The Transcription Factor Interferon Regulatory Factor 1 Is Expressed after Cerebral Ischemia and Contributes to Ischemic Brain Injury

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Summary

The transcription factor interferon regulatory factor 1 (IRF-1) is involved in the molecular mechanisms of inflammation and apoptosis, processes that contribute to ischemic brain injury. In this study, the induction of IRF-1 in response to cerebral ischemia and its role in ischemic brain injury were investigated. IRF-1 gene expression was markedly upregulated within 12 h of occlusion of the middle cerebral artery in C57BL/6 mice. The expression reached a peak 4 d after ischemia (6.0 ± 1.8-fold; $P < 0.001$) and was restricted to the ischemic regions of the brain. The volume of ischemic injury was reduced by 23 ± 3% in IRF-$1^{+/-}$ and by 46 ± 9% in IRF-$1^{-/-}$ mice ($P < 0.05$). The reduction in infarct volume was paralleled by a substantial attenuation in neurological deficits. Thus, IRF-1 is the first nuclear transacting factor demonstrated to contribute directly to cerebral ischemic damage and may be a novel therapeutic target in ischemic stroke.

Key words: cerebral ischemia • interferon regulatory factor 1 null mice • gene expression • neuroprotection • reverse transcription polymerase chain reaction

In experimental animals, as in humans, focal cerebral ischemia is associated with an intense inflammatory reaction that contributes to the secondary progression of ischemic brain injury (for review see references 1, 2). Within hours after the insult, cytokines and chemokines are produced in the ischemic brain (3–7). Cytokines contribute to the enhanced expression of adhesion molecules on cerebral endothelial cells, which, in turn, leads to adhesion of circulating neutrophils to cerebral endothelial cells (8–14). Neutrophils and, subsequently, monocytes migrate through the vessel wall and invade the injured brain (15–17). Inflammation-related enzymes, such as the "immunologic" isoform of nitric oxide synthase (iNOS) and cyclooxygenase-2, are induced in the postischemic brain (18–22). Furthermore, genes involved in programmed cell death, such as *p53*, *Bax*, and *bcl2*, are upregulated (23–26), whereas caspases, the cysteine proteases thought to be the effectors of programmed cell death, are activated (27, 28).

The molecular mechanisms by which this complex genomic response is orchestrated have not been elucidated. Although a number of transcription factors are activated after cerebral ischemia (29–32), no single transcription factor has been conclusively linked to the mechanisms that underlie tissue damage (for example see reference 33). One of the transcription factors that is important for gene expression during inflammation is interferon regulatory factor (IRF)-1[1] (for review see reference 34). IRF-1 was originally described as one of the transcriptional activators responsible for expression of interferon and interferon-inducible genes (35). More recently, the view has emerged that IRF-1 is involved in multiple functions including cellular responses to inflammation and programmed cell death (for review see reference 36). Therefore, it is conceivable that IRF-1 plays a role in gene expression after cerebral ischemia.

In this study, we investigated the potential contribution of IRF-1 to cerebral ischemic damage. Specifically, we sought to establish whether IRF-1 is upregulated after cerebral ischemia and, if so, whether its expression plays a central role in the mediation of tissue damage. Our studies demonstrate that occlusion of the middle cerebral artery

[1]*Abbreviations used in this paper:* CBF, cerebral blood flow; HPRT, hypoxanthine-guanine phosphoribosyl transferase; IRF-1, interferon regulatory factor 1, iNOS, inducible nitric oxide synthase; MCA, middle cerebral artery; NO, nitric oxide.

(MCA) in mice leads to upregulation of IRF-1 mRNA within the ischemic region. Furthermore, IRF-$1^{-/-}$ mice fail to express IRF-1 after cerebral ischemia and exhibit an attenuation of brain injury after MCA occlusion. The effect is more pronounced in IRF-$1^{-/-}$ than in IRF-$1^{+/-}$ mice, and is associated with improved neurological outcome. The findings demonstrate that IRF-1 is involved in the evolution of brain damage after cerebral ischemia. IRF-1 represents the first transcription factor for which a link with ischemic brain injury has been established.

Materials and Methods

Animals

All animal procedures were approved by the Animal Care Committee of the University of Minnesota. Mice with a targeted mutation in the IRF-1 gene (homozygous [−/−] mice and their heterozygous [+/−] littermates) were originally obtained from Dr. T.W. Mak (Amgen Institute, Toronto, Canada) and had been back-crossed to C57BL/6 mice three to five times. Mice were subsequently bred in a virus antibody-free facility at the Uniformed Services University of the Health Sciences. The IRF-$1^{-/-}$ colony was maintained by mating IRF-$1^{-/-}$ mice to either IRF-$1^{-/-}$ or IRF-$1^{+/-}$ mice. An IRF-1 wild-type (+/+) colony was maintained by mating IRF-$1^{+/+}$ mice that were derived from heterozygous mating of either IRF-$1^{+/+}$ or IRF-$1^{+/-}$ mice. To prevent the background of the IRF-$1^{-/-}$ and IRF-$1^{+/+}$ colonies from straying, all new breeding pairs were the progeny of IRF-$1^{+/-}$ to IRF-$1^{+/-}$ matings. The genotype of all IRF-1 mice was determined by PCR using primers and methods described previously (37). The IRF-1 primers amplify a 300-bp sequence from IRF-$1^{+/+}$ and IRF-$1^{+/-}$ genomic DNA, whereas neo primers amplify a product of 525 bp, present only in IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice. C57BL/6 mice were used as controls in some experiments and were obtained from The Jackson Laboratory.

Induction of Focal Cerebral Ischemia

Focal cerebral ischemia was produced by occlusion of the MCA as previously described (20). Mice were anesthetized with 2% halothane in 100% oxygen. Body temperature was maintained at 37 ± 0.5°C by a thermostatically controlled infrared lamp. A 2-mm hole was drilled in the inferior portion of the temporal bone to expose the left MCA. The MCA was elevated and cauterized distal to the origin of the lenticulostriate branches. Mice in which the MCA was exposed but not occluded served as sham-operated controls. Wounds were sutured and mice were allowed to recover and returned to their cages. Rectal temperature was controlled until mice regained full consciousness. Mice were killed at different time points after MCA occlusion for determination of IRF-1 mRNA or measurement of infarct size (see below).

Determination of IRF-1 mRNA by Reverse Transcription PCR

C57BL/6 and IRF-$1^{+/+}$, IRF-$1^{+/-}$, and IRF-$1^{-/-}$ mice were killed various times after MCA occlusion (n = 6–12/time point). As described in detail elsewhere (20), a 2-mm-thick coronal brain slice was cut at the level of the optic chiasm and the infarcted cortex was dissected using the corpus callosum as a ventral landmark. The homotopic region of the contralateral cortex was also sampled. Total RNA was extracted, and the integrity of the RNA was determined on denaturing formaldehyde gels. IRF-1 and hypoxanthine-guanine phosphoribosyl transferase (HPRT) mRNA were detected by reverse transcription PCR as described in detail previously (37, 38). The primer and probe sequences for IRF-1 and HPRT have been published previously (37–39). Cycle numbers for IRF-1 and HPRT were 29 and 23, respectively. The IRF-1 primers amplify a 148-bp fragment from cDNA. After agarose gel electrophoresis, amplified products were transferred to Hybond N+ membranes (Amersham) by standard Southern blotting techniques. Blots were hybridized with an internal oligonucleotide probe. Labeling of the probe and subsequent detection was carried out with the enhanced chemiluminescence system (ECL; Amersham) according to the manufacturer's instructions. Chemiluminescent signals were quantified using a scanner and imaging software (NIH Image; National Institutes of Health). The relative gene expression in test samples was assessed by linear regression analysis of a standard curve generated from a cDNA sample known to be positive for the gene of interest. Data were normalized to the housekeeping gene HPRT.

Determination of Infarct Volume

Mice were killed 4 d after MCA occlusion. Brains were removed and frozen in cooled isopentane (−30°C). Coronal forebrain sections (30-μm-thick) were serially cut in a cryostat, collected at 150-μm intervals, and stained with thionin for determination of infarct volume by an image analyzer (MCID, Imaging Research Inc.; reference 20). To factor out the contribution of ischemic edema to the total volume of the lesion, infarct volume in cerebral cortex was corrected for swelling (see reference 20 for details). The correction method is based on the determination of ischemic swelling by comparing the volume of ischemic and nonischemic hemispheres (40).

Determination of Neurological Deficits

Neurological deficits were assessed by a neurological scoring system widely used in mice (for examples see references 41, 42) that has been described previously (20). The examiner was not aware of the identity of the mice. The neurological scores were as follows: 0, normal motor function; 1, flexion of torso and contralateral forelimb upon lifting the mouse by the tail; 2, circling to the contralateral side when holding the mouse by the tail on a flat surface, but normal posture at rest; 3, leaning to the contralateral side at rest; 4, no spontaneous motor activity (20). Mice were evaluated before MCA occlusion and at 24-h intervals up to 4 d after MCA occlusion.

Monitoring of Cerebral Blood Flow

Techniques used for monitoring cerebral blood flow (CBF) in mice have been described in detail previously (20). Mice were anesthetized with halothane (maintenance 1%) and the femoral artery and trachea were cannulated. Mice were artificially ventilated with an oxygen-nitrogen mixture by a mechanical ventilator (SAR-830; CWI Inc.). The inspiration time was set at 0.1 s, the respiratory rate at 120/min, and the inspiratory flow at ~250 ml/min. The oxygen concentration in the mixture was adjusted to maintain arterial pO_2 between 150 and 170 mmHg. End-tidal CO_2 was continuously monitored using a CO_2 analyzer (Capstar-100; CWI Inc.) and maintained at 2.6–2.7%, which corresponds to a pCO_2 of 33–35 mmHg (20).

MCA Occlusion. For monitoring changes in CBF produced by MCA occlusion, two laser-Doppler flow probes (Vasamedic)

were placed through burr holes placed in the center (3.5 mm lateral to the midline and 1 caudal to bregma) and the periphery (1.5 mm lateral to the midline and 1.7 mm rostral to lambda) of the ischemic territory (20, 43). The location of the probe was selected in preliminary experiments to correspond to the region of brain that is spared from infarction in the IRF-1–deficient mice. After placement of the probes, the MCA was occluded and CBF was monitored for 90 min. CBF data are expressed as percentage of the preocclusion value.

Cerebrovascular Reactivity to Hypercapnia. Techniques for testing cerebral vascular reactivity to hypercapnia in mice were similar to those previously described (20). Mice were anesthetized and instrumented as described above. CBF was continuously monitored in the frontoparietal cortex with a laser-Doppler probe. After stabilization of arterial pressure and blood gases, CO_2 was introduced into the circuit of the ventilator and the increase in CBF produced by hypercapnia was monitored. CO_2 administration was discontinued after the CBF increase reached a plateau (usually 2–3 min).

Data Analysis

Data in text and figures are expressed as means ± SEM. Multiple comparisons were evaluated statistically by the analysis of variance and Tukey's test. Two-group comparisons were analyzed by the two-tailed Student's *t* test for independent samples. Neurological scores were analyzed by the Kruskal-Wallis analysis of variance followed by the Tukey-Kramer test (Systat) (20). For all procedures, probability values of <0.05 were considered statistically significant.

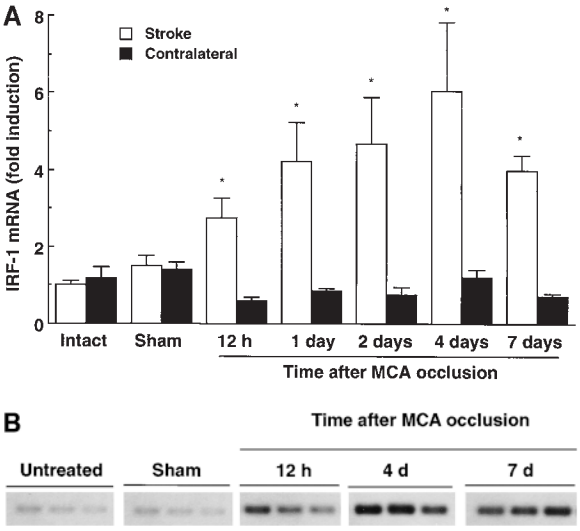

Figure 1. (A) Time course of IRF-1 mRNA expression in mouse cerebral cortex after MCA occlusion. Levels of IRF-1 mRNA were determined by reverse transcription PCR in samples of cerebral cortex ipsilateral (□) or contralateral to the occluded MCA (n = 6–12/time point). mRNA data were normalized to the housekeeping gene HPRT, and are expressed as fold-induction (mean ± SEM) relative to unoperated mice. Since no major differences in IRF-1 mRNA were observed in sham-operated mice killed 12 h and 1, 2, 4, and 7 d after sham operation (n = 1–2/time point), mRNA data from all sham-operated mice were averaged. After MCA occlusion IRF-1 mRNA was markedly upregulated in the ischemic cortex but not contralaterally (*$P < 0.05$ from contralateral side; Student's *t* test). (B) A representative Southern blot from the ischemic side of untreated, sham-operated, and MCA-occluded mice (n = 3/group) is shown. Analysis of mRNA for the housekeeping gene HPRT showed no difference among groups in the level of expression (data not shown; see also Fig. 2 A).

Results

Postischemic IRF-1 mRNA Expression in C57BL/6 and IRF-1 Mice. We first sought to determine if focal cerebral ischemia enhances IRF-1 mRNA expression. In C57BL/6 mice, MCA occlusion was associated with pronounced upregulation of IRF-1 mRNA in the postischemic brain (Fig. 1). IRF-1 mRNA expression was increased by 12 h and remained elevated 1–7 d after MCA occlusion ($P < 0.05$ from sham-operated mice; analysis of variance and Tukey's test). IRF-1 mRNA expression did not increase in the contralateral (nonischemic) cortex ($P > 0.05$) (Fig. 1). IRF-1 mRNA expression was reduced in IRF-$1^{+/-}$ mice and absent in IRF-$1^{-/-}$ mice (Fig. 2).

Volume of Ischemic Injury in C57BL/6 and IRF-1 Mice. In these experiments we used mice with a null mutation of IRF-1 to determine whether IRF-1 contributes to cerebral ischemic injury. In C57BL/6 mice (n = 6), MCA occlusion produced reproducible infarcts involving mainly the cerebral cortex (Figs. 3 and 4). Size and regional distribution of the infarct were comparable to those previously reported in mice from this and other laboratories (20, 41). The volume of the infarct in IRF-$1^{+/+}$ mice did not differ from that of C57BL/6 mice ($P > 0.05$; Fig. 4 A). Infarct volumes in IRF-1 knockout mice were smaller than those observed in C57BL/6 mice (Figs. 3 and 4). The reduction

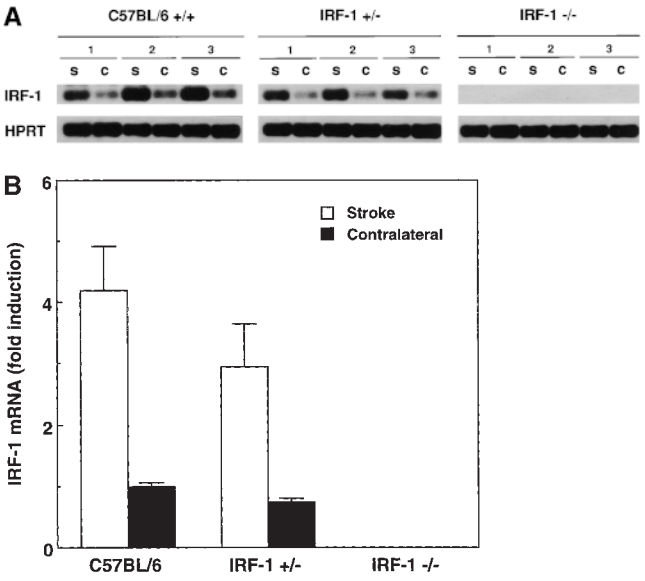

Figure 2. IRF-1 mRNA expression in cerebral cortex of C57BL/6, IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice 4 d after MCA occlusion. Levels of IRF-1 mRNA were determined as described in the legend to Fig. 1. (A) Representative Southern blot with three individual mice per group illustrating IRF-1 mRNA expression in wild-type, IRF-$1^{+/-}$, and IRF-$1^{-/-}$ mice. mRNA for the housekeeping gene HPRT is shown as a control. (B) Group data illustrating IRF-1 mRNA expression in C57BL/6, IRF-$1^{+/-}$, and IRF-$1^{-/-}$ mice. Means (n = 5/group) are expressed as fold increase relative to the response observed in the nonischemic side (■) of C57BL/6 mice. IRF-1 mRNA is reduced in IRF-$1^{+/-}$ mice and is absent in IRF-$1^{-/-}$.

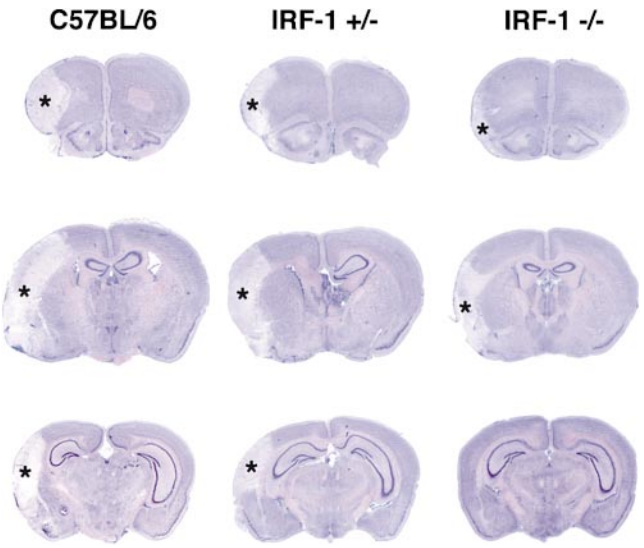

Figure 3. Distribution of the cerebral infarct produced by MCA occlusion in C57BL/6 mice and in IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice 4 d after MCA occlusion. Thionin-stained representative sections at three different rostrocaudal levels of the mouse brain are presented. The pale areas with asterisks represent the infarcted brain. The infarct size is smaller in IRF-$1^{-/-}$ than in IRF-$1^{+/-}$ or C57BL/6 at all rostrocaudal levels.

was less pronounced in IRF-$1^{+/-}$ (23 ± 3%; $P < 0.05$; $n = 5$), than in IRF-$1^{-/-}$ mice (46 ± 9%; $P < 0.05$; $n = 6$), and involved the infarct border throughout the entire rostrocaudal extent of the ischemic lesion (Figs. 3 and 4). The volume of postischemic brain swelling did not differ between C57BL/6 (6.6 ± 0.6 mm^3) and IRF-$1^{+/-}$ mice (5.7 ± 0.8; $P > 0.05$), but was significantly reduced in IRF-$1^{-/-}$ mice (3.5 ± 0.8; $P < 0.05$).

Neurological Deficits in C57BL/6 and IRF-1 Mice. To determine whether the reduction in infarct volume was associated with a better functional outcome, the neurological deficits produced by MCA occlusion were scored in C57BL/6 and IRF-$1^{-/-}$ mice. 24 h after MCA occlusion, neurological deficits did not differ between C57BL/6, IRF-$1^{+/-}$, and IRF-$1^{-/-}$ mice (Fig. 5). However, although the deficits in C57BL/6 remained stable throughout the monitoring period, the deficit in IRF-$1^{-/-}$ mice improved over time (Fig. 5). By 4 d after MCA occlusion, the motor impairment was less pronounced in the IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice than in C57BL/6 ($P < 0.05$), the improvement being more marked in IRF$^{-/-}$ than in IRF-$1^{+/-}$ mice (Fig. 5). These data indicate that the functional outcome of cerebral ischemia is improved in IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice.

Effect of MCA Occlusion and Hypercapnia on CBF in C57BL/6 and IRF-1 Mice. In these studies we investigated whether the reduction in CBF produced by MCA occlusion was comparable in IRF-$1^{-/-}$ mice and controls. As illustrated in Fig. 6 A, the reduction in CBF produced by MCA occlusion in IRF-$1^{-/-}$ mice was not different from that observed in C57BL/6 ($P > 0.05$; $n = 5$–8/group). Similarly, systemic arterial pressure did not differ between IRF-$1^{-/-}$ mice and controls (Fig. 6 B). We then studied the reactivity of the cerebral circulation to systemic

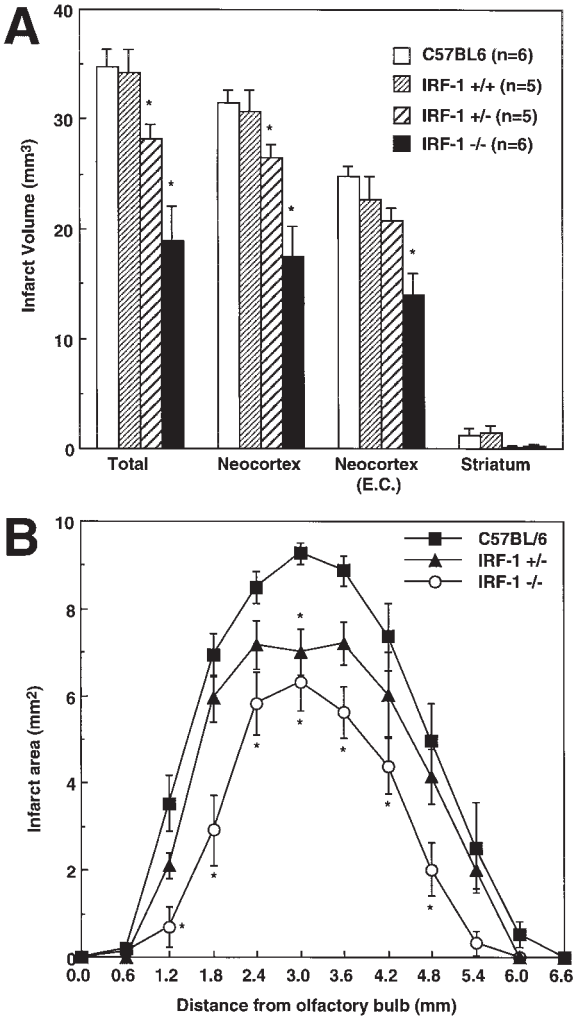

Figure 4. Infarct volume in C57BL/6 and IRF-1 mice 4 d after MCA occlusion. (A) Total infarct volume and infarct volume in neocortex and striatum are presented. Neocortex (E.C.) indicates neocortical infarct volume corrected for swelling (see Materials and Methods for details). Infarct volume (total, neocortex, and striatum) does not differ between C57BL/6 and IRF-$1^{+/+}$ mice ($P > 0.5$ analysis of variance and Tukey's test). However, infarct volume is smaller in IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice (*$P < 0.05$ from C57BL/6). (B) Rostrocaudal distribution of the area of infarction in the brain of C57BL/6 mice and IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice 4 d after MCA occlusion. The reduction in infarct area is greatest in IRF-$1^{-/-}$ and is distributed equally at all rostrocaudal levels (*$P < 0.05$ from C57BL/6; analysis of variance and Tukey's test).

hypercapnia in IRF-$1^{-/-}$ mice. The increase in CBF produced by hypercapnia (arterial pCO_2 = 50–60 mmHg) was 73 ± 12% of control in C57BL/6 and 61 ± 8% in IRF-$1^{-/-}$ mice ($P > 0.05$; $n = 5$/group). Thus, the degree of CBF reduction produced by MCA occlusion and the reactivity of the cerebral circulation to hypercapnia are not significantly altered in IRF-$1^{-/-}$ mice.

Discussion

We sought to determine whether IRF-1, a transcription factor involved in tissue responses to inflammation, cell proliferation, and programmed cell death, is involved in the

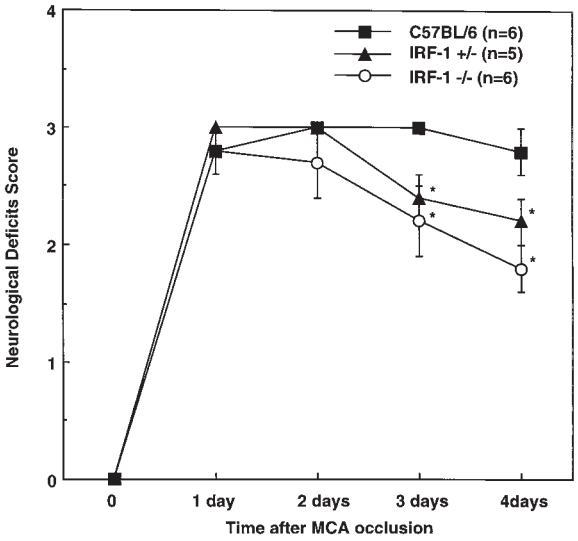

Figure 5. Neurological deficits in C57BL/6, IRF-$1^{+/-}$, and IRF-$1^{-/-}$ mice after MCA occlusion. Deficits were quantified according to a neurological scale widely used in mice (see Materials and Methods). Deficits were identical in all groups 24 h after MCA occlusion. At 3 and 4 d after MCA occlusion deficits were significantly smaller in IRF-$1^{+/-}$ and IRF-$1^{-/-}$ mice than in C57BL/6 mice (*$P < 0.05$, Kruskal-Wallis analysis of variance and Tukey's test).

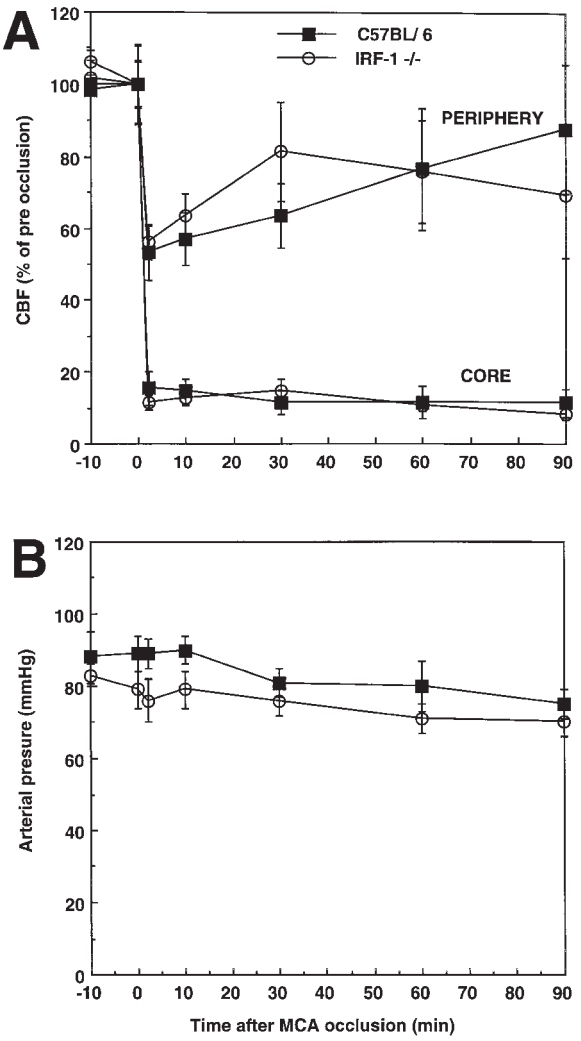

Figure 6. (A) Effect of MCA occlusion on CBF in C57BL/6 mice and in IRF-$1^{-/-}$. CBF was measured by laser-Doppler flowmetry in the cerebral cortex of anesthetized artificially ventilated mice (n = 5–8/group) with monitoring of arterial pressure and controlled arterial blood gases (see Materials and Methods for details). CBF recordings were made in the center of the ischemic territory, both where the CBF reduction was greatest (core) and toward the edge of the ischemic area (periphery). MCA occlusion produces reduction in CBF that are comparable in C57BL/6 and in IRF-$1^{-/-}$ mice in both the ischemic core and the periphery ($P > 0.05$). (B) Mean arterial pressure in wild-type and IRF-$1^{-/-}$ mice before and after MCA occlusion. No significant differences in arterial pressure were observed ($P > 0.05$).

mechanisms of cerebral ischemic injury. We found that IRF-1 mRNA expression is selectively upregulated in the ischemic brain after occlusion of the mouse MCA. The upregulation is observed within 12 h of induction of ischemia and is still present at 7 d. To determine whether the IRF-1 mRNA expression contributes to ischemic brain damage, the infarct volume produced by MCA occlusion was assessed in mice with a null mutation of the IRF-1 gene. We found that the volume of brain injury produced by MCA occlusion is markedly reduced in mice with a null mutation of the IRF-1 gene. The reduction is more pronounced in IRF-$1^{-/-}$ than in IRF-$1^{+/-}$ mice and, importantly, is associated with an improvement of the neurological deficits produced by the ischemic insult. The findings demonstrate that IRF-1 is upregulated after induction of cerebral ischemia and that such upregulation plays a critical role in the mechanisms of ischemic brain injury.

Several transcription factors have been shown to be activated after cerebral ischemia. For example, activator protein 1, factors encoded by early genes, Stat3, hypoxia inducible factor 1, and nuclear factor κb are upregulated after focal or global cerebral ischemia (29–32). However, direct evidence for an involvement of these transcription factors in the mechanisms of ischemic brain injury is lacking (for example see reference 33). In the present study, we have demonstrated that deletion of IRF-1 reduces the magnitude of cerebral ischemic damage and improves neurological recovery. Therefore, IRF-1 is the first transcription factor that has been shown to be linked directly to the development of ischemic brain injury.

Like most mice generated by homologous recombination, the genetic background of IRF-$1^{-/-}$ is heterogeneous, including both C57BL/6 and SV129 genes (44). However, such genetic heterogeneity is unlikely to contribute to the observed reduction in infarct volume. This conclusion is supported by the following observations. First, the infarct volume of IRF-$1^{+/+}$ mice, bred from the same heterozygotic stock from which the homozygous knockouts were derived, was not different from that of inbred wild-type C57BL/6 controls. Second, a clear gene dosage effect is observed when wild-type, IRF-$1^{+/-}$, and IRF-$1^{-/-}$ mice are compared for IRF-1 mRNA or infarct volume. The reduction in infarct volume cannot be attributed to differences in the degree of cerebral ischemia

between C57BL/6 and IRF-1$^{-/-}$ mice: MCA occlusion produced comparable decreases in CBF in wild-type and IRF-1$^{-/-}$ mice. Furthermore, arterial pressure and the reactivity of the cerebral circulation to the vasodilation produced by hypercapnia were not altered in IRF-1$^{-/-}$ mice. Therefore, genetic factors affecting the susceptibility of the brain to cerebral ischemia or vascular factors influencing the degree of ischemia cannot be responsible for the protection observed in IRF-1$^{-/-}$ mice.

After induction of cerebral ischemia, the temporal evolution of the ensuing brain damage is heterogeneous. In the center of the ischemic territory, where the ischemic insult is most severe, irreversible neuronal damage occurs rapidly (45, 46). Pathogenic factors involved in the rapid phase of the damage include energy failure, glutamate excitotoxicity, and reactive oxygen species (47–49). In the peripheral regions of the ischemic territory, the damage progresses at a slower pace, reaching a maximum 2–3 d after induction of ischemia (46, 50). Factors contributing to the delayed evolution of the damage include postischemic inflammation and apoptosis (1, 51). The observation that mice lacking the IRF-1 gene are relatively protected from ischemic infarction suggests that IRF-1 is involved in the mechanisms of ischemic brain injury. However, the delayed time course of its expression suggests that this transcription factor is involved mainly in events occurring in the late stages of cerebral ischemia. The observation that neurological deficits in IRF-1$^{-/-}$ mice are not different from those of wild-type mice 24 h after ischemia and improve 3–4 d later is also consistent with the hypothesis that IRF-1 contributes to the progression, rather than the initiation, of tissue damage.

IRF-1 deletion could attenuate the progression of ischemic brain injury by different mechanisms. One possibility is that IRF-1 deletion interferes with the inflammatory response associated with cerebral ischemia. Cerebral ischemia induces an inflammatory reaction in the injured brain (15–17). Cytokines produced in the ischemic tissue are one of the factors that induce the expression of adhesion molecules in endothelial cells (9–12). Blood-borne neutrophils adhere to the cerebral endothelium, cross the blood vessel wall, and migrate into the brain parenchyma (13, 14, 17). Neutrophils contribute to ischemic brain injury because tissue damage is reduced if their invasion of the brain parenchyma is prevented (52–55; for review see reference 1). After cerebral ischemia, neutrophils express iNOS, an enzyme that produces toxic amounts of NO (18–20). Evidence suggests that NO produced by iNOS participates in ischemic brain injury. Administration of inhibitors of iNOS reduces ischemic damage, whereas iNOS$^{-/-}$ mice have smaller infarcts and a better neurological outcome after MCA occlusion (19, 20, 56). Because IRF-1 plays a role in iNOS transcription (37, 57, 58), it is possible that iNOS is not expressed in IRF-1$^{-/-}$ mice. Therefore, reduced iNOS expression could be one of the mechanisms by which IRF-1$^{-/-}$ mice are protected from cerebral ischemia. On the other hand, the possibility that postischemic iNOS expression is driven by nuclear factor κB and hypoxia inducible factor 1, transcription factors that are also upregulated after cerebral ischemia (30, 32), cannot be ruled out.

Another potential mechanism for the reduction of ischemic injury in IRF-1$^{-/-}$ mice is inhibition of programmed cell death. There is increasing evidence that programmed cell death occurs after focal cerebral ischemia (for review see reference 51). Genes involved in the apoptotic cascade are upregulated after cerebral ischemia (23–26), and internucleosomal DNA fragmentation, one of the hallmarks of apoptosis, occurs (59–62). Several lines of evidence suggest that apoptosis plays a pivotal role in the mechanisms of brain injury. Transgenic mice that overexpress the anti-apoptotic protein bcl2 or mice with a null mutation of p53, a protein that promotes apoptosis, are relatively protected from the effects of focal ischemia (63, 64). In addition, inhibition of the caspase family of cysteine proteases, one of the effectors of apoptotic cell death, reduces cerebral ischemic damage (65–67). There is evidence that IRF-1 is involved in the molecular mechanisms of apoptosis. For example, DNA damage-induced apoptosis is attenuated in T-lymphocytes and in macrophages lacking IRF-1 (68, 69). Therefore, it is conceivable that postischemic apoptotic neuronal death is attenuated in IRF-1$^{-/-}$ mice resulting in less brain damage.

The mechanisms of IRF-1 expression after cerebral ischemia and its cellular localization have not been elucidated. In other systems, IRF-1 is induced by a wide variety of factors including IFNs and cytokines (34). Some of these factors, such as IL-1, IL-6 and TNF-α, are also expressed after cerebral ischemia (4–6) and could conceivably trigger IRF-1 mRNA expression. Furthermore, intracellular second messengers, such as calcium and protein kinase C, may also induce IRF-1 expression (70). These pathways are activated after cerebral ischemia (for examples see references 71, 72) and could contribute to trigger IRF-1 expression. Therefore, multiple mechanisms are likely to contribute to IRF-1 upregulation. Further studies will be required to address these issues.

In conclusion, we have demonstrated that the mRNA encoding for the transcription factor IRF-1 is markedly upregulated after focal cerebral ischemia. We have also shown that IRF-1$^{-/-}$ mice are substantially protected from the brain damage and neurological deficits produced by MCA occlusion. The protection develops 2–3 d after induction of ischemia. The findings suggest that IRF-1–inducible genes contribute to the late stages of tissue damage after cerebral ischemia. Thus, IRF-1 is a critical transcription factor in the molecular mechanisms of ischemic brain injury and, as such, might be a new target for therapeutic interventions in ischemic stroke.

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