Central Gene Transfer of Interleukin-10 Reduces Hypothalamic Inflammation and Evidence of Heart Failure in Rats After Myocardial Infarction

Yang Yu, Zhi-Hua Zhang, Shun-Guang Wei, Yi Chu, Robert M. Weiss, Donald D. Heistad, Robert B. Felder

Abstract—The expression of proinflammatory cytokines increases in hypothalamus of rats with myocardial infarction (MI) and heart failure. We used central gene transfer of human interleukin (IL)-10, a potent antiinflammatory cytokine, to counter the effects of brain proinflammatory cytokines and examine their functional significance. Sprague–Dawley rats underwent coronary ligation to induce MI or sham surgery (SHAM). One week later, adenoviral vectors encoding human IL-10 (AdIL-10) or β-galactosidase (βGal) were injected (30 μL over 30 minutes) into lateral ventricle. One week after injection, there was abundant expression of human IL-10 in the brain of MI + AdIL-10 and SHAM + AdIL-10 rats. Compared with SHAM + βGal, MI + βGal had increased (P<0.05) IL-1β and cyclooxygenase-2 mRNA and protein and nuclear factor κB activity in the hypothalamus, cyclooxygenase-2 fluorescence in perivascular cells of the paraventricular nucleus of hypothalamus, prostaglandin E₂ in cerebrospinal fluid, and Fra-like activity (indicating neuronal excitation) in paraventricular nucleus. Plasma norepinephrine levels, lung/body weight, right ventricle/body weight, and left ventricular end-diastolic pressure were increased and maximal left ventricular dP/dt was decreased. All of these findings were ameliorated in MI rats treated with AdIL-10. Hypothalamic tumor necrosis factor-α and circulating tumor necrosis factor-α and IL-1β levels, also increased in MI + βGal, were not affected by AdIL-10 treatment. Rat native IL-10 was not affected by MI or AdIL-10. AdIL-10 had no effects on SHAM rats. The results demonstrate that cardiovascular and autonomic mechanisms leading to heart failure after MI can be modulated by manipulating the balance between proinflammatory and antiinflammatory cytokines in the brain. (Circ Res. 2007;101:304-312.)

Key Words: brain ■ proinflammatory cytokines ■ adenoviral vector ■ autonomic regulation

Myocardial infarction (MI)-induced heart failure (HF) is a systemic inflammatory state characterized by elevated circulating proinflammatory cytokines.¹⁻⁵ Circulating proinflammatory cytokines induce cyclooxygenase-2 (COX-2) activity in the cerebral vasculature, increasing the production of prostaglandins E₂ (PGE₂)⁶ and indirectly activating the hypothalamic–pituitary–adrenal axis.⁷ Recent studies in rats with HF suggest that circulating proinflammatory cytokines also induce the expression of proinflammatory cytokines in cardiovascular regulatory regions of the brain.⁸⁻¹⁰ Interleukin (IL)-1β and tumor necrosis factor (TNF)-α appear in the rat hypothalamus early after MI⁴⁻⁵ and remain present at increased levels in rats with established HF.⁴ Emerging evidence suggests that proinflammatory cytokines in the brain upregulate the activity of the brain renin–angiotensin system (RAS)¹¹ and contribute to oxidative stress.¹⁰ All of these central neural mechanisms may increase sympathetic nerve activity and contribute to the progression of HF.

IL-10 is a potent antiinflammatory and immune regulatory cytokine that inhibits production of several proinflammatory mediators, including IL-1, IL-6, IL-8, granulocyte colony-stimulating factor, and TNF-α, and upregulates the expression of the naturally occurring IL-1 receptor antagonist.¹²,¹³ Increasing evidence indicates that IL-10 can modulate the production and/or action of proinflammatory cytokines in the central nervous system. Intracerebroventricular or intravenous administration of an adenovirus encoding the human IL-10 gene completely inhibits brain IL-1β and TNF-α production in response to intracerebroventricular lipopolysaccharide in mice¹⁴ and blocks lipopolysaccharide-induced behavioral effects and brain damage by downregulating the inflammatory response.¹⁵,¹⁶

The present study sought to determine whether brain proinflammatory cytokines contribute to the progression of HF in rats following MI. We tested the hypothesis that the antiinflammatory influence of IL-10 would modulate the
MI-induced hypothalamic expression of inflammatory mediators (IL-1β, TNF-α, COX-2, PGE2) and ameliorate the peripheral manifestations of HF after MI.

Materials and Methods

Animals
Adult male Sprague–Dawley rats weighing 250 to 300 g were obtained from Harlan Sprague–Dawley (Indianapolis, Ind). They were housed in temperature- (23±2°C) and light-controlled animal quarters and were provided with rat chow ad libitum. These studies were performed in accordance with the Guiding Principles for Research Involving Animals and Human Beings. The experimental procedures were approved by the University of Iowa, Institutional Animal Care and Use Committee.

Induction of MI or SHAM
Rats were anesthetized (ketamine 90 mg/kg + xylazine 10 mg/kg IP) and underwent coronary ligation to induce MI (n=56) or sham coronary ligation (SHAM, n=30) as described. Nineteen rats died within 24 hours of coronary ligation. All of the surviving rats underwent echocardiography under sedation (ketamine 25 mg/kg IP) within 24 hours of coronary ligation to confirm the extent of ischemic injury. Measurements of ischemic zone as a percentage of left ventricular (LV) circumference, LV ejection fraction, and LV end-diastolic volume were made as previously described. Only animals with large infarctions (≥39%; n=33) were used in the study. Four rats with small MI were excluded.

Injection of Adenoviral Vector
One week after MI, rats were anesthetized (ketamine 90 mg/kg + xylazine 10 mg/kg IP) and a small hole was drilled in the skull to permit placement of a 27-gauge needle connected to a Hamilton syringe in the right lateral ventricle (stereotaxic coordinates: 1.5-mm lateral and 1 mm posterior to the bregma, 4.0 mm in depth). The adenoviral vectors encoding human IL-10 (AdIL-10) or β-galactosidase (βGal, control) were then injected (30 µL; 3×1010 plaque-forming units/mL) over 30 minutes in MI rats (MI+AdIL-10, n=16; MI+βGal, n=17) and SHAM (SHAM + AdIL-10, n=15; SHAM + βGal, n=15). The human IL-10 and βGal viral vectors were constructed at the Gene Transfer Vector Core of the University of Iowa. Dose and method for injection of the adenoviral vectors were derived from a previous study.

Hemodynamic/Anatomical Measurements and Sample Collection
Two weeks after MI, rats (n=7 to 8 for each group) were anesthetized (pentobarbital, 50 mg/kg IP) for terminal study. Systolic blood pressure, diastolic blood pressure, LV end-diastolic pressure, LV peak systolic pressure, maximum rate of rise of LV pressure (dP/dtmax), and heart rate were measured as described previously. Blood samples were collected from the right carotid artery immediately following the hemodynamic measurements, and cerebrospinal fluid (CSF) was withdrawn from the cisterna magna. Rats were then euthanized with an overdose of pentobarbital, and heart, lung, and brain tissues were harvested. The brain tissue was divided into 2 parts (left and right). Half of hypothalamus, a piece of brain cortex from the same side and a piece of noninfarcted LV were homogenized in TRI Reagent (Molecular Research Center Inc, Cincinnati, Ohio) to extract total RNA for gene analysis. The other half of hypothalamus and a piece of brain cortex from the same side were homogenized in cell lysis buffer (Cell Signaling Technology Inc., Beverly, Mass) to extract protein for Western blotting analysis. Wet lung weight and right ventricular (RV) weight, with respect to body weight (BW), were measured as indices of pulmonary congestion and RV remodeling.

Detection of Human IL-10 mRNA Expression After Gene Transfer
RT-PCR was used to determine expression of human IL-10 mRNA in hypothalamus, brain cortex, and noninfarcted left ventricle after gene transfer with AdIL-10. Total RNA was reverse transcribed into cDNA; 200 ng of RNA equivalent of cDNA was used for a PCR. The sequence of primers for human IL-10 was as follows: forward primer, 5′-CTTCCTGTTTTTACAGGAAG-3′; and reverse primer, 5′-AGTCTGAGAAACAGCTGAC-3′. For an internal control, we used GAPDH (glyceraldehyde-3-phosphate dehydrogenase) primers; forward primer, 5′-TGAACGGGAACTCCTAGTG-3′; and reverse primer, 5′-TCCACCACCTGTGTCGTGA-3′. The intensity of each band was analyzed by the NIH image analysis system (version 1.62).

Quantification of mRNA Levels of Inflammatory Mediators
mRNA levels for COX-2, COX-1, IL-1β, and TNF-α in hypothalamus and brain cortex were measured with real-time PCR following reverse transcription of total RNA. The sequences for primers and probe used were as follows: COX-2: forward primer, 5′-CGCTGTACAAAGCATGGAAGAGG-3′; reverse primer, 5′-GCGTTGCGTGCTACCTAGGA-3′; probe, 5′-CCTCCTATTGA-CAGAGCAGCAGAGATGAAA-3′; COX-1: forward primer, 5′-TCTCGTGAAGATGCGCTACC-3′; reverse primer, 5′-AACACCTCTGGGCGCACAG-3′; probe, 5′-CCAGGTTGGCTCCGCGCAGGAG-3′; TNF-α: forward primer, 5′-CCAGGAGAAGATCAGCCCTCT-3′; reverse primer, 5′-TCTAACCAGGGCGTGGACTCA-3′; probe, 5′-AGAGCCCCCTGGCGCTAAGGACACCCCT-3′. Primers and probes for IL-1β and GAPDH were purchased from Applied Biosystems (Foster City, Calif). Real-time PCR was performed using the ABI prism 7900 Sequence Detection System (Applied Biosystems, Foster City, Calif.). The final results of real-time PCR are expressed as the ratio of mRNA of interest to GAPDH.

Protein Analysis for Inflammatory Mediators
Protein levels for inflammatory mediators were measured with Western blotting analysis, as previously described, using polyclonal primary antibodies to COX-2 and COX-1 (Cayman Chemical Co, Ann Arbor, Mich) and IL-1β and TNF-α (Santa Cruz Biotechnology Inc, Santa Cruz, Calif).

Immunohistochemistry
At 2 weeks after MI, 4 rats from each group were used for immunohistochemical studies to assess paraventricular nucleus (PVN) neuronal activity or COX-2 immunoreactivity in the PVN as described before. A Fra-like (Fra-LI; fos family gene) activity, a marker of neuronal activation, was detected using a rabbit polyclonal antibody (c-fos K-25; Santa Cruz Biotechnology). Fra-LI–positive cells were counted across the entire expanse of PVN and analyzed as described before. Thus, parvocellular and magnocellular neurons are included in the counts reported.

COX-2 immunoreactivity in the PVN was determined by immunofluorescent staining using antibody for COX-2 (1:1000, Cayman Chemical Co, Ann Arbor, Mich). Perivascular cells were identified with an antibody for ED2 (1:1000, Serotec Inc, Raleigh, NC), an established marker for perivascular cells. Nuclear stain for TUNEL (1:2000; Molecular Probes, Eugene, Ore) was used to visualize cell nuclei. The sections stained with fluorescent dyes were recorded with a confocal laser-scanning microscope (Zeiss LSM 510, Carl Zeiss Inc, Minneapolis, Minn). The fluorescence of COX-2 immunoreactivity was measured with NIH image analysis software. For each rat, cross-sections of no less than seven blood vessels in the PVN were examined and an average value was reported.

Analysis of Blood and CSF
The levels of cytokines (human IL-10 and native rat IL-10, IL-1β, and TNF-α) in plasma and CSF; norepinephrine (NE) in plasma and PGE2, in CSF were measured using high sensitivity ELISA kits (Biosource International Inc [Camparillo, Calif], Rocky Mountain

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Diagnostics Inc (Colorado Springs, Colo), and R&D Systems Inc (Minneapolis, Minn), respectively.

Analysis of Nuclear Factor κB Activation
Nuclear extracts for Nuclear factor (NF)-κB DNA binding and cytoplasmic extracts for NF-κB inhibitory protein (IκB) were prepared from hypothalamus (n=4 to 5 for each group) using a Nuclear Extract Kit (Active Motif, Carlsbad, Calif). NF-κB p65 DNA binding activity was detected by ELISA (Pierce Biotechnology Inc, Rockford, Ill). Cytoplasmic IκB level was measured with Western blotting as described above, using polyclonal antibody to IκBα (Santa Cruz Biotechnology).

Statistical Analysis
All data are expressed as means±SEM. The significance of differences in mean values was analyzed by 2-way, repeated-measure ANOVA followed by post hoc Fischer’s least-significant difference test. Echocardiographic parameters were analyzed using 1-way ANOVA followed by Fischer’s least significant difference test.

Results
Echocardiography
Rats assigned to treatment with AdIL-10 and βGal were well matched with regard to echocardiographically defined LV function. LV ejection fraction was significantly (P<0.05) reduced in MI rats assigned to AdIL-10 (0.41±0.03) or βGal (0.44±0.04), compared with SHAM rats assigned to those same treatments (0.83±0.04 and 0.85±0.01, respectively). LV end-diastolic volume was significantly (P<0.05) increased in MI rats assigned to AdIL-10 (0.81±0.11 mL) or βGal (0.80±0.13 mL), compared with SHAM rats assigned to those same treatments (0.37±0.05 and 0.35±0.04 mL, respectively). There was no difference in ischemic zone among MI rats assigned to AdIL-10 (49±3%) versus βGal (47±2%) treatment. For further details, see Table I in the online data supplement at http://circres.ahajournals.org.

Transgene Expression
One week after gene transfer with human AdIL-10, there was abundant expression of human IL-10 mRNA by RT-PCR in the hypothalamus and brain cortex but not in LV from both MI and SHAM rats (Figure 1A and 1B). No PCR products for human IL-10 were observed in the hypothalamus, brain cortex, or left ventricle of MI or SHAM rats transfected with βGal. Human IL-10 was detectable by ELISA in CSF but not
in plasma of rats treated with AdIL-10. There was no detectable human IL-10 in CSF or plasma of rats treated with \( \beta \)Gal (Figure 1C). Rat native IL-10 was detectable in CSF and plasma of all groups, with no significant difference among groups (Figure 1D).

**Effects of IL-10 on Indices of HF**

Compared with the SHAM rats, the MI + \( \beta \)Gal rats had significantly (\( P<0.05 \)) higher LV end-diastolic pressure (21 ± 2 versus 4 ± 1 mm Hg) and RV/BW (0.85 ± 0.04 versus 0.59 ± 0.03) and lung/BW (6.83 ± 0.38 versus 4.25 ± 0.18) ratios and significantly (\( P<0.05 \)) lower dP/dt (4459 ± 314 versus 8224 ± 434 mm Hg/sec). Systolic blood pressure (111 ± 4 versus 122 ± 4 mm Hg), LV peak systolic pressure (100 ± 5 versus 110 ± 3 mm Hg), and BW (306 ± 6 versus 330 ± 6 g) were also significantly (\( P<0.05 \)) lower in MI + \( \beta \)Gal than SHAM + \( \beta \)Gal rats. MI rats treated with AdIL-10 had significantly (\( P<0.05 \)) higher dP/dt (5794 ± 263 mm Hg/sec) and lower LV end-diastolic pressure (9 ± 1 mm Hg) and RV/BW (0.70 ± 0.03) and lung/BW (5.28 ± 0.42) ratios than MI + \( \beta \)Gal rats, but these values were all still significantly (\( P<0.05 \)) different from SHAM + \( \beta \)Gal rats. Systolic blood pressure and LV peak systolic pressure were not affected. There were no significant differences in diastolic blood pressure or heart rate across the experiment groups. See supplemental Table I available online at http://circres.ahajournals.org for further details.

**Effects of IL-10 on Fra-Like Activity**

The expression of Fra-like (Fra-LI) (fos family gene) activity has been used as a marker for chronic neuronal activation in studies of rats or mice with MI-induced HF.\(^2\)\(^1\),\(^2\)\(^2\). Immunohistochemical study revealed that Fra-LI activity increased in the PVN of MI + \( \beta \)Gal rats compared with SHAM + \( \beta \)Gal rats at

![Figure 2. Expression of Fra-LI activity in the PVN of hypothalamus. A, Representative examples of Fra-LI immunoreactive positive neurons in the PVN of rats from each treatment group. Dark dots indicate Fra-LI-positive activated neurons. Scale bar=200 \( \mu \)m. B, Quantification of Fra-LI-positive neurons in the PVN of each group. Values are expressed as means±SEM (n=4 for each group) and relative to SHAM + \( \beta \)Gal. *P<0.05 vs SHAM + \( \beta \)Gal, †P<0.05 MI + \( \beta \)Gal vs MI + AdIL-10.](http://circres.ahajournals.org/)

![Figure 3. Quantitative comparison of the mRNA expression for COX-2 (A), COX-1 (B), IL-1\( \beta \) (C), and TNF-\( \alpha \) (D) in the hypothalamus of MI or SHAM rats after gene transfer with AdIL-10 or \( \beta \)Gal. Values were expressed as mean±SEM (n=7 to 8 for each group) and relative to SHAM + \( \beta \)Gal. *P<0.05 vs SHAM + \( \beta \)Gal, †P<0.05 MI + AdIL-10 vs MI + \( \beta \)Gal.](http://circres.ahajournals.org/)
2 weeks after MI (Figure 2). There were fewer Fra-LI–positive PVN neurons in MI+AdIL-10 than MI+βGal rats. However, there was no difference in expression of Fra-LI activity between SHAM+βGal and SHAM+AdIL-10 rats.

**Effects of IL-10 on mRNA Expression of Inflammatory Mediators**

The expression of COX-2, IL-1β, and TNF-α mRNA was higher in hypothalamus of MI+βGal compared with SHAM+βGal rats (Figure 3). After gene transfer of AdIL-10, hypothalamic expression of COX-2 and IL-1β mRNA was significantly lower in MI rats but hypothalamic expression of TNF-α mRNA was not affected. There was no difference for COX-1 mRNA across the four experimental groups. Gene transfer of AdIL-10 had no effects on COX-2, IL-1β, and TNF-α mRNA expression in hypothalamus in SHAM rats. Neither MI nor treatment with AdIL-10 or βGal affected mRNA expression of COX-2, COX-1, IL-1β, and TNF-α in brain cortex (data not shown).

**Effects of IL-10 on Protein Expression of Inflammatory Mediators**

Western blot analysis showed that COX-2, IL-1β, and TNF-α proteins were markedly upregulated in hypothalamus of MI+βGal rats compared with SHAM+βGal rats. There was significantly less protein expression of COX-2 and IL-1β, but not TNF-α, in hypothalamus of MI+AdIL-10 compared with MI+βGal rats (Figure 4). The expression of COX-1 protein was not different among the 4 groups, and AdIL-10 treatment did not change protein expression of COX-2, IL-1β, or TNF-α protein in SHAM rats. Thus, the effect of IL-10 on protein expression paralleled its effect on mRNA induction (Figure 3).

**COX-2 Immunostaining**

Laser confocal microscopy showed that COX-2 immunoreactivity in the PVN was localized primarily in the cytoplasm of perivascular cells (Figure 5A), as we have previously demonstrated. COX-2 immunoreactivity was present in the PVN of both MI and SHAM rats but was most abundant in the PVN of MI+βGal rats (Figure 5B and 5C). COX-2 immunoreactivity in the PVN was reduced by gene transfer with AdIL-10 in MI rats but not in SHAM rats (Figure 5B and 5C).

**Humoral Effects of IL-10**

CSF PGE2, a product of COX-2 activity, and plasma NE, a marker of sympathetic nerve activity, were higher in MI+βGal compared with SHAM+βGal rats (Figure 6). Gene transfer of IL-10 significantly reduced CSF PGE2; and plasma NE levels in MI but not in SHAM rats. Plasma IL-1β and TNF-α levels were also significantly increased in MI+βGal compared with SHAM+βGal rats (Figure 6), but treatment with AdIL-10 had no effects on these circulating proinflammatory cytokines.

**Effects of IL-10 on NF-κB Activation**

There was greater NF-κB p65 DNA binding activity and less cytoplasmic IκB protein in the hypothalamus of MI+βGal rats compared with SHAM+βGal rats (Figure 7A and 7B). Cytoplasmic IκB protein was higher and NF-κB p65 DNA binding activity was lower in the hypothalamus of MI rats treated with AdIL-10 (Figure 7A and 7B).

**Discussion**

The important new finding of this study is that the peripheral manifestations of HF following MI in rats can be ameliorated by reducing the intensity of the inflammatory/immune response in the brain. A shift in the balance between pro- and antiinflammatory cytokines in the brain induced by the central administration of an adenoviral vector encoding the gene for human IL-10 had beneficial effects on indices of the severity of HF following MI. LV
function improved, plasma NE decreased, and pulmonary vascular congestion and RV remodeling were less prominent. This report is the first to attribute pathophysiological significance to the appearance of proinflammatory cytokines in the brain following MI.

A significant effect of brain proinflammatory cytokines on the peripheral manifestations of HF might well have been anticipated. The proinflammatory cytokines interact with several neurochemical systems that act within the brain to influence cardiovascular regulation. Recent studies have identified reactive oxygen species (ROS) in the brain as the likely ultimate mediator of sympathetic nervous system activity in HF. Although the extant HF literature has emphasized the association between the brain RAS and NAD(P)H oxidase dependent generation of ROS, the proinflammatory cytokines are another well-recognized potent stimulus to NAD(P)H oxidase. In addition, the proinflammatory cytokines may activate the nuclear transcription factor NF-κB in a ROS dependent manner. Potential gene products of cytokine-induced NF-κB activation include angiotensinogen and the angiotensin type 1 receptor, key components of the RAS, and COX-2, which regulates the synthesis of PGE2, in addition to TNF-α and IL-1β. Thus, by activating NF-κB, the proinflammatory cytokines may prime at least two central systems that regulate autonomic function. The proinflammatory cytokines have been reported to facilitate the actions of RAS in peripheral tissues, and preliminary data from our laboratory suggest a similar influence in brain tissue. PGE2 is generally sympathoexcitatory in the brain, and more specifically activates the hypothalamic-pituitary-adrenal axis to increase sympathetic nerve activity and circulating catecholamines and glucocorticoids.

In the present study, AdIL-10 treated MI rats had significant reductions in NF-κB and downstream indicators of NF-κB activity, COX-2 and PGE2. In the PVN, a major center for cardiovascular autonomic regulation that is activated in rats with HF, there was less COX-2 expression in perivascular cells and less neuronal excitation (ie, Fra-LI activity) in MI rats treated with AdIL-10 than in MI rats treated with βGal. In the periphery, the AdIL-10 treated MI rats had significantly less circulating NE and improved indices of volume regulation and LV function. Compared with systemically administered eplerenone, which reduced both circulating and brain proinflammatory cytokines in our previous study, centrally administered AdIL-10 had a greater effect to lower LV end-diastolic pressure. However, differences in the nature of the injected substances, the route of administration,
and the time points at which treatment and functional testing occurred obviate any attempt to attribute pathophysiological significance to that observation.

Central interventions that reduce sympathetic nerve activity in rats with HF have been shown to reduce LV remodeling and improve renal regulation of extracellular fluid balance. In those studies, the functional assessments of cardiac and renal performance were made after 3 to 4 weeks of continuous drug treatment. In the present study, rats were treated with a single central injection of AdIL-10 one week after MI. Considering the time required for synthesis of human IL-10, it is surprising that significant improvements in LV contractility, LV filling pressures, pulmonary congestion, and RV remodeling were observed only one week later. A significant reduction in preload related to increased sodium and water excretion could explain these early findings. In

![Figure 6](image_url) Plasm IL-1β (A), TNF-α (B), NE (C), and CSF PGE₂ (D) level in MI or SHAM rats after gene transfer with AdIL-10 or βGal. Values are expressed as means±SEM (n=7 to 8 for each group). *P<0.05 vs SHAM+βGal, †P<0.05 MI+AdIL-10 vs MI+βGal.

![Figure 7](image_url) Hypothalamic NF-κB p65 DNA binding activity expressed as relative light units (A), and cytoplasmic IκB protein expression (B) in MI and SHAM rats after gene transfer with AdIL-10 or βGal. Representative Western blots are aligned with the matching grouped data. Values are expressed as mean±SEM (n=4 to 5 for each group) and relative to SHAM+βGal. *P<0.05 vs SHAM+βGal, †P<0.05 MI+AdIL-10 vs MI+βGal.
unpublished work we have observed improved volume regulation in HF rats as early as one day after a central intervention (ie, spironolactone) that reduces sympathetic outflow. Inhibition or reversal of LV remodeling is another possible explanation. Biochemical markers of LV remodeling respond rapidly to appropriate stimuli but cardiac function is typically examined weeks rather than days after a therapeutic intervention. In the present study, functional and anatomical studies of the effect of centrally administered AdIL-10 on LV myocardium were not undertaken, and so a potential role for early cardiac remodeling cannot be excluded.

Native rat IL-10 was not affected by MI, suggesting that MI does not elicit a protective antiinflammatory IL-10 response, at least at the time point at which these data were acquired. In the AdIL-10-treated rats, there was robust expression of human IL-10 in hypothalamic tissues, with associated significant reductions in the hypothalamic expression of IL-1β, NF-κB, and COX-2. There are several possible explanations for these effects. IL-10 may inhibit the production of IL-1β secondarily affecting downstream NF-κB activity, and COX-2 activity. Alternatively, IL-10 may directly inhibit NADPH oxidase or NF-κB activity. The results of the present study do not discriminate among these possibilities.

AdIL-10 treatment had no measurable effect on hypothalamic TNF-α. In a comparable study of spontaneously hypertensive rats with brain ischemia, IL-1β levels in CSF decreased but TNF-α levels actually increased in response to treatment with intracerebroventricular AdIL-10. These observations are not inconsistent with an overall effect of IL-10 to quench the inflammatory response in the brain. IL-10 is a potent antiinflammatory and immune regulatory cytokine that inhibits the production of a variety of proinflammatory mediators and upregulates the expression of the naturally occurring IL-1 receptor antagonist. Thus, a shift in the balance of inflammatory and antiinflammatory cytokines in the brain may have blunted the influence of TNF-α without reducing tissue levels. An effect of altered circulating proinflammatory cytokines was excluded in this study.

The effect of AdIL-10 treatment on COX-2 expression by perivascular cells in the PVN deserves further comment. In normal rats, COX-2 activity can be induced in perivascular cells by systemic injections of IL-1β. In rats with established HF, in which TNF-α and IL-1β are chronically elevated, COX-2 activity in PVN can be reduced by interventions that lower the circulating levels of these cytokines or reduce the activity of NF-κB, a transcription factor known to regulate the expression COX-2. The present study clearly demonstrates that these perivascular cells also respond to changes in the proinflammatory cytokines content inside the blood-brain barrier. Thus, as previously pointed out, these resident macrophages are situated at the interface between blood and brain and appear to monitor conditions on both sides of the divide.

A technical limitation to be considered in interpreting the peripheral measures of HF is that the hemodynamic measurements and blood sampling were performed under pentobarbital anesthesia. Pentobarbital anesthesia is known to blunt sympathetic and cardiovascular responses, and that effect may have diminished the differences between groups. Nonetheless, significant differences were observed in hemodynamic measurements and in plasma NE values in MI versus SHAM rats, and in MI rats treated with AdIL-10 versus βGal. Thus, treatment effects were clearly present despite pentobarbital anesthesia.

This study demonstrates that central gene transfer of the antiinflammatory cytokine IL-10 provides effective transgene expression in the hypothalamus in rats after MI. Overexpression of human IL-10 in brain reduces hypothalamic IL-1β and COX-2 expression, CSF PGE2 level, sympathetic excitation, and evidence of volume accumulation in these rats. Previous studies from our laboratory and others have demonstrated that inhibiting the activity of the brain RAS or blocking brain mineralocorticoid receptors can substantially reduce the peripheral manifestations of HF. This study demonstrates a similar beneficial effect of reducing (or counterbalancing) inflammatory mediators in the brain. We speculate that the common mechanism linking these excitatory systems is NADPH oxidase or NF-κB activity in a feed-forward manner. However, the precise mechanisms linking the central actions of these systems remain to be determined.

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Disclosures
None.

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## Online Table I. Echocardiographic, Hemodynamic and Anatomical Measurements

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<th>SHAM + βGal (n=15)</th>
<th>SHAM + AdIL-10 (n=15)</th>
<th>MI + βGal (n=17)</th>
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<td>dP/dt (mmHg/s)</td>
<td>8224 ± 434</td>
<td>8404 ± 418</td>
<td>4459 ± 314*</td>
<td>5794 ± 263* †</td>
</tr>
</tbody>
</table>

SHAM: sham-operated control; MI: myocardial infarction. LVEDV: left ventricular end-diastolic volume; LVEF: LV ejection fraction; %IZ: percent ischemic zone. BW: body weight; RV: right ventricular; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; LVPSP: LV peak systolic pressure; LVEDP: LV end-diastolic pressure; dP/dt: maximum rate of rise of LV pressure. Values are expressed as mean ± SEM. *P<0.05 versus SHAM + βGal, †P<0.05 versus MI + βGal.