Tumor-Necrosis-Factor-\( \alpha \)-Gene-Deficient Mice Have Improved Cardiac Function Through Reduction of Intercellular Adhesion Molecule-1 in Myocardial Infarction

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Background  Tumor necrosis factor (TNF-\( \alpha \))-KO is linked to the pathogenesis of cardiovascular diseases, but how it affects myocardial infarction (MI), so the present study examined the effects of TNF-\( \alpha \) and the involvement of intercellular adhesion molecule (ICAM)-1 on MI.

Methods and Results  Left coronary arteries of C57BL/6 wild type (WT) and TNF-\( \alpha \) knockout (KO) mice were ligated and the mice were killed 1, 3, and 7 days later. Fractional shortening on echocardiography of the KO mice was significantly higher than that of the WT mice from day 1 to 7 (\( p<0.01 \)). The ICAM-1 mRNA in the infarcted area of the KO mice was significantly lower than that of the WT from day 1 (\( p<0.01 \)) to 7. In immunohistochemistry, the expression of ICAM-1 was weaker in the KO than in the WT mice. The number of neutrophils in the KO mice peaked at day 1, but even this peak level failed to reach the levels in the infarcted (\( p<0.01 \)) and peri-infarcted areas (\( p<0.05 \)) in the WT. The number of macrophages in the KO mice peaked at day 7, but this peak level failed to reach the levels in the infarcted (\( p<0.01 \)) and peri-infarcted areas (\( p<0.05 \)) in the WT.

Conclusion  In a permanent occlusion model of MI TNF-\( \alpha \) decreased cardiac function and ameliorated myocardial remodeling through the induction of ICAM-1.  (Circ J 2006; 70: 1635 – 1642)

Key Words: Intercellular adhesion molecule-1; Myocardial infarction; Nitric oxide; Tumor necrosis factor-\( \alpha \)

Tumor necrosis factor (TNF-\( \alpha \)) is a pro-inflammatory cytokine with pleiotropic biological effects and an established role in the initiation and continuation of inflammation. TNF-\( \alpha \) has been implicated in the pathogenesis of various cardiovascular diseases, such as acute myocardial infarction (AMI), chronic heart failure, electrical remodeling of the diseased heart, and atherosclerosis. The cytokine is secreted mainly by infiltrating cells and myocytes after injury, but it also facilitates the adhesion and transmigration of leukocytes, a group of cells that mediate cardiac injury themselves through the release of oxygen free radicals, proteases, and nitric oxide (NO). Severe chronic heart failure develops in transgenic mice that have constitutive overexpression of TNF-\( \alpha \) in cardiac myocytes and histological examination of these hearts reveals neutrophilic, mononuclear, and lymphocytic infiltration consistent with transmural myocarditis.

There are some reports of a role of TNF-\( \alpha \) in an ischemia–reperfusion model of myocardial infarction (MI)13–17 Treatment with anti-TNF-\( \alpha \) antibody reportedly improved myocardial recovery after ischemia–reperfusion13 and in vivo gene transfer of soluble TNF-\( \alpha \) receptor 1 has been shown to alleviate the size of the infarct in rats undergoing ischemia–reperfusion. Maekawa et al observed a smaller infarct size, decreased frequency of arrhythmia, and improvement of cardiac function in TNF-\( \alpha \) knockout (KO) mice than in the wild type (WT) mice in an ischemia–reperfusion model. In permanent occlusion models of MI there is only one report that TNF KO mice had a significantly reduced acute cardiac rupture rate and improved chronic left ventricular (LV) function. However, involvement of inflammation, such as neutrophil and macrophage infiltration, from a very early stage and the important factors related to the inflammatory process have not been fully elucidated. On the other hand, TNF-\( \alpha \) conferred protective effects against heart damage in a model of ischemia–reperfusion by inducing protective proteins such as manganese superoxide dismutase. Furthermore, TNF-\( \alpha \) receptor KO mice show accelerated myocardial apoptosis after MI, indicating that TNF-\( \alpha \) also has protective effects against ischemia. It remains to be clarified whether TNF-\( \alpha \) has deleterious effects in AMI.

Intercellular adhesion molecule (ICAM)-1 is required for the transmigration of neutrophils and monocytes/macrophages and recruitment of leukocytes from the circulation by the endothelium is essential for the initiation and targeting of an inflammatory response. Firm adhesion of leukocytes is mediated by binding of the \( \alpha \)2-integrin family to ICAM-1 and ICAM-2 expressed in the vascular endothelium. Interestingly, TNF-\( \alpha \) transgenic mice with targeted disruption of ICAM-1 and P-selectin exhibit improved
cardiac function and prolonged survival. On the basis of those results, we presumed that the TNF-α-induced upregulation of ICAM-1 plays an important role as an initial process of TNF-α-related cardiac injury. In a TNF-α KO mouse model of ischemia–reperfusion reduction of nuclear-factor-kappa-activation and ICAM-1 mRNA expression has been shown but in a permanent occlusion model of MI, no studies have elucidated the involvement of ICAM-1 and cellular infiltration in the inhibition of TNF-α.

In the present study we examined the time-course and semiquantitative changes of ICAM-1 mRNA expression and cellular infiltration from the very acute to subacute phases of MI in a permanent occlusion model using TNF-α KO mice to elucidate the effects of TNF-α and the involvement of ICAM-1.

Methods

Animals, Microsurgery and Tissue Preparation

This study was performed according to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). The experimental protocol was approved by The Animal Care and Use Committee of Showa University. The experiments were performed using male C57BL/6 WT and TNF-α KO mice, ranging in age from 6 to 8 weeks and ranging in body weight from 20 to 24 g. The WT mice were obtained from the Saitama Experimental Animal Center (Saitama, Japan) and the KO mice were produced by gene disruption in embryonic stem cells using homologous recombination, as described previously. The mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (100 mg/kg) and fixed in a supine position. The trachea was intubated with a 20 G intravenous catheter and ventilated with a mixture of oxygen and sevoflurane at a rate of 2 L/min. The stroke volume was 1.0 ml and the respiratory rate was 120 breaths/min. After a median sternotomy, the left anterior descending (LAD) coronary artery was identified and tied off. Occlusion of the LAD was confirmed by a change in the color of the involved LV wall and ST-segment elevation on the electrocardiograph monitoring. The chest was closed and the skin was sutured with 5-0 silk. The animals recovered on a heating pad for small animals at a temperature of 36°C. Sham-operated animals underwent a similar procedure without ligature of the LAD.

In experiment 1, WT (n=20) and KO mice (n=19) were monitored after surgery for mortality. Because technical failure to create MI causes acute death, any mice that survived at least 12 h after coronary ligation were included in the analysis.

In experiment 2, WT and KO mice were randomized into 3 groups of 12 mice each for euthanasia on days 1, 3 and 7 after surgery. The sham-operated animals were also randomized and killed at the same time points (n=6). The mice were deeply anesthetized with an overdose of injected pentobarbital sodium and a median sternotomy was performed. The hearts of 6 animals in each group were rapidly excised and used for histology and immunohistochemistry. The other 6 animals were used for reverse transcription-polymerase chain reaction (RT-PCR) analysis and immunohistochemistry. After the immediate separation of infarcted areas from non-infarcted areas, each area was stored in liquid nitrogen until the RT-PCR. For immunohistochemistry, the heart was immersed in 4% formalin and embedded in paraffin.

Echocardiography

Transthoracic echocardiography was performed before coronary ligation (baseline n=12) and before death at each time point, using an EUB-6000 ultrasound machine (HITACHI Medical Corp, Tokyo, Japan) with a 10-MHz phased array transducer under anesthesia with mechanical ventilation as mentioned earlier. The transducer was placed in gentle contact with the mid-precordial area through a transmission medium. M-mode echocardiograms, guided by 2-dimensional long-axis images, were obtained through the anterior and posterior LV walls at the level of the papillary muscles. The LV end-diastolic diameter (LVEDd), fractional shortening (FS), and the interventricular septal wall thickness in diastole (IVSTd) were measured from the M-mode tracing by the leading-edge method of the American Society for Echocardiography.

RT-PCR for ICAM-1

The RT-PCR analyses for ICAM-1 were performed as follows. Total RNA was extracted from the frozen samples by the modified acid guanidinium thiocyanate/phenol/chloroform method and the RNA concentration and purity were estimated spectrophotometrically, with the quality further assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Total RNA was subjected to RT-PCR analysis.

cDNA was synthesized from RNA as follows. Total RNA (1 μg), random hexamer (Applied Biosystems, Foster City, CA, USA), and the manufacturer’s recommended buffer were mixed and incubated at 95°C for 2 min, cooled to 37°C, and incubated with Superscript II (Invitrogen, Carlsbad, CA, USA), 10 mmol/L dithiothreitol, 0.5 mmol/L of each dNTP, and 20 U RNAsin (Promega, Madison, WI, USA) at 42°C for 50 min. Primer sequences for the PCR and the PCR method were performed as previously described.

Detection of Amplification Products by Microchip Electrophoresis

PCR product sizes and quantities were detected by the Agilent 2100 bioanalyzer (Agilent Technologies). This bioanalyzer sizes and quantitates 12 samples on a disposable microchip in approximately 30 min. Each glass microchip holds 16 wells interconnected via a network of microchannels filled with a gel-dye mix. Twelve of the 16 wells are used for experimental samples and 1 is used as a sizing standard (ladder). The chip was prepared and samples were loaded as recommended by the manufacturer. In brief, the microchannels were filled with 9 ml of gel-dye mix and the mixture was then forced into the channel network. Two additional buffer wells were also filled with the 9 ml gel-dye mix and the sample and ladder wells were filled with a 5 ml marker mix before the addition of a 1 ml DNA sizing ladder or sample. The prepared microchip was vortexed and placed into the bioanalyzer for analysis. All analyses were performed using Agilent biosizing software (Version A.02.12).

Immunohistochemistry

Paraffin-embedded sections of 5 μm thickness were stained with hematoxylin-eosin and Azan-Mallory for histopathological evaluation. Deparaffinized and hydrated sections were prepared for immunohistochemistry by a 10-
min incubation in 0.3% H2O2 in methanol to quench endogenous peroxidase activity, a 15-min rinse with phosphate-buffered saline, and an overnight incubation with each of primary antibodies (goat anti-human ICAM-1 polyclonal antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA), rabbit anti-nitrotyrosine antibody (Upstate, Lake Placid, NY, USA), goat anti-mouse myeloperoxidase (MPO) polyclonal antibody for neutrophils (Santa Cruz Biotechnology), rat anti-mouse Mac-3 monoclonal antibody for macrophages (BD Biosciences Pharmingen, San Jose, CA USA)). Immune complexes were detected with biotinylated goat antibodies to each host immunoglobulin G followed by avidin-biotin-horseradish peroxidase, then incubated for 5 min with 0.1% 3,3’-diaminobenzidine and 0.02% hydrogen peroxide. Negative control was obtained by replacing the primary antiserum with normal serum.

Morphometric Analysis of Invasive Neutrophils and Macrophages Into Myocardium

The number of neutrophils and macrophages invading the myocardium in each infarcted, non-infarcted and perifasciated area was manually counted in randomly selected high-power fields (×400) using immunostained sections of anti-MPO and anti-macrophage antibodies. Measurements were taken at 10 fields of each area in each animal (n=6 at each time point in both groups).

Statistical Analysis
Results are expressed as mean±SEM. A multiple Student’s t-test or a 1-way ANOVA was used for comparisons. Kaplan-Meier lifetime analysis was used for survival comparison between WT and KO mice. A value of p<0.05 was considered to be significant.

Results
Survival Rate
The survival rate for WT mice at 3 days after coronary ligation was 75.0%, compared with 84.2% of the KO mice. Survival rate at 7 days tended to be higher in the KO mice (73.7%) than in the WT mice (45.0%) (p=NS).

Echocardiography
The LVEDd was similar in the WT and KO mice at baseline (p=NS) (Table 1), and remained unchanged throughout the time course in the sham-operated mice of both groups (data not shown). At day 1 after MI, the LVEDd of the KO mice was slightly less than that of the WT mice, but by day 3, the difference had become significant (p<0.05). Thereafter, the LVEDd gradually increased and although it still

Table 1  Echocardiographic Data

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<th>Baseline</th>
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<tr>
<td></td>
<td>WT</td>
<td>KO</td>
<td>WT</td>
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<tr>
<td>LVEDd (mm)</td>
<td>3.2±0.22</td>
<td>3.4±0.21</td>
<td>4.0±0.16*</td>
<td>3.8±0.21</td>
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<td>FS (%)</td>
<td>34.1±2.1</td>
<td>32.8±1.7</td>
<td>14.8±1.0**</td>
<td>18.8±1.5**</td>
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<tr>
<td>IVSTd (mm)</td>
<td>0.68±0.07</td>
<td>0.66±0.08</td>
<td>0.64±0.06</td>
<td>0.65±0.08</td>
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WT, wild type; KO, knockout; LVEDd, left ventricular end-diastolic diameter; FS, fractional shortening; IVSTd, interventricular septum wall thickness in diastole.

*p<0.05, **p<0.01 vs corresponding baseline; †p<0.05, ‡p<0.01 vs WT.

Fig 1. Representative macroscopic photographs of transverse sections of the hearts of WT and KO mice at 3 and 7 days after myocardial infarction (H&E). WT, wild type; KO, tumor necrosis factor-α knockout. Bar=1.0 mm.
fell short of its peak in both groups at 7 days, the significant difference between the groups remained (p<0.01). The LVEDd remained significantly higher than the corresponding baseline values in the WT mice from 1 to 7 days (day 1 p<0.05; day 3, 7 p<0.01) and in the KO mice from day 3 to 7 (p<0.05).

Likewise, FS was similar between the WT and KO mice at baseline (p=NS), and remained unchanged throughout the time course in the sham-operated mice of both groups (data not shown). At day 1 after MI, the FS was significantly higher in the KO mice than in the WT mice (p<0.05) and it gradually decreased thereafter in both groups. It did not reach its minimum by day 7 in either groups; however, the significant difference between the groups was maintained (p<0.01). The FS remained significantly lower than the corresponding baseline values from day 1 to 7 in both groups (p<0.01).

The IVSTd was also similar between the WT and KO mice at baseline (p=NS), and remained unchanged throughout the time course in the sham-operated mice of both groups (data not shown). At day 1 after MI, the IVSTd of the KO mice was still similar to that of the WT mice (p=NS), but by 3 days the IVSTd of the KO mice was significantly greater than that of the WT mice (p<0.05). The IVSTd decreased gradually thereafter in both groups and although it did not reach its minimum by day 7 in either groups, the significant difference between the groups was maintained (p<0.05). The IVSTds remained significantly lower than the corresponding baseline values from day 3 to 7 in both the WT mice (p<0.01) and the KO mice (p<0.05).
Macroscopic Examination

The LV cavity was slightly dilated in both groups at day 1 after MI, but the thickness of the LV wall was almost the same as that of the controls. By day 3, the wall had thinned in both groups and the LV cavity was dilated. By day 7, the LV cavity had dilated more in both groups and wall thinning was more apparent than it had been after 3 days (Fig 1A).

RT-PCR for ICAM-1

The ICAM-1 mRNA expression in the control hearts was similar between the WT and KO mice (WT: 0.14±0.02%; KO: 0.20±0.03%; p=NS) (Fig 2). The ICAM-1 mRNA in the infarcted area of the WT mice peaked on day 1 and gradually decreased thereafter, whereas in the infarcted area of the KO mice it peaked on day 3. The ICAM-1 mRNA in the infarcted area of the KO mice was significantly lower than that of the WT mice at day 1 after MI (WT: 1.62±0.5%; KO: 0.38±0.1%; p<0.01), and the significant difference remained at day 3 and 7 (p<0.05). The ICAM-mRNA in the non-infarcted area of the WT mice peaked on day 7 and differed significantly from that observed in the KO mice at the same time point (WT: 0.55±0.2%; KO: 0.15±0.04%; p<0.05).

Immunohistochemistry

The expression of ICAM-1 was very low in both groups at day 1 after MI (data not shown). At day 3, the expression in the WT mice was localized to infiltrating inflammatory cells and some cardiomyocytes in the peri-infarct and infarcted areas (Fig 3A). The expression was much weaker in the KO mice (Fig 3B). The expression weakened in both groups at day 7, and the localization in expression was almost same at day 3 (data not shown).

The expression of 3-nitrotyrosine, a marker of peroxynitrite, was very low in both groups at day 1 after MI (data not shown) and by day 3, the expression in the WT mice was localized to infiltrating inflammatory cells, presumably neutrophils, cardiomyocytes, and capillary endothelial cells of the peri-infarct and infarcted areas (Fig 4A). The expression was weaker in the KO mice (Fig 4B). The expression weakened in both groups at day 7 and the localization in expression was almost same at day 3 (data not shown).

Morphometric Analysis of Invasive Neutrophils and Macrophages Into Myocardium

Many inflammatory cells were observed in the infarcted and peri-infarcted areas at day 1 after MI, and most of those were positively stained with MPO (Fig 5A). The MPO-positive cells had decreased in number by day 3, but the number of macrophage-positive cells had increased. The macrophages were further increased in number by day 7 (Fig 6A), whereas the MPO-positive cells had almost entirely disappeared.

The results of the manual counts of invasive neutrophils are shown in Fig 5B. Invasive neutrophils were observed in both groups at day 1, and there were significant differences in the numbers counted in the infarcted (WT: 78.2±5.2 positive cells/HPF; KO: 54.0±8.1 positive cells/HPF; p<0.01) and peri-infarcted areas (WT: 43.3±3.3 positive cells/HPF; KO: 33.5±4.1 positive cells/HPF; p<0.05). There were significant differences between WT and KO mice in both the infarcted and peri-infarcted areas at day 3 (infarcted area: WT 55.4±3.4 positive cells/HPF; KO: 44.1±4.1 positive cells/HPF; p<0.01).
The cell counts had decreased in the infarcted and peri-infarcted areas by day 7 and no significant difference was observed between the groups.

The results of the manual counts of invasive macrophages are shown in Fig 6B. There were fewer macrophages invading the infarcted area than in the peri-infarcted area in both groups at day 1. The macrophages had started to invade the myocardium by day 3. There were significantly more invading macrophages in the WT mice than in the KO mice in both the infarcted and peri-infarcted areas (infarcted: WT 23.7±2.0 positive cells/HPF; KO 19.3±1.5 positive cells/HPF, p<0.05; peri-infarcted: WT 10.6±2.4 positive cells/HPF; KO 7.3±3.3 positive cells/HPF, p<0.05). The differences between the groups widened further in both the infarcted and peri-infarcted areas at day 7 (infarcted: WT 41.5±3.5 positive cells/HPF; KO 27.9±2.0 positive cells/HPF, p<0.01; peri-infarcted: WT 13.7±4.3 positive cells/HPF; KO 8.4±4.0 positive cells/HPF, p<0.05) (Figs 6A,B).

Discussion

In this study, echocardiography clearly demonstrated better cardiac function in TNF-α KO mice than in the WT mice after experimental acute MI. The infiltration of inflammatory cells and expression of ICAM-1 mRNA were both significantly lower in the KO than in WT mice. Furthermore, the immunohistochemical expression of ICAM-1 and 3-nitrotyrosine in the infarcted myocardium was weaker in the KO than in WT mice. This study is the first to demonstrate that TNF-α contributes to myocardial dysfunction and the progression of remodeling in mice after MI through an inflammatory reaction via ICAM-1 expression and the subsequent production of NO. No earlier studies demonstrate neutrophil and macrophage infiltration from a very early stage to subacute stage in a permanent occlusion model of MI.

**TNF-α and ICAM-1**

Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes. Neutrophil migration and adhesion to cardiac myocytes are mediated mainly by the interaction between neutrophil Mac-1 (CD11b/CD18) and cardiac myocytes.
been demonstrated as compared to WT mice.\textsuperscript{30}

**TNF-\(\alpha\) and NO**

TNF-\(\alpha\) can produce LV dysfunction\textsuperscript{31} and the negative inotropic effects of TNF-\(\alpha\) responsible for LV dysfunction are mediated through myocardial NO synthase.\textsuperscript{32} In in-vitro experiments, cardiac myocytes have been found to produce inducible NO synthase within several hours of treatment with TNF-\(\alpha\).\textsuperscript{33} After MI, TNF-\(\alpha\) is induced by invading inflammatory cells and the infiltrated and non-infarcted cardiac myocytes produce a high level of NO and NO products, such as peroxynitrite, especially in the infiltrated and border zone areas.\textsuperscript{34,35} The concentrations of NO and TNF-\(\alpha\) in patients with congestive heart failure increase in proportion to the severity of heart failure, indicating a role of TNF-\(\alpha\) in enhanced systemic and local production of NO.\textsuperscript{36} Our study is the first to demonstrate weaker expression of 3-nitrotyrosine in infarcted myocardium in KO mice than in WT mice, and this attenuation of 3-nitrotyrosine expression in the KO mice is at least partly attributed to less inflammatory cells invading after MI. Our study results therefore suggest that TNF-\(\alpha\) induces NO in the invading cells and myocardium, and thereby ultimately contributes to the LV dysfunction after MI.

**TNF-\(\alpha\) and Myocardial Remodeling**

LV remodeling occurring after AMI still limits the long-term prognosis and remains an important problem despite developments in coronary interventions\textsuperscript{37} and pharmacological therapy.\textsuperscript{38} In a rat model TNF-\(\alpha\) mRNA was upregulated in both the infarcted and non-infarcted areas in the early stage after MI, but remained upregulated in the non-infarcted area for nearly 28 days.\textsuperscript{39} This expression of the TNF-\(\alpha\) gene in the non-infarcted area suggests that the cytokine may be involved in the signaling process leading to myocardial remodeling. A study by Sun et al demonstrated that LV chamber dilatation and decreased LV developed pressure were less in TNF-KO mice than in WT mice at 28 days after coronary ligation,\textsuperscript{40} and in the present study the LVEDd was significantly smaller in the KO mice than in the WT mice at each time point after MI. It thus appears that LV remodeling was more strongly inhibited in the KO mice than in the WT mice.

The early inflammatory response induced by ICAM-1 may have long-term consequences in ventricular function and remodeling. Expression of ICAM-1 mRNA in the WT mice peaked on day 1 after MI and thereafter decreased in the infarcted area until day 7, which almost corresponded to the time course of the number of infiltrating neutrophils. On the other hand, the mRNA expression of ICAM-1 in the infarcted area and the number of neutrophils infiltrating both the infarcted and peri-infarcted areas were inhibited in the KO mice. TNF-\(\alpha\) could be responsible for the induction of ICAM-1 and subsequent infiltration of the neutrophils to the infarcted myocardium; however, the mRNA expression of ICAM-1 in the non-infarcted area peaked at day 7, suggesting that prolonged expression of ICAM-1 in the non-infarcted area may play a role in myocardial remodeling. The expression of ICAM-1 mRNA in the non-infarcted area of the KO mice was the same from day 1 to 7 after MI.

**Conclusions**

TNF-\(\alpha\) decreased cardiac function and ameliorated myocardial remodeling through the induction of ICAM-1 and NO in a permanent occlusion model of AMI. Anti-TNF-\(\alpha\) therapy may have beneficial effects after AMI.

**Acknowledgments**

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**References**