Contribution of Microglia/Macrophages to Expansion of Infarction and Response of Oligodendrocytes After Focal Cerebral Ischemia in Rats

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Background and Purpose—The purpose of this study was (1) to examine the contribution of microglia and macrophages with their interleukin-1β production and (2) to assess the vulnerability and response of oligodendrocytes in cerebral infarction.

Methods—Male Wistar rats were subjected to permanent occlusion of the left middle cerebral artery. Expansion of ischemic infarction and response of oligodendrocytes were investigated together with accumulation of inflammatory cells, production of interleukin-1β, and disruption of the blood-brain barrier. Apoptotic cell death was inferred from fragmented DNA and the expression of proapoptotic Bax protein.

Results—During expansion of infarction, amoeboid microglia and extravasation of serum albumin were observed not only in the infarcted area but also in the adjacent surviving area, whereas macrophages accumulated along the boundary and granulocytes migrated into the center of the infarction. Both amoeboid microglia and macrophages produced interleukin-1β, an inflammatory cytokine, during an early ischemic period. Furthermore, macrophages within the infarcted tissue expressed Bax protein and subsequently showed fragmented nuclear DNA. Oligodendrocytes were detected in the infarcted area even after 24 hours following middle cerebral artery occlusion, but they subsequently developed fragmented DNA. A week after onset of ischemia, oligodendrocytes were found to be accumulated in the intact area bordered with the infarct together with reactive astrocytes.

Conclusions—Our results suggest the importance of amoeboid microglia, macrophages, and their interleukin-1β production in gradual expansion of cerebral infarction. Resident oligodendrocytes may be resistant to ischemic insults, and oligodendrocytes accumulated at the border of the infarction may participate in tissue repair after cerebral infarction. (Stroke. 2000;31:1735-1743.)

Key Words: apoptosis ■ cerebral infarction ■ interleukins ■ macrophages ■ microglia ■ oligodendrocytes ■ rats

It is known that cerebral infarction expands slowly over 24 hours from the core to the peripheral area after occlusion of a middle cerebral artery (MCA) in the rodent,1 where the peripheral area is a potential target for therapeutic intervention.2,3 However, the molecular mechanism of the expansion of infarction to the peripheral area remains unclear, although several studies have focused on the role of inflammatory cells, including granulocytes and microglia.4–6 To clarify the type of inflammatory cells directly involved in the expansion of infarction, we first examined the relationship between expansion of the ischemic lesion and the appearance of inflammatory cells together with inflammatory cytokines in the rat brain after occlusion of the left MCA. In the mouse brain, oligodendrocytes have been shown to be more resistant to ischemic insults than neurons,7 but it is still unknown whether oligodendrocytes are resistant to ischemia in other rodent brains because they are considered susceptible to hypoxia and ischemia, at least in the developing brain.8,9 It has been difficult to detect oligodendrocytes with conventional histological technique. However, we have used a sensitive in situ hybridization method and succeeded in finding oligodendrocytes in paraffin-embedded sections of adult rodent brains.7 We therefore investigated the vulnerability of oligodendrocytes in ischemic mouse brain by using the in situ hybridization technique.

Another purpose of this study was to investigate the dynamics of tissue repair after cerebral infarction. After the process of cerebral infarction is completed in 2 to 3 days after

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vessel occlusion, the process of tissue repair or remodeling is activated, and the infarction is eventually encircled by glial scar a few weeks later. For resorption of infarcted tissue, macrophages and granulocytes invading the infarcted tissue have to succumb following their efforts in the injured tissue. We used in situ end labeling of fragmented DNA and immunohistochemistry for Bax, an inducer of apoptosis, to investigate whether those macrophages and granulocytes were removed through apoptosis after they invaded the infarction. Another important aspect of tissue repair is the formation of glial scar that reestablishes the interface in the glial-pial boundary. While participation of astrocytes is well known in formation of glial scar, the role of oligodendrocytes has not drawn attention. We therefore investigated whether oligodendrocytes were involved in this process by using the in situ hybridization technique.

Materials and Methods

Animals

Forty-seven adult male Wistar rats weighing 250 to 300 g (Charles River Laboratory, Kanagawa, Japan) were used. They were fed standard laboratory chow and had free access to water before and after the surgical procedure. Animals were cared for according to the guidelines of the Animal Center of the Osaka University Graduate School of Medicine.

Surgical Procedure

Each animal was anesthetized with halothane, and occlusion of the left MCA was accomplished according to Koizumi et al. Briefly, the left common carotid artery was exposed through a midline incision, and the internal carotid artery was isolated and carefully separated. A 4-0 nylon monofilament, whose tip was rounded by heating, was introduced from the bifurcation of the internal carotid artery and advanced until resistance was felt. Rats were observed postoperatively, and those with spastic paralysis of the right forelimb and circling to the right during the ischemic period were taken for histological examination. Rectal temperature was monitored routinely to maintain it at 37.0±0.5°C during the surgical procedure.

Immunohistochemistry

For morphological examinations with paraffin sections, 3 nonoperated control rats and 33 ischemic rats at 3 hours (n=3), 6 hours (n=5), 12 hours (n=5), 24 hours (n=5), 48 hours (n=5), 4 days (n=4), 96 hours (n=3), and 7 days (n=3) after MCA occlusion were perfusion-fixed with saline and then Zamboni’s solution or 4% paraformaldehyde solution under deep pentobarbital anesthesia. Brains were removed and postfixed in the same fixative for 5 hours at 4°C. Then each tissue block was dehydrated, embedded in paraffin, and cut into 4-μm-thick coronal sections. Another 11 rats were used for examinations with frozen sections. In nonoperated control rats (n=3), 24-hour ischemia rats (n=4), and 48-hour ischemia rats (n=4), the brain was cryoprotected after perfusion-fixation and cut into 10-μm-thick coronal sections with a cryostat. The peroxidase-antiperoxidase method for light microscopy was used for the immunohistochemical procedure for albumin, microtubule-associated protein 2 (MAP2), glial fibrillary acidic protein (GFAP), and myeloperoxidase (MPO). Briefly, each deparaffinized and rehydrated section was incubated with 10% nonimmunized goat serum, with anti-MAP2 (Sigma-Aldrich Co; 1:200), anti-albumin (Organon Teknik Co; 1:400), anti-GFAP (Dako; 1:400), or anti-MPO (Dako; 1:100) antibody at 4°C overnight, with an appropriate antiserum (1:20) for 1 hour, and with the peroxidase-antiperoxidase complex (Miles-Yeda Ltd; 1:100) for 1 hour. Proliferating cells were detected by using a monoclonal mouse anti-proliferating cell nuclear antigen (PCNA) coupled with horseradish peroxidase (HRP) (Dako). Immunoreactions for interleukin-1β (IL-1β) and Bax, a Bcl-2 family protein, were also performed with an anti-IL-1β antibody (NIBSC; 1:200) to detect the production of IL-1β and with an anti-Bax antibody (1:200) to detect apoptotic cell death of microglia/macrophages, respectively, by using an avidin-biotin complex method. Monoclonal anti-rat CD11b/c antibody (OX-42, Serotec; 1:500) was used to detect microglia as well as macrophages in the frozen section. The paraffin sections were finally reacted with 0.05% 3'-3-diaminobenzidine in the presence of 0.01% H2O2. The frozen sections used for double staining with fragmented DNA were finally reacted with 3-amino-9-ethylcarbazole for visualization of immunoreaction for OX-42. The control sections were incubated with nonimmune serum or ascites.

Lectin Histochemistry

We used griffonia simplicifolia B4 isoelectin (GSA I-B4) for detection of resident and reactive microglia/macrophages. The rehydrated sections were incubated in PBS containing cations (0.1 mmol/L of CaCl2, MgCl2, and MnCl2) for at least 10 minutes before application of GSA I-B4 HRP conjugates (Sigma-Aldrich Co). Aliquots of the conjugates were diluted 1:10 in PBS containing the cations and 0.1% Triton X-100. After incubation overnight at 4°C, the slides were washed with PBS, and the peroxidase reaction was accomplished by incubation in the 3'-3-diaminobenzidine-H2O2 substrate medium.

In Situ Hybridization Histochemistry

Rat cDNA encoding proteolipid protein (PLP) mRNA in pGEM3 plasmid was used, and RNA probes were transcribed by RNA polymerases from appropriate linearized plasmids containing T3 or SP6 promoters with digoxigenin-UTP (DIG RNA Labeling Mixture, Boehringer Mannheim). RNA probes were generated in both antisense and sense directions, and sense probes were used as controls. The deparaffinized and rehydrated sections were treated with 10 μg/mL of proteinase K in Tris-EDTA buffer for 10 minutes at 37°C, followed by the second fixation with 4% paraformaldehyde. To quench the endogenous alkaline phosphatase activity, the sections were immersed in 0.2N HCl and then were acetylated in 0.1 mol/L triethanolamine-HCl with acetic anhydride. The sections were finally dehydrated with ethanol and chloroform. The probes in the hybridization solution, containing 50% deionized formamide, 10% dextran sulfate, 1× Denhardt’s solution, 200 μg/mL tRNA, 10 mmol/L Tris-HCl (pH 8.0), 0.6 mol/L NaCl, and 0.25% SDS, were denatured by heating up to 85°C for 3 minutes. Each section was covered with the hybridization solution and then with a cover glass, placed on a hotplate at 85°C for 3 minutes, and incubated at 55°C overnight. After hybridization, the sections were immersed in 5× SSC at 55°C to dislodge cover glasses, then washed in 50% formamide and 2× SSC at 65°C for 30 minutes. The sections were then treated with RNase A (Boehringer Mannheim) for 30 minutes at 37°C and washed in 50% formamide with 2× SSC at 65°C for 30 minutes. After that, the sections were incubated with the blocking buffer for 1 hour and then reacted with alkaline phosphatase–conjugated Fab fragment derived from an antiserum to digoxigenin (Boehringer Mannheim, 1:500) overnight. To visualize the reaction product, the sections were incubated with 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate for 24 to 48 hours at 4°C in a dark, moist chamber.

Terminal Deoxynucleotidyltransferase–Mediated dUTP-Biotin Nick End-Labeling Staining

The terminal deoxynucleotidyltransferase (TdT)–mediated dUTP-biotin nick end-labeling (TUNEL) procedure was performed according to the method of Gavrieli et al. In brief, deparaffinized and rehydrated sections were treated with proteinase K (10 μg/mL in Tris-EDTA buffer), immersed in 2% H2O2 for 5 minutes to inactivate endogenous peroxidase, and then, after a rinse with water, immersed in TdT with biotin-11-dUTP (Sigma-Aldrich Co) and incubated at 37°C for 1 hour. The sections were incubated with 2% bovine serum albumin for 10 minutes for blocking, which was followed by reaction.
Figure 1. Gradual expansion of infarction in focal cerebral ischemia. The immunohistochemical reaction for MAP2 (A, C through F) and extravasated serum albumin (B) in the normal (A) and ischemic parietal cortex at 6 (C), 16 (B, D), 24 (E), and 48 hours (F) after MCA occlusion is shown. Note gradual expansion of the ischemic lesion with loss of MAP2 immunoreactivity from the ischemic core to the periphery (A, C, D, E, F). Also note that extravasated albumin was detected not only in the ischemic lesion but also in the area where MAP2 immunoreactivity was still preserved (B, D). This area subsequently lost MAP2 (E, F). A broken line in (B, D) indicates the borderline between the areas with and without extravasated albumin. Bar=500 μm.

Statistical Analysis
All values are presented as mean±SD. The number of cells was compared between different time points with repeated-measures ANOVA and Fisher’s protected least significant difference post hoc test. P<0.05 was considered statistically significant.

Results
Inflammatory Cells in Gradual Expansion of the Ischemic Lesion to the Periphery of the MCA Territory
In the center of the MCA territory including the striatum and the frontal cortex, the immunoreactivity for MAP2 was already lost 3 hours after MCA occlusion. In the periphery of the MCA territory, however, ischemic neuronal damage detected as loss of the MAP2 immunoreactivity gradually expanded from 6 to 48 hours to the border between the MCA and anterior cerebral artery territory (Figure 1). This gradual expansion of the ischemic lesion was typically observed in the parietal cortex. Therefore, the sections including parietal cortex were used for investigation of the contribution of microglia/macrophages to expansion of cerebral infarction. In the sham-operated rats, extravasation of albumin was absent and microglial cells were not observed in the cerebral cortex. In the core of the infarction in the cortex, extravasation of albumin was first observed 3 hours after MCA occlusion and expanded into the whole ischemic hemisphere 16 hours after MCA occlusion. Microglial cells were first detected 6 hours after MCA occlusion; they were of the resting type with ramified shape (ramified microglia) and persisted in the ischemic hemisphere for 7 days. However, at 16 and 24 hours after MCA occlusion, activated microglial cells of the amoeboid type (amoeboid microglia), as identified by their enlarged size, stout processes, and intense staining, were observed not only in the infarcted tissue but also in the area adjacent to infarction with preserved immunoreactivity for MAP2 (Figure 2). The lectin binding to microglia and macrophages was confirmed by the immunohistochemical reaction with OX-42 (Figure 2E1 and 2E2). The amoeboid microglia showed positive immunoreaction for PCNA in the adjacent sections (Figure 3). It seems that PCNA-positive cells were mostly microglia/macrophages, but a few PCNA-positive cells existed in the microvessels, as reported by Abumiya et al.17 Those areas, adjacent to the infarction at 16 and 24 hours, subsequently lost the immunoreaction for MAP2 and became infarcted at 48 hours after MCA occlusion (Figure 1). Macrophages that were morphologically discrim-
inated from microglia by their large nuclei and granulocytes that were detected with the immunoreaction for MPO were always absent in the area with preserved immunoreaction for MAP2 (Figure 2). Between 16 and 24 hours after MCA occlusion, however, both macrophages and granulocytes started to emerge in the infarcted tissue. Macrophages were mainly accumulated in the boundary within the infarction, whereas granulocytes were distributed widely from the infarct border to the core during expansion of the ischemic lesion. However, macrophages (○) and granulocytes (●) appeared mainly within the infarction. Also note that more macrophages accumulated close to the infarct border and granulocytes were distributed from the infarct border to the core during expansion of the ischemic lesion from 16 to 48 hours. B, Changes in the number (per square millimeter) of microglia/macrophages and granulocytes in the peripheral area adjacent to the infarct border with preserved immunoreaction for MAP2 (top) and inside the infarction (bottom). Note that amoeboid-shaped microglia were increased in number in the peripheral area during expansion of the ischemic lesion for up to 48 hours, while in the infarcted area, macrophages and granulocytes were increased at 16 hours after MCA occlusion. Data are mean±SD. *P<0.05 vs 16 hours.

Figure 4. A, Relationship between expansion of the ischemic lesion and appearance of inflammatory responses. The area with dark shadow (area A) lost the immunoreaction for MAP2. Note gradual expansion of the ischemic lesion from 6 to 48 hours after MCA occlusion, with gradual reduction in the area with preserved immunoreaction for MAP2. The area with light shadow (area B) indicates the area showing extravasation of serum albumin in the area with preserved immunoreaction for MAP2. During expansion of the ischemic lesion, microglia were activated in the surrounding intact area (+ indicates resting microglia with ramified shape; ●, reactive microglia with amoeboid shape), preceding expansion of the ischemic lesion. However, macrophages (○) and granulocytes (●) appeared mainly within the infarction. Also note that more macrophages accumulated close to the infarct border and granulocytes were distributed from the infarct border to the core during expansion of the ischemic lesion from 16 to 48 hours. B, Changes in the number (per square millimeter) of microglia/macrophages and granulocytes in the peripheral area adjacent to the infarct border with preserved immunoreaction for MAP2 (top) and inside the infarction (bottom). Note that amoeboid-shaped microglia were increased in number in the peripheral area during expansion of the ischemic lesion for up to 48 hours, while in the infarcted area, macrophages and granulocytes were increased at 16 hours after MCA occlusion.
Consistent with our previous observation,7 the mRNA signals for PLP were present in the oval-shaped cells, oligodendrocytes, mainly in the white matter. However, the mRNA signals for PLP started to decrease in the center of the MCA territory, including the lateral caudoputamen, at 16 hours after MCA occlusion, but the signals were still present and colocalized with macrophages and granulocytes in the infarcted tissue for up to 48 hours after MCA occlusion (Figure 5). Up to 24 hours after MCA occlusion, cells with fragmented DNA detected by the TUNEL method largely appeared to be damaged neurons, but scattered oligodendrocytes were also clearly shown to have DNA fragmentation within the infarction (Figure 5). The mRNA signals for PLP completely disappeared in the infarcted tissue 96 hours after MCA occlusion; however, they were still present in the corpus callosum even at 7 days after MCA occlusion (Figure 6).

Glial Responses in the Tissue Repair Process After Cerebral Infarction
Expansion of cerebral infarction was complete by 48 hours after MCA occlusion. At this point, the infarcted tissue consisted of damaged cells and invading cells, including microglia/macrophages and granulocytes. The immunohistochemical reaction for Bax protein and the TUNEL method showed abundant positive nuclei in the infarct. Macrophages in the center of the infarct started to express Bax protein at 24 hours and began to exhibit DNA fragmentation at 48 hours after MCA occlusion (Figure 7). Double staining with TUNEL and immunoreaction for OX-42 revealed that the ratio of macrophages to TUNEL-positive cells was 27.0±5.4% in the center of the infarction at 48 hours after MCA occlusion. Reactive astrocytes started to increase in the peripheral area of infarction at 24 hours after MCA occlusion, but they were rarely detected in the center of the infarcted tissue. At the rim of the infarct, ramified microglia and oligodendrocytes were also accumulated and colocalized with reactive astrocytes at 7 days after MCA occlusion (Figure 8).

Discussion
The penumbral area has been considered a therapeutic target in focal cerebral ischemia.2–3 The concept of penumbra was based on functional and biochemical changes,18 but progressive vascular compromise may also be involved in expansion of the lesion.19,20 It takes hours to days to detect ischemic damage in the penumbral area with the conventional histological method.21 In the present study we used the immunoreaction for MAP2 to detect expansion of the ischemic lesion after MCA occlusion because our previous studies demonstrated that MAP2 was a specific and sensitive marker for ischemic neuronal damage.7,13 On the basis of the present findings in the periphery of the MCA territory of the cerebral cortex, we concluded that macrophages and granulocytes did not play major roles in the progression of ischemic neuronal damage for up to 16 hours. During this early ischemic period after MCA occlusion, neuronal insults, including glutamate toxicity22 and spreading depolarization23 may be more important than inflammatory cells for progression of the ischemic lesion. However, expansion of the ischemic lesion was still observed from 16 to 48 hours after MCA occlusion. During this period, amoeboid microglia became visible in the area where the immunoreaction for MAP2 was still preserved, but this area subsequently became infarcted. Immunoreaction for PCNA suggested the activation and proliferative response of
amoeboid microglia. Furthermore, the accumulation of macrophages was observed mainly along the border within the infarction. Both amoeboid microglia and macrophages produce IL-1β, an inflammatory cytokine, and it is likely that those inflammatory cells contributed to expansion of infarct, especially in the late ischemic period after MCA occlusion. The presence of IL-1β in the ischemic brain and aggravation of ischemic damage have been reported.24,25 Several recent studies also suggested that inflammatory cytokines such as IL-1β and tumor necrosis factor α (TNF-α) were expressed mainly in microglia/macrophages,14,26 but there is a possibility that neurons and astrocytes generate those cytokines after ischemia.27 The contribution of inflammatory cells in expansion of infarction at 16 and 24 hours after MCA occlusion is also consistent with the report emphasizing the pathogenic role of inducible nitric oxide synthase expressed in inflammatory cells after focal cerebral ischemia.28 However, it should be taken into account that the contribution of inflammatory cells may be different in non-human primates, in which the delimiting lesion is fixed relatively early.29 Furthermore, the effect of suppression of activated microglia/macrophages in cerebral infarction remains unclear, although depletion of granulocytes has been shown to reduce infarct size.30

Consistent with our previous findings in mice,7 the present study with in situ hybridization histochemistry suggested that oligodendrocytes were resistant against ischemic insult, although some reports have shown opposite findings.9 Because mRNA for PLP is exclusively localized in the perinuclear region of oligodendrocytes in the brain,31 the preservation of mRNA signals for PLP at least indicates preservation of the integrity of the cytoplasmic membrane. This technique has been applied for identification of oligodendrocytes in human brain sections32 and in murine brain sections subjected to demyelination and remyelination.33 Oligodendrocytes were often detected in the infarct 24 and 48 hours after MCA occlusion. Colocalization of oligodendrocytes with macrophages and granulocytes in the infarcted tissue suggested the possibility that oligodendrocytes were destroyed by invading inflammatory cells in the infarcted tissue through an apoptotic mechanism, as suggested by the presence of fragmented DNA in oligodendrocytes. It has been reported that cytokines such as TNF-α and IL-1β were involved in microglia-
mediated apoptosis in cultured oligodendrocytes. Apoptotic death of oligodendrocytes has been also observed after spinal cord injury. After completion of the expansion of infarction, both resorption of the infarcted tissue and glial scar formation occur for tissue repair or remodeling. Two major cellular components that migrated into the infarcted area to eliminate tissue debris were macrophages and granulocytes. However, these cells also had to be destroyed or recruited for tissue resorption. After a peripheral nerve injury, activated proliferating microglia have been reported to exhibit nuclear DNA fragmentation by 7 days after injury. In the present study we suspect that macrophages accumulated in the infarction underwent apoptosis after expressing Bax protein and DNA fragmentation. Surprisingly, approximately a quarter of TUNEL-positive cells within the infarction showed microglia/macrophages phenotype with double staining. Although the TUNEL approach does not unequivocally define apoptosis, accumulation of Bax protein in macrophages has been reported to mediate apoptotic cell death induced by nitric oxide and morphine. Whether those macrophages in infarction express caspase activity, a better marker for the presence of apoptosis, remains to be determined. It remains unclear how granulocytes were removed from the infarcted tissue before resorption. It has long been believed that glial cells participate in the glial scar formation at the rim of the infarct. Reactive astrocytes and activated microglia have been shown to participate in the formation of glial scars. Consistent with our previous findings in mice, the present study clearly showed that oligodendrocytes also accumulated, together with astrocytes and microglia, along the rim of the infarct in the rat after MCA occlusion. Although controversy exists regarding whether or not all astroglial reactions are beneficial for regeneration, our findings suggested that oligodendrocytes colocalized with reactive astrocytes could contribute to the tissue repair process, glial scar formation, and nerve regeneration. It is also possible that accumulation of oligodendrocytes is an attempt to provide myelin when axonal regeneration occurs.

In conclusion, we demonstrated the possibilities that (1) microglia/macrophages contributed to the gradual expansion of cerebral infarction through production of IL-1β; (2) oligodendrocytes were resistant to ischemic insult; (3) once macrophages invaded the infarct, they eventually underwent apoptosis; and (4) oligodendrocytes were a component of glial scar formation together with astrocytes and microglia. The possibility of therapeutic control of activated microglia/macrophages to limit cerebral infarction will be pursued in the future.

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References


**Editorial Comment**

Cellular inflammation, triggered by ischemia, is a significant contributor to the final pathology of ischemic stroke. Garcia and colleagues1,2 identified the early involvement of polymorphonuclear (PMN) leukocytes and the subsequent invasion of mono- cytes into ischemic brain after middle cerebral artery occlusion (MCAO). But, the potential contributions of resident cells of the ischemic brain to the “secondary injury” triggered by ischemia has received little attention until recently. Mabuchi and colleagues, in their very fine survey, examined the responses of resident microglia and oligodendrocytes in the ischemic territory to MCAO in the Wistar rat. By 16 to 24 hours after MCAO, ameboid microglia and macrophages within the ischemic tissue expressed the cytokine IL-1β, while oligodendrocytes were found in the central injured region and were apparently increased in number in the peripheral regions by 7 days. Activation of microglia, and the accumulation of the relatively ischemia-resis- tant oligodendrocytes to the ischemic territory, suggest their participation in the injury process.

Microglia, primary immune-competent cells of the central nervous system, are derived from cells of the monocyte/macrophage lineage in the bone marrow.3,4 With breakdown of the blood brain barrier which accompanies cerebral ischemia, brain inflammation, trauma, and toxic injury, perivascular microglia and infiltrating monocytes/macrophage from the circulation are activated. These cells share in common many phenotypic mark- ers and responses.3,4 Furthermore, following MCAO, and after transmigration of PMN leukocytes from the circulation has begun, mononuclear cells accumulate within the injured territory by 24 hours, in response to specific chemokines.2,7

Microglial cell activation and proliferation is stimulated by macrophage colony-stimulating factor 1 (CSF-1),8 an astrocyte- derived growth factor,9 and by transforming growth factor β2 (TGF-β2), a neuron-derived factor, in the presence of CSF-1.10 Certain chemokines can recruit and activate specific subsets of inflammatory cells, including mononuclear cells, to sites of tissue damage, which may enhance progression of endothelial cell, astrocyte, and neuronal injury.11,12 Tumor necrosis factor α and interleukin-1β, derived from neurons, astrocytes, and acti- vated inflammatory cells, contribute to the ischemic injury in experimental preparations.13 Chemokines and their receptors have been detected in microglia, neurons, and astrocytes under physiologic conditions, and following hypoxia microglial generation of chemokines is stimulated by excitotoxic stress, CSF-1, or TGF-β2.11 The present data suggest that microglial activation in this setting may occur during the evolution of the injury and the expansion of the infarct.

Similarly, the topographical distribution of oligodendrocytes implies potential participation in reactive processes. Microglia/mac- rophages may also participate in the repair and remodeling of the tissue after or during development of the infarct.14,15

The panorama of microglial and oligodendrocytic response to focal ischemia following central nervous system ischemia sug-gests that detectable activation is not immediate but requires preparatory steps that include cytokine and chemokine secretion, and undoubtedly more. The contributions that activated micro- glia may make to the development of the injury have not been explored. It will be of interest to determine whether microglia with genetically altered phenotypes may also alter the evolution of the injury after MCAO. This is particularly interesting, as microglia may serve immune and other functions, belonging to a family of cells with potentially diverse functional roles. The precise derivation of microglia in the ischemic regions, whether they arrive from the circulation and when, are important ques-
tions. This information and the reactivity of oligodendrocytes to the ischemic insult, could provide leads for limiting expansion of the injury. To what degree these cells contribute to later attempts at repair of the tissue will provide ample basis for future studies.

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