the same insult. These were fully restored in $Rag1^{-/-}$ mice that received WT splenocytes. The infarct that developed in

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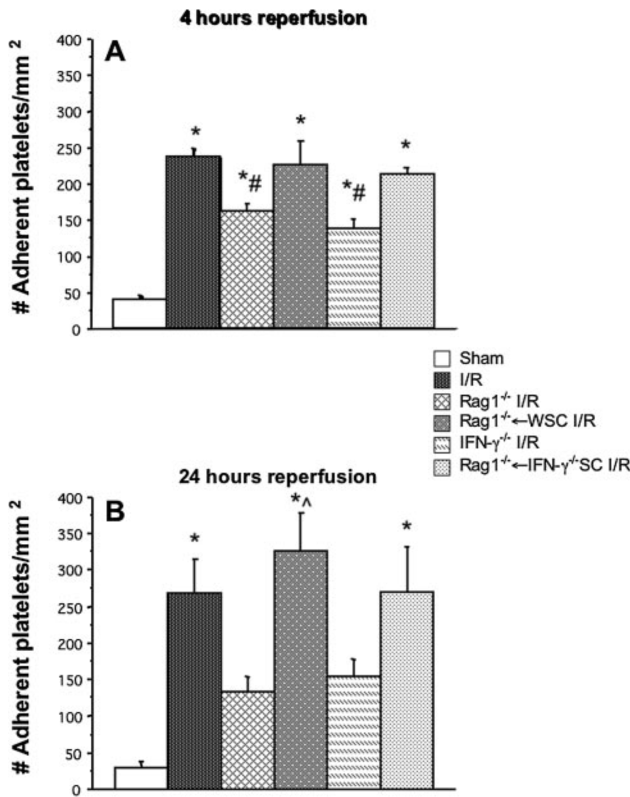

Figure 5. Adherent platelets in cerebral venules after MCAO followed by either 4 (A) or 24 (B) hours of reperfusion in WT mice and mice deficient in T and B lymphocytes ($Rag1^{-/-}$) or IFN-$\gamma^{-/-}$. Separate groups of $Rag1^{-/-}$ mice received whole splenocytes isolated from WT ($Rag1^{-/-}$←WSC) or IFN-$\gamma^{-/-}$ ($Rag1^{-/-}$←IFN-$\gamma^{-/-}$SC) animals 5 days before induction of stroke. *$P<0.01$ vs shams; #$P<0.05$ vs WT I/R; ^$P<0.05$ vs $Rag1^{-/-}$.

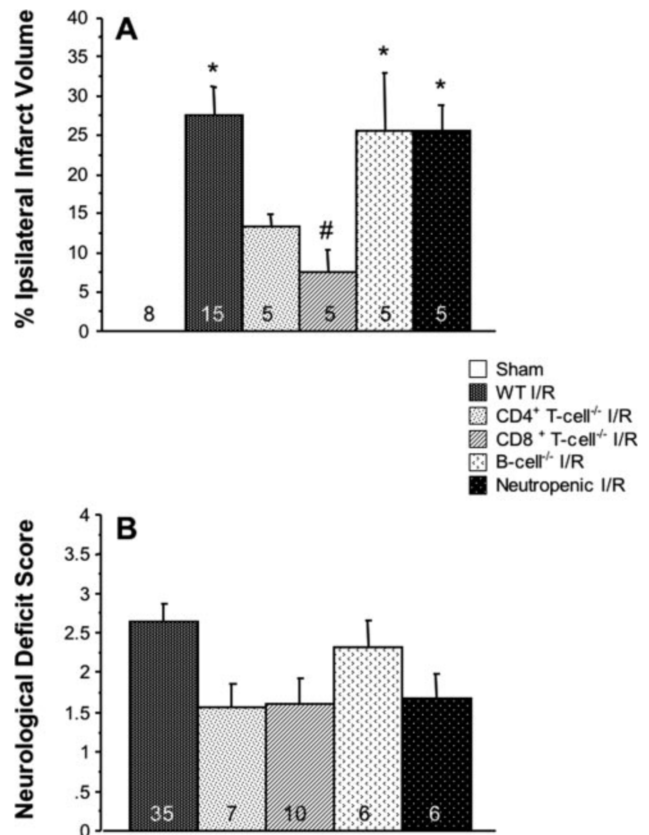

Figure 6. Ischemic infarct volume (A) and neurological score (B) in WT mice and mice deficient in $CD4^{+}$ T cells, $CD8^{+}$ T cells, B cells, or neutrophils exposed to 1 hour of MCAO followed by 24 hours of reperfusion. Numbers of mice are given on bars. *$P<0.01$ vs shams; #$P<0.05$ vs WT I/R.

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IFN-$\gamma^{-/-}$ mice was significantly smaller than in WT mice but comparable to that in $Rag1^{-/-}$ mice (Figure 7A). The neurological deficit was moderately (but not significantly) reduced in the group. Reconstitution of $Rag1^{-/-}$ mice with splenocytes from IFN-$\gamma^{-/-}$ mice restored the tissue injury and behavioral impairment toward those detected in WT and $Rag1^{-/-}$←WSC groups.

Discussion

Although the contribution of neutrophils to cerebral I/R injury has received considerable attention, less effort has been devoted to defining the role of other subsets of leukocytes in this disease process. The revelation that T lymphocytes participate in the early neutrophil recruitment and tissue damage after I/R injury in other organs[7–11] raises the question of whether T cells also contribute to stroke-induced microvascular dysfunction and tissue damage. Furthermore, although the T-cell–associated cytokine IFN-γ plays a pivotal role in the pathogenesis of T-cell–mediated responses to I/R in other organs,[7] its potential involvement in cerebral I/R injury has received little attention. The present study strongly implicates both T lymphocytes and IFN-γ as key participants in the microvascular dysfunction and tissue injury that result from transient focal ischemia and reperfusion of mouse brain. In addition, our findings suggest that T lymphocytes and IFN-γ may mediate these responses by causing the cerebral microvasculature to assume a proinflammatory and prothrombogenic phenotype, although T lymphocytes do not appear to be the primary source of this IFN-γ.

The early accumulation of neutrophils in the postischemic brain has been demonstrated in animal models and humans.[20–22] The present work extends our previous observations that neutrophil depletion resulted in a 50% to 60% reduction of both leukocyte and platelet recruitment observed in murine cerebral venules at 4 hours of reperfusion after transient focal ischemia,[2] and it addresses whether neutrophil accumulation contributes to the deleterious effects (tissue necrosis, neurological deficit) of ischemic stroke. Despite the marked neutropenia achieved in our model, the I/R-induced adhesion of leukocytes was attenuated only slightly and platelet recruitment was unaltered by depletion of neutrophils at 24 hours of reperfusion. Furthermore, the neurological deficits at 24 hours of reperfusion were reduced only partially by neutropenia, and there was no reduction in the volume of infarcted tissue. Taken together with the previous observations at 4 hours of reperfusion,[2] our findings suggest that leukocyte subsets other than neutrophils are trafficking through the brain microcirculation, perhaps as early as 4 hours after MCAO.

Lymphocytes contribute to the pathogenesis of I/R injury in several vascular beds[7–11] by promoting the recruitment of

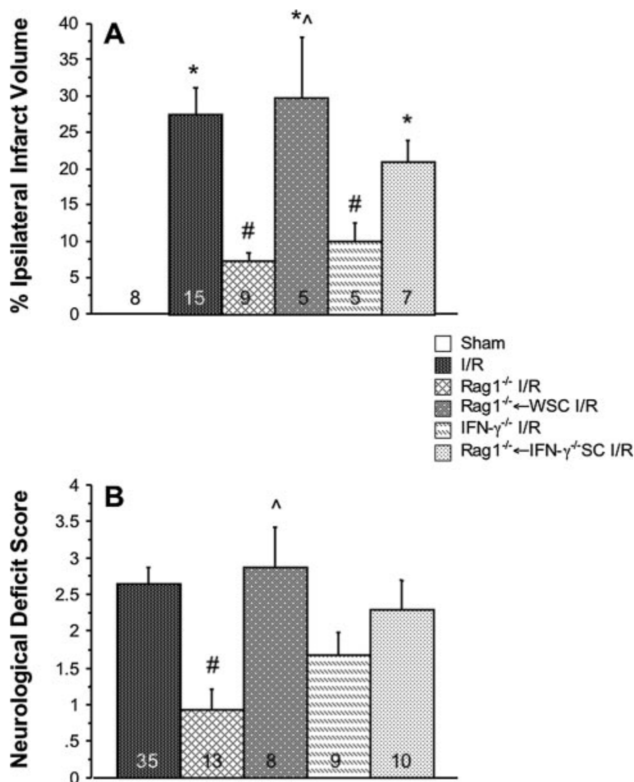

Figure 7. Ischemic infarct volume (A) and neurological score (B) in WT mice and mice deficient in T and B lymphocytes ($Rag1^{-/-}$) or IFN-$\gamma^{-/-}$ exposed to 1 hour of MCAO followed by 24 hours of reperfusion. Separate groups of $Rag1^{-/-}$ mice received whole splenocytes isolated from WT ($Rag1^{-/-}$←WSC) or IFN-$\gamma^{-/-}$ ($Rag1^{-/-}$←IFN-$\gamma^{-/-}$SC) animals 5 days before induction of stroke. Numbers of mice are given on bars. *$P<0.01$ vs sham mice; #$P<0.05$ vs WT I/R mice; ^$P<0.01$ vs $Rag1^{-/-}$ group.

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other leukocyte subsets and by enhancing the microvascular dysfunction induced by I/R.[10] The findings of the present study clearly indicate that lymphocytes also play a major role in the inflammatory responses elicited in the cerebral microvasculature after I/R. An assessment of the role of different lymphocyte subsets in our cerebral I/R model provided the first direct evidence that T lymphocytes, specifically $CD4^+$ and $CD8^+$ T cells, are major contributors to brain inflammation induced by MCAO/reperfusion. We also demonstrated a dependence of platelet adhesion in postischemic venules on $CD4^+$ and $CD8^+$ T lymphocytes. Although previous findings have demonstrated that neutrophil depletion is effective at abrogating platelet recruitment at 4 hours of transient cerebral ischemia,[2] the lack of effect of antineutrophil serum in reducing platelet adhesion at 24 hours suggests that lymphocytes are not exerting their effect on platelet recruitment through neutrophils. Of relevance in this regard is published evidence that lymphocytes do not bind platelets nearly as avidly as neutrophils and monocytes[23]; rather, they may be acting via a soluble mediator or through activation of other cell types to induce the prothrombogenic phenotype that is assumed by postischemic cerebral venules.

Previous studies in the liver and kidney have demonstrated that T lymphocytes, in particular $CD4^+$ T cells, contribute to the organ dysfunction induced by I/R.[7,9] Although comparable analyses of lymphocyte involvement in ischemic brain injury have not previously been performed, some circumstantial evidence derived from studies using immunosuppressive agents such as FK506[24] supports the potential involvement of these immune cells in the pathogenesis of ischemic stroke, whereas other data do not.[25,26] Our findings of moderately reduced infarct volume and neurological deficit after I/R in mice lacking $CD4^+$ T cells strongly support a role for T lymphocytes in the brain injury responses to I/R and are consistent with the protection afforded by $CD4^+$ T-cell deficiency in other organs.[9,10] However, contrary to other tissues, $CD8^+$ T cells appear to be as important as $CD4^+$ T cells in mediating I/R-induced brain responses. Interestingly, the lack of either T-cell subset alone was as effective as deficiency of all lymphocytes ($Rag1^{-/-}$ mice) in protecting the brain, suggesting that these $CD4^+$ and $CD8^+$ T cells act through a common pathway to produce brain injury after MCAO and, in fact, require each other to generate the inflammatory response. Exactly how these 2 T-cell populations may influence each other remains unclear, although both $CD4^+$ and $CD8^+$ T cells have been located in the brain after stroke[4,6] and both subsets can produce similar cytokines that may act to propagate their responses. An alternative explanation for our findings is that these lymphocyte-deficient mice ultimately suffer comparable changes in infarct volume, but the damage is simply delayed. However, this possibility appears unlikely because a significant reduction in infarct volume also was evident in lymphocyte-deficient $Rag1^{-/-}$ mice even at 72 hours of reperfusion.

T cells can use several potential mechanisms/pathways to promote the adhesion of leukocytes and platelets and the subsequent tissue injury/organ dysfunction after ischemic stroke. One possibility is that the T cells per se adhere to venular endothelium, enter the tissue, and directly elicit cell necrosis. This possibility is supported by reports describing the presence of T cells (particularly $CD8^+$ T cells) in the brain early after reperfusion,[4,6] and by our observation of only a partial reduction in leukocyte adhesion in mice treated with antineutrophil serum. Alternatively, lymphocytes could initiate or propagate the inflammatory responses that occur within the brain after stroke by releasing mediators that increase the expression of endothelial cell adhesion molecules and/or activate resident macrophages or other populations of leukocytes, which in turn may create a proinflammatory and prothrombogenic environment in the brain. This possibility is supported by the general view that lymphocytes enter the brain in very small numbers early after reperfusion and by reports describing the presence of activated circulating T lymphocytes in blood within the first 9 hours of reperfusion after stroke in humans.[27]

An inflammatory mediator released from both $CD4^+$ and $CD8^+$ T cells is IFN-γ. IFN-γ is considered a key regulator of immune and inflammatory responses and is absent from normal brain parenchyma. During inflammatory conditions such as multiple sclerosis, IFN-γ is produced by infiltrating T cells and NK cells. The role of IFN-γ in permanent MCAO is controversial, with 1 group demonstrating an elevated IFN-γ mRNA and another study unable to corroborate this response.

The present study provides the first evidence that IFN-γ is a mediator of the inflammatory and thrombogenic responses in the postischemic brain microvasculature. Of interest is our observation that IFN-γ exerted a comparable influence on the I/R-induced cerebral responses at 24 hours (compared with 4 hours) of reperfusion, suggesting that the cytokine contributes to the maintenance, and perhaps the initiation, of inflammatory changes in the postischemic brain. Moreover, IFN-γ appears to exert a significant influence on the development of tissue necrosis and a modest effect on the neurological deficit that was associated with brain I/R. These findings may reflect the pluripotent inflammatory properties of IFN-γ that initiate several pathways implicated in cerebral I/R injury such as induction of adhesion molecule expression, stimulation of NAD(P)H oxidase, and activation of microglial cells.[28–31]

Although our findings in $Rag1^{-/-}$ mice reconstituted with IFN-$\gamma^{-/-}$ splenocytes reveal that the source of IFN-γ is unlikely to be T lymphocytes in the early postreperfusion period, the requirement of $Rag1^{-/-}$ mice (which can produce IFN-γ, for example, from NK cells) to have lymphocytes to generate the full inflammatory and thrombogenic response to I/R suggests that T cells and IFN-γ share a common pathway of injury in this model. This contention is supported by the comparable level of protection observed in IFN-$\gamma^{-/-}$ and lymphocyte-deficient mice. However, by 24 hours after reperfusion, T cells appear to be one of several sources of the IFN-γ that is acting to promote leukocyte adhesion and perhaps tissue necrosis and neurological deficit. Although the exact cell type generating the majority of the IFN-γ in this model remains to be elucidated, NK cells, which have been shown to infiltrate the brain alongside T lymphocytes during permanent MCAO,[4] also may contribute to the tissue responses to transient cerebral ischemia through IFN-γ release.

T lymphocytes also could participate in stroke-induced brain injury by inducing an immune response in concert with B lymphocytes. Although relatively little information is available on the role of B cells in I/R injury, a recent study suggested that B lymphocytes make a significant contribution to I/R injury in the kidney through the release of soluble factors and independently of an effect on neutrophil, macrophage, or $CD4^+$ T-cell recruitment.[13] The results of our study failed to reveal a role for B lymphocytes in modulating the accumulation of leukocytes and platelets in the postischemic brain, and we found that B-cell–deficient mice did not show significant improvement in the I/R-induced infarct volume or neurological deficit, suggesting that B cells do not contribute to stroke-induced inflammation and tissue injury.

In summary, our study provides the first evidence for a specific and significant role for $CD4^+$ and $CD8^+$ T lymphocytes in the pathogenesis of ischemic stroke. Whether the lymphocytes are acting directly on brain tissue or indirectly through activation of other circulating blood cells and/or extravascular cells (eg, resident macrophages) remains unclear. However, our findings reveal a novel role for IFN-γ, released primarily from a non–T-cell source, with a smaller contribution from T cells, in the inflammatory and prothrombogenic responses, as well as in the tissue necrosis and behavioral deficits caused by cerebral I/R. Although our results suggest that T cells and IFN-γ should be considered therapeutic targets for ischemic stroke, it should be noted that infection is the leading cause of mortality in the postacute phase of ischemic stroke, which results largely from immunodepression caused by depletion of circulating T-cell and NK-cell populations and therefore the antibacterial cytokine IFN-γ in the early reperfusion period.[32] Alternatively, it is conceivable that the inflammatory responses described here may contribute to the generation of an immunodepressed state; consequently, early intervention directed at these inflammatory events may protect against subsequent infections by maintaining an adequate level of immune defense. Thus, although our findings extend current knowledge about the mechanisms underlying ischemic stroke, the important roles of the T-cell/IFN-γ pathway in preventing infection must be considered when the therapeutic potential of inhibiting these targets in stroke patients is assessed.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Current therapies for ischemic stroke are limited to thrombolysis, anticoagulation, and medical support. Although neutrophils have been implicated in the tissue injury and microvascular dysfunction associated with ischemic stroke, relatively little is known about the contribution of lymphocytes to this pathological process. In this study, we assessed the relative roles of T cells, B cells, neutrophils, and IFN-γ in a mouse model of focal ischemic stroke. Our findings indicate that in the early period (24 hours) after an ischemic stroke, $CD4^+$ and $CD8^+$ T lymphocytes are the principal inflammatory cells that contribute to the microvascular dysfunction and brain injury. The inflammatory cytokine IFN-γ also appears to make a major contribution to this injury process. Our study suggests that immunomodulation of T-cell function during the acute phase of ischemic stroke may represent a novel neuroprotective target for the treatment of ischemic stroke.

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