Absence of the Chemokine Receptor CCR2 Protects Against Cerebral Ischemia/Reperfusion Injury in Mice

Oliver B. Dimitrijevic, MD; Svetlana M. Stamatovic, MD, PhD; Richard F. Keep, PhD; Anuska V. Andjelkovic, MD, PhD

Background and Purpose—The chemokine, monocyte chemoattractant protein-1 (CCL2), is a major factor driving leukocyte infiltration into the brain parenchyma in a variety of neuropathologic conditions associated with inflammation, including stroke. In addition, recent studies indicate that CCL2 and its receptor (CCR2) could have an important role in regulating blood-brain barrier (BBB) permeability. This study evaluated the role of the CCL2/CCR2 axis in regulating postischemic inflammation, BBB breakdown, and vasogenic edema formation.

Methods—CCR2−/− and CCR2+/+ mice were subjected to focal transient cerebral ischemia. BBB permeability and brain edema formation were observed at days 1 and 5 of reperfusion by evaluating the product surface area for fluorescein isothiocyanate–albumin and measuring water and electrolyte contents. Immunohistochemistry was used to assess leukocyte infiltration. cDNA gene and protein arrays for inflammatory cytokines were used to assess inflammatory profiles in CCR2+/+ and CCR2−/− mice.

Results—CCR2−/− mice had reduced infarct sizes and significantly reduced BBB permeability and brain edema formation in the affected ischemic hemisphere compared with CCR2+/+ mice. This reduction in injury was closely associated with reduced infiltration of not only monocytes but also neutrophils (7- and 4-fold decreases, respectively). In addition, CCR2−/− mice had reduced expression/production of inflammatory cytokines during reperfusion.

Conclusions—These data suggest that inhibiting the CCL2/CCR2 axis affects brain reperfusion outcome by reducing brain edema, leukocyte infiltration, and inflammatory mediator expression. (Stroke. 2007;38:1345-1353.)

Key Words: blood-brain barrier permeability ■ CCL2 ■ chemokines ■ inflammation ■ stroke

Postischemic inflammation is considered a significant contributor to ischemic brain injury.1–3 The central event in postischemic inflammation is recruitment of leukocytes, first neutrophils, and then an influx of cells of the monocyte/macrophage lineage.2,3 This is a multifactorial process involving chemotactic signals that promote directed migration of leukocytes, adhesion receptor/ligand interaction at the microvascular endothelial surface, and matrix metalloproteinase production needed for breakdown of the extracellular matrix and leukocyte extravasation.2,4 The presence of destructive leukocytes in the brain parenchyma can enhance ischemic injury, but they also further amplify the inflammatory response. Therefore, a critical event to control postischemic injury is regulating leukocyte entry. Crucial mediators of these events are the chemokines, multifunctional mediators of cellular communication in the ischemic brain and key molecules in leukocyte recruitment.

Chemokines (chemoattractant cytokines) are a superfamily of structurally related proinflammatory peptides that mediate cell-specific, directed migration of leukocytes into sites of inflammation.5 There are 4 different subfamilies (CC, CXC, CX3C, and C) based on biochemical, structural, and functional differences.6 During ischemia/reperfusion, the brain produces chemokines such as CXCL8 (also known as interleukin [IL]-8), CCL2 (MCP-1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL7 (MCP-3), CX3CL1 (fractalkine), and CXCL10.7–14 These contribute to neutrophil and monocyte recruitment into ischemic tissue. Some chemokines, such as IL-8 (or its murine closest equivalent, CXCL5), CCL2, or fractalkine, are critical factors regulating postischemic inflammation and inducing not only leukocyte recruitment but also blood-brain barrier (BBB) disruption and leukocyte adhesion to brain endothelial cells.7,8,13 These chemokines are found in the ischemic penumbra as early as 6 hours of reperfusion, with peak expression within 24 to 48 hours and lasting up to 5 days.7–14 Chemokine expression is closely correlated with infarct size and the progression of ischemic lesions. Manipulation of chemokine expression/activity affects ischemic brain injury. Thus, deletion of the CCL2 or CXCL1 gene in mice or treatment of rabbits with a neutralizing CXC8 antibody during reperfusion significantly attenuates infarct size and decreases the inflammatory response.
and brain edema formation.\textsuperscript{7,8,15} Chemokines may, therefore, be a target in stroke therapy.

The present study assessed the issue of how excluding the activity of 1 widely expressed chemokine, CCL2, affects BBB permeability and inflammation development during central nervous system ischemia/reperfusion injury in mice. Owing to the fact that CCL2 exerts its activity via binding to CCR2 receptors, inhibition of CCL2 activity by deleting CCR2 in our experiments offers insight into new prospective stroke therapies wherein CCR2 antagonists might be used to reduce brain injury.

**Materials and Methods**

All procedures were performed in strict accordance with the National Institute of Health’s Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee of the University of Michigan.

**Middle Cerebral Artery Occlusion**

Experiments were performed on male CCR2\textsuperscript{+/+} and CCR2\textsuperscript{−/−} mice (22 to 25 g) on a C57BL/6\times129Sv background. CCR2 knockout (KO, −/−) mice were crossed with wild-type (+/+ or +/−) CCR2 mice to produce heterozygous (±) CCR2 mice. These were crossedbred to produce additional CCR2 KO mice. This backcrossing was repeated for at least 10 generations to ensure that the generated CCR2 KO mice were almost completely on the same C57BL/6\times129Sv background as the wild-type mice. All breeding and husbandry of animals were undertaken under specific pathogen-free conditions at the University of Michigan. Genotypes were determined by polymerase chain reaction on tail biopsy specimens with the use of CCR2 primer pairs (SuperArray Bioscience Co, Inc).

Mice were anesthetized by injection of ketamine (100 mg/kg IP) and xylazine (10 mg/kg IP). Body temperature was maintained at 37±0.5°C by means of a heating blanket and a heating lamp during the entire experimental procedure. Focal cerebral ischemia was induced by left middle cerebral artery occlusion (MCAO) with an intraluminal filament technique. In brief, the right common carotid artery was exposed through a midline incision in the neck. Next, a 6–0 silicone suture was introduced into the external carotid artery and advanced into the internal carotid artery for a distance of 10 to 11 mm from the common carotid artery bifurcation, according to animal weight. MCAO was confirmed by a laser Doppler flow probe (model BPM System, Vasomedics) positioned 3 mm posterior and 5 mm lateral to the bregma. After 30 minutes of MCAO, the mice were reperfused by suture withdrawal and then allowed to awaken from anesthesia. Sham-operated animals underwent all procedures except MCAO.

Reperfusion periods were 1 to 5 days. During reperfusion, neurologic deficits were evaluated with the following scoring scheme: 0, no visible neurologic deficits; 1, forelimb flexion; 2, contralateral forelimb grips weakly (the operator places the animal on an absorbent pad and gently pulls the tail); 3, circling to the parietic side only when pulled by the tail (the animal was allowed to move about freely on the absorbent pad); and 4, spontaneous circling.

**Brain Water Content and Electrolytes**

Brain water content was measured by the wet/dry weight method. Samples were taken from ischemic and nonischemic hemispheres. After decapitation under deep isoflurane anesthesia, brains were weighed wet and then oven-dried at 100°C for 48 hours and reweighed. Brain water content (%) was calculated as (wet weight−dry weight)/wet weight\times100. After the tissue was dried, electrolytes were extracted with nitric acid, and sodium and potassium contents were measured by flame photometry (Corning Corp).

**Morphometric Measurement of Infarct Volume**

Animals were humanely killed 1 and 5 days hours after transient MCAO, and the brain was removed and sliced. Slices of brain tissue were incubated in 2% 2,3,5-triphenyltetrazolium chloride (TTC) solution for 1 hour at 37°C. The area of infarction in each slice was determined by a computerized image analysis system, and the volume of infarction was calculated by multiplying the distance between sections. In addition, to account for cerebral edema or resolution of the infarct, an indirect measurement of infarction was performed. Infarct volume was calculated as [contralateral hemisphere volume−(ipsilateral hemisphere volume−measured injury volume)].\textsuperscript{16} Cresyl violet staining of 200-μm-thick serial sections was also used to examine infarct size after 5 days of reperfusion.

**In Vivo Permeability Assay**

BBB integrity in mice was assessed by measurement of the blood brain transfer coefficient (\(K_i\)) for fluorescein isothiocyanate (FITC)–albumin with a method based on a previously described procedure.\textsuperscript{17,18} In brief, FITC-albumin was injected as a bolus into a femoral vein 20 minutes before the end of the experiment. Serial arterial blood samples were taken from a femoral artery every 5 minutes from 0 to 20 minutes to determine the FITC-albumin plasma profile. At the end of the experiment, the mice were killed by decapitation. Brains were rapidly removed and dissected into right and left hemispheres. These were weighed and homogenized in 50 mmol/L Tris buffer solution (pH 7.4). The homogenates were centrifuged at 3000 rpm for 30 minutes and the supernatant was collected. Methanol was added to the collected supernatant (1:1, vol/vol), and the mixture was centrifuged again under the same conditions. The fluorescence intensity of the supernatant as well as that of the plasma samples was measured with a fluorescence reader (Bio-Tek Instruments, Inc; emission 485 nm and excitation 540 nm), and concentration was calculated by using a standard curve.\textsuperscript{19} The \(K_i\) for FITC-albumin was determined according to the following equation developed by Ohno and colleagues\textsuperscript{19}:

\[
K_i = \frac{C_{pl} - V_d C_{br}}{\int C_{pl} \cdot dt}
\]

where \(C_{br}\) is the concentration of FITC-albumin in brain tissue at the time of decapitation (ng/g), \(C_{pl}\) is the concentration of FITC-albumin in plasma at the time of decapitation (ng/mL), \(V_d\) is the volume of distribution of FITC-albumin, and \(C_{pl} \cdot dt\) represents the area under the concentration-time curve of FITC-albumin in plasma.

**Physiologic Variables in the Experimental Mouse Groups**

\begin{table}
<table>
<thead>
<tr>
<th>Variables</th>
<th>CCR2\textsuperscript{+/+}</th>
<th>CCR2\textsuperscript{−/−}</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Before 7.34±0.036</td>
<td>7.34±0.023</td>
</tr>
<tr>
<td></td>
<td>After 7.26±0.045</td>
<td>7.28±0.13</td>
</tr>
<tr>
<td>PO\textsubscript{2}</td>
<td>Before 34.4±2.9</td>
<td>34.5±2.5</td>
</tr>
<tr>
<td></td>
<td>After 39.4±1.1</td>
<td>38.4±1.9</td>
</tr>
<tr>
<td>PO\textsubscript{2}</td>
<td>Before 124.05±7</td>
<td>118.45±8.1</td>
</tr>
<tr>
<td></td>
<td>After 120.25±3.2</td>
<td>116.05±2.6</td>
</tr>
<tr>
<td>Blood glucose, mg/dL</td>
<td>Before 137.5±10.6</td>
<td>134.5±6.36</td>
</tr>
<tr>
<td></td>
<td>After 127.14±24</td>
<td>122.5±5.65</td>
</tr>
<tr>
<td>rCBF, %</td>
<td>Before 100±0</td>
<td>100±0</td>
</tr>
<tr>
<td></td>
<td>During 18±3</td>
<td>17±1</td>
</tr>
<tr>
<td></td>
<td>After 91±2</td>
<td>91±3</td>
</tr>
<tr>
<td>rCBF indicates regional cerebral blood flow. Other abbreviations are as defined in text.</td>
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FITC-albumin (ng/mL) in the last blood sample, $V_0$ is regional blood volume (mL/g), and $\int C_p \, dt$ is the integral of the arterial concentration of FITC-albumin over time t. $V_0$ was determined in a second set of animals in which mice were decapitated 1 minute after intravenous injection of FITC-albumin. If one assumes no extravasation during this short circulation time, $V_0 = C_{br}/C_{bl}$. $V_0$, $C_{pl}$, and $C_{bl}$ were determined for CCR2$^{+/+}$ and CCR2$^{-/-}$ mice separately, but there were no significant differences in these values between the 2 groups.

**Immunohistochemistry**

Brain samples were fixed in 4% paraformaldehyde for 18 hours, cryoprotected with sequential immersions in 10% and 20% sucrose solutions, and then cut into 50-μm-thick coronal sections with a freezing microtome. Afterward, the samples were preincubated in blocking solution (5% bovine serum albumin, 5% normal goat serum, 0.05% Tween, and phosphate-buffered saline) and then incubated overnight with the primary antibody, rat anti-mouse Ly6G (BD Bioscience) and anti-myeloperoxidase antibody (HyCult Biotechnology) at 4°C. Reaction was visualized by Texas red–conjugated anti-rat (Sigma-Aldrich) or anti-rabbit (Vector Laboratories) antibody. All samples were viewed on a confocal microscope (LSM 510, Zeiss). For quantification, 50 coronal brain slices (25 slices in front and 25 behind the “middle line” of the visible lesion). Microscope data were acquired with a 10× objective with constant laser power (45% of laser power), pinhole, zoom, focus, gain, and duration of image capture. A total of 20 images were randomly selected and captured per slide. The immunolabeled cells were counted in areas surrounding the ischemic lesion. Five mouse brains per group were analyzed. In the sham-operated group, brain areas were analyzed corresponding to those analyzed in ischemic mice. Slides were coded so that the counter was blinded to the identity of the slides being counted.

**cDNA Array**

Brain was sampled from the area around the ischemic lesion with a “pinch-out” method, whereby the visible ischemic lesion was removed and the surrounding area (1 mm thick) was collected. The corresponding contralateral region was also collected. Total RNA...
was prepared with Trizol (Invitrogen). Biotinylated cDNA probes were synthesized with an Ampho-LPR labeling kit (SuperArray). After labeling, the resulting cDNA probe was denaturated and hybridized to the GEArray Q Series mouse inflammatory cytokine gene array (SuperArray). The hybridized membrane was incubated with a chemiluminescent substrate and exposed to x-ray film. The images were scanned and the relative expression level of each gene was analyzed with software provided by SuperArray Bioscience. In brief, the relative value of every gene was obtained by subtracting the background and comparing with the positive control gene. Brain samples, with 5 animals per group, were analyzed. In addition, reverse transcription–polymerase chain reaction was performed for selected genes (CXCL5, CCL2, IL-1β, tumor necrosis factor [TNF]-α, and IL-13). All primer sets were purchased from SuperArray.

Cytokine Antibody Array
A mouse cytokine antibody array (Raybiotech Inc) was used to simultaneously detect and quantify 22 cytokines in samples collected from the ischemic lesion of the ipsilateral hemisphere and from the corresponding contralateral side. Tissue samples were homogenized in 1.8 mL Tris buffer solution (pH 8.5) supplemented with Triton X-100 at a final concentration of 1% and stirred for 12 hours at 4°C. Samples were centrifuged at 100 000 g for 60 minutes at 4°C to remove cell debris. The supernatant was collected and protein levels were evaluated with a bicinchoninic acid protein assay (Pierce). Total protein level was adjusted for every sample to 2 μg/mL. The cytokine antibody array was performed according to the manufacturer’s instructions. Brain samples from 5 mice per experimental group (MCAO operated and sham control CCR2+/+ and CCR2−/− mice) were analyzed.

Statistical Analysis
All values are expressed as mean±SD. One-way ANOVA was used, followed by Bonferroni post hoc analysis as well as a χ² test with the use of Prism analysis software. P<0.05 was regarded as statistically significant.

Results
Physiologic parameters (pH, PO₂, PCO₂, glucose level, and regional cerebral blood flow) before MCAO and after 30 minutes of reperfusion were not significantly different between wild-type CCR2+/+ and CCR2−/− (KO) mice (Table). At 1 and 5 days after reperfusion, the infarct volume in CCR2−/− mice was smaller than that in CCR2+/+ animals (day 1: CCR2+/+ 61±9 vs CCR2−/− 145±3 mm³; day 5: CCR2+/+ 64±5 vs CCR2−/− 164±6; P<0.001; Figure 1A through 1C). Similar statistically significant differences were present by indirect measurement of infarct volume to correct for brain edema and/or infarct resolution (P<0.001, Figure 1C). There were no significant differences in infarct size measured after TTC and cresyl violet staining at days 1 and 5 of reperfusion. Analyzing the regional distribution of the infarct, we found that in our all experimental groups, infarct lesion was present in the cortex and striatum. However, a reduced infarct volume in CCR2−/− mice was associated with parallel reductions in both structures (cortex and striatum) at days 1 and 5 of reperfusion (Figure 1D).
reduction in infarct size in CCR2−/− mice was associated with less severe neurologic impairment (Figure 1E). There were also significant differences in mortality (P<0.001 by \chi^2). Four of 20 CCR2−/− mice died before neurologic scoring, whereas all 20 CCR2−/− mice survived.

To evaluate brain edema development, brain water, sodium and potassium contents, and the BBB Ki for FITC-albumin were measured. Transient MCAO in CCR2+/+ mice led to an increase in BBB permeability for FITC-albumin during reperfusion (Figure 2A). The BBB disruption was much less in CCR2−/− mice (FITC-albumin Ki at day 1: 0.041±0.0007 vs 0.158±0.017 \mu Lg^{−1}min^{−1} in CCR2+/+ mice, P<0.01; at day 5: 0.034±0.015 vs 0.192±0.051 \mu Lg^{−1}min^{−1} in CCR2+/+ mice, P<0.001, Figure 2A). This difference in BBB permeability was associated with reduced brain edema (Figure 2B) in the ischemic hemisphere in CCR2−/− mice compared with CCR2+/+ mice at days 1 and 5. There were no significant differences in brain water content in the contralateral hemisphere. The decreased edema formation in CCR2−/− mice was associated with less sodium accumulation in the ischemic hemisphere (Figure 2C) and reduced potassium loss (Figure 2C). The data presented indicate that the absence of CCR2 and the activity of its ligand CCL2 have protective effects in BBB disruption and brain edema development associated with ischemic/reperfusion injury.

In CCR2+/+ mice, there was a massive infiltration of neutrophils (myeloperoxidase-positive cells) at days 1 and 5 after MCAO. In addition, at day 5, monocytes were present in brain parenchyma around the ischemic lesion. This pattern of leukocyte infiltration was significantly changed in CCR2−/− mice. Monocyte migration (CCL2 is a potent monocyte chemoattractant) was markedly reduced, but also there was much less neutrophil infiltration (Figure 3).

To address how blocking the CCL2/CCR2 axis could have such broad effects on the inflammatory response, cDNA gene arrays and protein microarrays were used to examine the ischemic penumbra at days 1 and 5 of reperfusion. Results are shown in Figure 4. In CCR+/+ mice, in a comparison of the expression profile of proinflammatory cytokines and chemokines and their receptors at day 5 of reperfusion, there was significant mRNA overexpression of a variety of proinflammatory cytokines (eg, IL-1α, IL-1β, IL-6, IL-18, IL-17, and TNF-α; ≥2.5-fold increases) and CC chemokines (eg, CCL2, CCL22, CCL24, CCL4, CCL6, CCL7, CCL8, and CCL9; >2-fold increases). On the other hand, at day 1, CCR2+/+ mice had significant upregulation of the CXC chemokines (CXCL2,
CXCL5, CXCL9, and CXCL12 as well as CX3CL1). A similar pattern was found in protein expression. As shown in Figure 5, there was significant upregulation of proinflammatory cytokines like IL6, IL-12, TNF-α, and the chemokine CC at day 5 compared with day 1, whereas the CXC chemokine family showed increased activity at day 1 only.

This inflammatory response during reperfusion was significantly reduced in CCR2−/− mice. Most proinflammatory cytokines and chemokines (and their receptors) were underexpressed in the CCR2−/− group at the level of mRNA and protein. Exceptions were a group anti-inflammatory cytokines (IL-4, IL-5, and IL-13) whose transcript and protein...
expressions were upregulated in CCR2−/− mice (2- to 3-fold increase, \( P<0.001 \)). Thus, the absence of CCR2 has a profound effect on the inflammatory response during reperfusion as well as affecting BBB permeability and brain edema formation.

**Discussion**

The present study investigated how blocking the CCL2/CCR2 axis with a CCR2 KO affects ischemia/reperfusion injury. Our results indicate that a lack of CCR2 greatly reduces brain edema formation and BBB disruption, as well as decreasing leukocyte infiltration. This study also showed that CCR2−/− mice had decreased expression of a wide range of proinflammatory cytokines during reperfusion. The implications of these findings are discussed next.

Brain edema is a leading complication in ischemia and ischemia/reperfusion-induced brain injury. The mechanisms of brain edema formation after focal ischemia are complex. The classic, simplistic pathobiology of ischemia-evoked cerebral edema includes a cytotoxic component (secondary to postischemic energy failure) and a vasogenic component (secondary to breakdown of the BBB and extravasation of protein-rich plasma filtrate), although some other mechanisms, such as intrahemispheric diaschisis or hypome-
Proinflammatory mediators are considered potential causes of vasogenic brain edema. Their effect could be (1) direct, by disrupting junctional complexes between endothelial cells of the BBB and/or (2) indirect, by inducing leukocyte migration and production of a secondary wave of proinflammatory mediators.24–25 Thus, proinflammatory cytokines (eg, IL-1α, IL-1β, TNF-α, IL-6, and granulocyte macrophage–colony stimulating factor), mediators of oxidative injury (peroxinitrite, superoxide, or H2O2), and adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and selectins) regulate the magnitude and persistence of inflammation but may also directly reduce tight junction integrity and enhance edema formation.3,4,19,21,22,26,27 Chemokines may have similar effects. Neutralizing antibodies against IL-8 and CINC1 prevent BBB breakdown and reduce brain edema formation.8,28,29 The current study has shown that the CCL2/CCR2 axis also has an effect on BBB disruption and edema formation during ischemia/reperfusion as well as regulating leukocyte migration.

How does the CCL2/CCR2 axis modulate BBB permeability and edema? In our opinion, CCL2 also has direct and indirect effects on the BBB. Our recent in vitro studies on ischemia/reperfusion injury to brain endothelial cells showed that CCL2 via endothelial CCR2 directly altered brain endothelial tight junction proteins.30 In addition, prolonged exposure to exogenous CCL2 in vivo also disrupts the BBB and causes brain edema formation. The depletion of monocytes only partially ameliorated this effect, suggesting a direct effect of CCL2 on BBB integrity.31 In the current experiments, we cannot exclude the possibility that the reduction in CCL2-induced infiltration of leukocytes in CCR2−/− mice might also contribute to reduced BBB disruption and brain edema formation, but it is likely that direct effects on barrier tight junction proteins also contribute.

An intriguing finding in this study is that the absence of CCR2 reduced neutrophil as well as monocyte infiltration. Two possible reasons for this are as follows: (1) CCL2 diminishes expression of adhesion molecules on endothelial cells and, in this case, prevents leukocyte–endothelial cell interaction; and (2) CCL2 may directly modulate cytokines and CXC chemokines that may alter the inflammatory response. For example, in hindlimb ischemia and renal ischemia/reperfusion, the absence of CCR2 is closely associated with decreased leukocyte adhesion, expression of adhesion molecules (intercellular adhesion molecule), and selectin expression.31 Analysis of chemokine expression also indicates that CCL2 can reduce the expression of chemoattractants for neutrophils, such as MIP-2 (CXCL2).11 CCR2 is also detected on neutrophils under in vivo conditions.32 CCL2 is also a very important factor for modulation of the Th1/Th2 response.33 Specifically, CCL2 regulates the Th1 type of response and expression of associated cytokines. An absence of CCR2 and diminished CCL2/CCR2 axis activity change the balance to the Th2 type of response and the associated Th2 cytokine profile (IL-4, IL-5, and IL-13). For example, CCR2−/− mice have increased levels of IL-4, IL-5, and IL-13 in renal reperfusion injury or in allergic responses to Aspergillus and a significantly reduced inflammatory response and lesion size.32,33 As such, it may not be surprising that diminishing CCL2/CCR2 axis activity by abolishing CCR2 receptors in brain ischemia/reperfusion injury affects the cytokine expression profile, switching it in the direction of an anti-inflammatory phenotype.

What is the significance of our findings? This study indicates that the CCL2/CCR2 axis plays a pivotal role in ischemia/reperfusion injury. This axis not only regulates leukocyte entry but also has a major role in regulating the expression of other proinflammatory mediators. Furthermore, CCL2 via CCR2 could also be involved directly or indirectly in inducing vasogenic edema formation. Taken together, it appears that CCL2 could be a good marker of secondary brain ischemia/reperfusion injury, owing to the fact that the CCL2 level can be measured in cerebrospinal fluid or plasma in patients. These results in particular indicate that CCR2 might be a good therapeutic target for inhibiting the effects of CCL2 during cerebral ischemia/reperfusion and ameliorating brain injury in stroke.

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Disclosures
None.

References


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