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Excessive Tumor Necrosis Factor Activation After Infarction Contributes to Susceptibility of Myocardial Rupture and Left Ventricular Dysfunction

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Background—We investigated the potential contributions of tumor necrosis factor-α (TNF-α) on the incidence of acute myocardial rupture and subsequent chronic cardiac dysfunction after myocardial infarction (MI) in TNF knockout ($TNF^{-/-}$) mice compared with C57/BL wild-type (WT) mice.

Methods and Results—Animals were randomized to left anterior descending ligation or sham operation and killed on days 3, 7, 14, and 28. We monitored cardiac rupture rate, cardiac function, inflammatory response, collagen degradation, and net collagen formation. We found the following: (1) within 1 week after MI, 53.3% (n=120) of WT mice died of cardiac rupture, in contrast to 2.5% (n=80) of $TNF^{-/-}$ mice; (2) inflammatory cell infiltration and cytokine expression were significantly higher in the infarct zone in WT than $TNF^{-/-}$ mice on day 3; (3) matrix metalloproteinase-9 and -2 activity in the infarcted myocardium was significantly higher in WT than in $TNF^{-/-}$ mice on day 3; (4) on day 28 after MI compared with sham, there was a significant decrease in LV developed pressure (74%) and $\pm dP/dt_{max}$ (68.3%/65.3%) in WT mice but a less significant decrease in $\pm dP/dt_{max}$ (25.8%/28.8%) in $TNF^{-/-}$ mice; (5) cardiac collagen volume fraction was lower in WT than in $TNF^{-/-}$ mice on days 3 and 7 but higher on day 28 compared with $TNF^{-/-}$ mice; and (6) a reduction in myocyte apoptosis in $TNF^{-/-}$ mice occurred on day 28 compared with WT mice.

Conclusions—Elevated local TNF-α in the infarcted myocardium contributes to acute myocardial rupture and chronic left ventricle dysfunction by inducing exuberant local inflammatory response, matrix and collagen degradation, increased matrix metalloproteinase activity, and apoptosis. **(*Circulation*. 2004;110:3221-3228.)**

Key Words: myocardial infarction ■ tumor necrosis factor ■ metalloproteinases ■ remodeling

After acute myocardial infarction (MI), a host of molecular, cellular, and physiological responses are triggered in the heart in response to injury, which can lead to acute myocardial wall rupture, life-threatening arrhythmias, and, in survivors, remodeling and transition to heart failure. Even though the incidence of myocardial rupture after infarction is now likely much lower than the previously published rates of 5% to 31%, it remains a serious acute complication after infarction.[1] Chronically after MI, heart failure has an escalating cardiovascular burden worldwide, and the 1-year mortality remains high at 25% to 40%.[2]

Accumulated evidence indicates that cytokines are important mediators of wound healing and remodeling after MI. They are elaborated by immune, vascular, and interstitial tissues to regulate important biological processes of cell growth, migration, repair, and fibrosis. Tumor necrosis factor-α (TNF-α) is secreted primarily by myocytes and macrophages after injury.[3] TNF binds to cell surface receptors and triggers intracellular signaling cascades that can result in activation of transcription factors such as nuclear factor-κB (NF-κB) or death domain effectors such as caspases. They modulate host cell gene expression[4] and can amplify inflammation, facilitate growth and survival, or promote apoptosis. TNF also has direct effects on the matrix and collagen framework and is a potential major contributor to cardiac remodeling.[5]

In this study we tested the hypothesis that elevation of local TNF after MI in the susceptible host contributes to acute cardiac rupture and chronic heart failure after MI. Using a well-established model of experimental MI in TNF knockout ($TNF^{-/-}$) mice, we monitored the rate of acute cardiac rupture and chronic ventricular function. We studied these events in

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The online-only Data Supplement, which contains additional information about Methods and additional figures, can be found with this article at http://www.circulationaha.org.

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reference to local inflammation, matrix alterations, and rates of apoptosis.

Methods

Animals

Left ventricular (LV) MI was created in 12-week-old male TNF$^{-/-}$ and wild-type (WT) mice (C57BL/6-TNFtm1Gk1, Jackson Laboratory) by left anterior descending coronary artery ligation, as previously described by our laboratory.[6]

In experiment 1, TNF$^{-/-}$ (n=80) and WT mice (n=120) underwent coronary artery ligation and were monitored rigorously for morbidity and mortality. After death in each animal, autopsy was immediately performed to determine the cause of death, particularly with reference to cardiac rupture.

In experiment 2, TNF$^{-/-}$ and WT mice were randomized into sham-operated controls (n=10) or infarction group, with preassigned euthanasia on postoperative days 3, 7, 14, and 28 (n=10 surviving animals per time point). Hearts were harvested, rinsed with PBS, frozen, and stored at −80°C until analysis.

In experiment 3, TNF$^{-/-}$ and WT mice with MI (n=10 per group) or sham (n=10) were killed on day 28. Hearts were collected to determine cardiac function by the Langendorff technique.

For details regarding methodology of evaluation of LV function, cardiac morphometry, Western blot analysis, and in situ hybridization, please refer to the online-only Data Supplement.

Immunohistochemistry and Collagen Content

Cryostat sections (4 μm) were cut from hearts and immunolabeled with antibodies recognizing CD45 (BD PharMingen), matrix metalloproteinase (MMP)-9 (Chemicon), and NF-κB p65 (Santa Cruz). Protocol and tests for specificity are described elsewhere in detail.[6] Quantification of immunoreactive cells (20 optical fields) was done with the use of a Quantimet 600 image analysis system (Leica).

To determine collagen content, sections (6 μm thickness) were cut and stained with sirius red 3BA in saturated picric acid solution.[7] With the use of an image analysis system (Leica Q500, Leica Imaging Systems), these sections were analyzed morphometrically. Fibrillar collagen was identified in the picrosirius-stained sections by its red appearance.

MMP Zymography

Extracted proteins in nonreducing conditions were mixed with matched volume of sample buffer (Invitrogen Novex), and equal amounts of protein (20 μg) were loaded in each lane of 10% zymogram gelatin gels (Novex). After electrophoresis, the gel was incubated in 2.5% Triton-X 100 and further incubated for 16 hours in 50 mmol/L Tris-HCl buffer, pH 7.5, containing 200 mmol/L NaCl and 10 mmol/L $CaCl_2$ at 37°C, and the gels were stained with Coomassie blue and destained in 30% methanol/10% acetic acid. White bands on a blue background indicated zones of digestion corresponding to the presence of specific MMPs identified on the basis of their molecular weight. Bands were scanned (GS-700 Bio-Rad), and quantification was performed with the use of Multi-analyst software(Bio-Rad).

Apoptosis Index Determination

The terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay was used to monitor the extent of DNA fragmentation as a measure of apoptosis in cryostat sections. The assay was performed according to the recommendations of the manufacturer (Boehringer Mannheim). Fluorescein-conjugated dUTP incorporated in nucleotide polymers was detected and quantified by fluorescence microscopy. TUNEL-positive nuclei were distinguished from the TUNEL-negative nuclei by counterstaining with Hoechst 33258 and were counted after being photographed. The count was done blindly. The percentage of nuclei labeled by TUNEL per unit of cells stained with Hoechst nuclear dye reflected the apoptotic index.

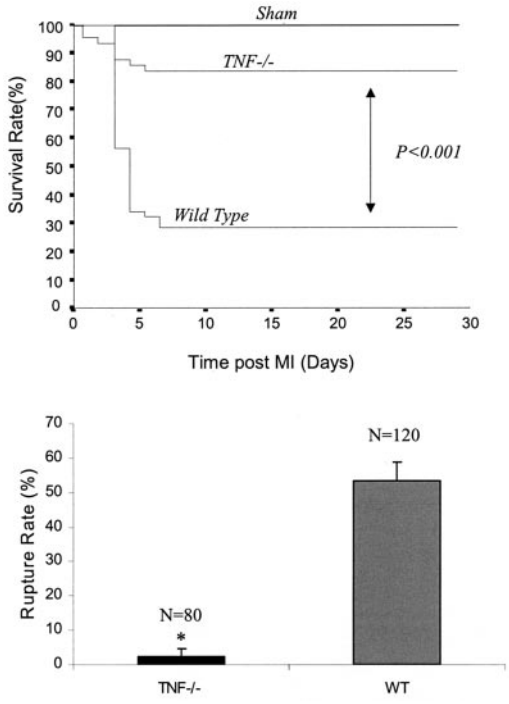

Figure 1. A, Kaplan-Meier survival curves in WT (n=120) and TNF$^{-/-}$ (n=80) mice after MI. *$P<0.001$ vs WT. B, Rupture rate of mice. *$P<0.001$ vs WT.

Statistical Analysis

Statistical analysis of cardiac rupture rate, cardiac function, in situ hybridization, MMP activity, collagen content findings, and cell number was performed with the use of ANOVA with subgroup testing. Values are expressed as mean±SEM, with $P<0.05$ considered significant.

Results

Contribution of TNF in Acute Phase After MI

TNF$^{-/-}$ Improved Survival Rate and Decreased Cardiac Rupture

Ligation of the left anterior descending coronary artery produced large infarcts averaging 45±6.4% of LV wall in both WT and TNF$^{-/-}$ mice. Survival rate after MI was compared between WT and TNF$^{-/-}$ mice. In WT mice, 31.6% survived within the first week after MI, whereas 84% of TNF$^{-/-}$ mice survived (Figure 1A). Among the WT mice, 53.3% died of fatal cardiac rupture of the LV wall (accounting for 95% of the acute deaths), in contrast to only 2.5% of TNF$^{-/-}$ mice that died of cardiac rupture ($P<0.05$) (Figure 1B).

TNF$^{-/-}$ Reduced Tissue Inflammation

As shown in Figure 2A, nuclear immunolocalization of activated NF-κB was not observed in sham-operated WT and TNF$^{-/-}$ hearts (a, c). However, after MI, inflammatory cells, interstitial cells, and myocytes were positively labeled by antibody against activated NF-κB (b, d) in the infarcted myocardium in WT mice on day 3. In contrast, a significant reduction (47%) in the immunostaining of activated nuclear NF-κB ($P<0.01$) was observed in the day 3 infarcted myocardium of the TNF$^{-/-}$ mice (Figure 2B).

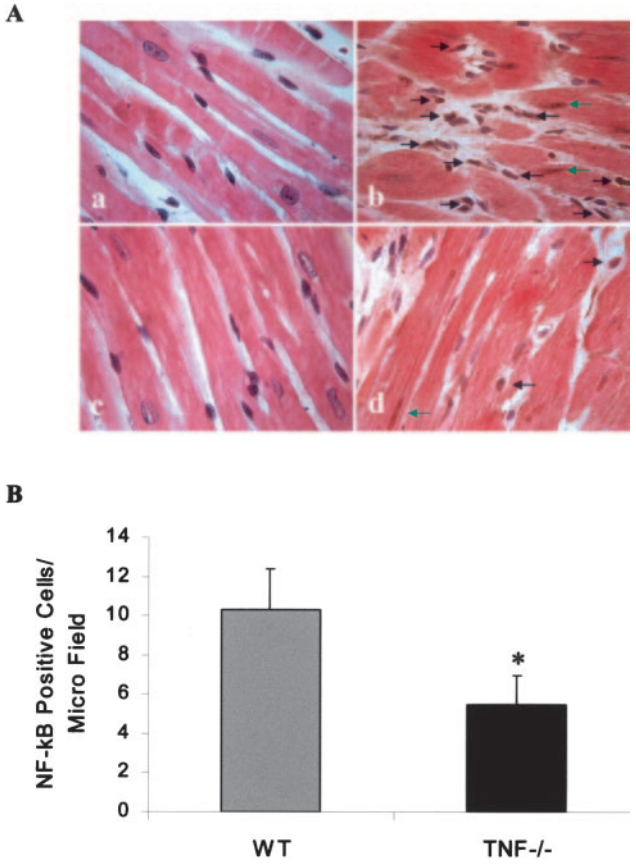

Figure 2. A, Activated NF-κB expression in hearts of WT (a, b) and TNF$^{-/-}$ (c, d) mice. No NF-κB–positive cells were found in sham-operated hearts (a, c); b and d show NF-κB expression in infarcted hearts in inflammatory cells, interstitial cells (black arrow), and myocytes (green arrow). Magnification ×1000. B, Quantification of NF-κB–positive cells. *P<0.01 vs WT.

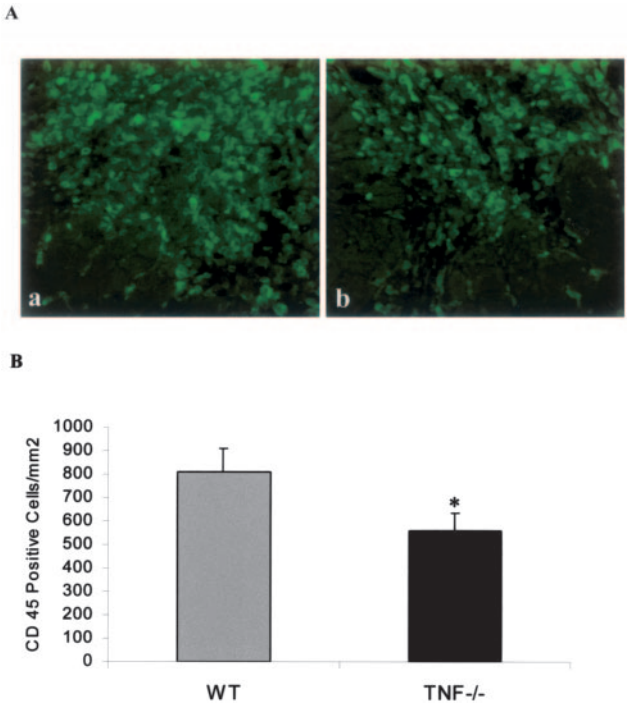

Figure 3. Accumulation of CD45-immunoreactive leukocytes in infarcted hearts. A, WT (a) and TNF$^{-/-}$ (b) mice hearts (magnification ×400). B, Quantification of leukocytes in infarcted myocardium on day 3 after MI. *P<0.05 vs WT.

Inflammatory cytokines interlukin-1β (IL-1β) and interlukin-6 (IL-6) were detected by Western blot analysis (Figure I online). The relative levels of IL-1β and IL-6 proteins in the infarcted myocardium increased on days 3 and 7 in both groups (Figure IA). Between WT and TNF$^{-/-}$ mice, IL-1β and IL-6 expression in TNF$^{-/-}$ mice was significantly decreased by 36% (IL-1β) and 50% (IL-6) at day 3 and 54% and 32% at day 7, respectively (P<0.01) (Figure IB).

Representative immunofluorescent images of tissue sections from WT and TNF$^{-/-}$ mice stained for CD45 (Figure 3A) indicated a large inflammatory infiltrate of cells not only in the infarcted zone but also in the noninfarcted region. In contrast, no evidence of inflammatory cell infiltrate was observed in the noninfarcted region of the TNF$^{-/-}$ mice (data not shown). Inflammatory cell infiltration was observed in the infarcted myocardium of both WT and TNF$^{-/-}$ mice at day 3 after MI. Compared with WT mice, the number of inflammatory cells in TNF$^{-/-}$ mice was decreased by 31% (P<0.05) (Figure 3B).

TNF$^{-/-}$ Decreased MMP Expression and Activation

As shown by immunofluorescence microscopy (Figure 4A), the number of MMP-9–positive cells was predominately localized to the peri-infarct and infarcted regions in both WT and TNF$^{-/-}$ mice. However, the prevalence of MMP-9–positive cells was reduced in TNF$^{-/-}$ mice (b) compared with WT mice (a). This was concordant with zymography data that indicated that MMP activity was increased in the infarcted heart on day 3 and peaked before cardiac rupture. Compared with the WT sham group, which displayed low levels of cardiac MMP-9, MMP-9 activity was significantly increased in the peri-infarct, infarcted, and noninfarcted myocardium of WT mice with MI. In TNF$^{-/-}$ mice with MI, MMP-9 activity was significantly less (44%) than that in WT mice (P<0.05) (Figure 4B, 4C). The changes in MMP-2 activity were not statistically different between TNF$^{-/-}$ and WT mice.

Collagen mRNA Expression

Type I collagen mRNA level was assessed by quantitative in situ hybridization (Figure II online). Low-level type I collagen gene expression was observed in the normal myocardium. After MI, type I collagen mRNA was markedly increased at the site of MI on day 3, reached a peak at day 7, and declined thereafter but remained higher than controls in both WT and TNF$^{-/-}$ mice. No significant difference in type I collagen mRNA was observed at the infarct site between WT and TNF$^{-/-}$ mice at all time points evaluated (Figure IIB).

TNF$^{-/-}$ Protected Against Collagen Degradation

The picrosirius red staining revealed that collagen fraction was decreased at the infarct site at day 3 after MI in both WT and TNF$^{-/-}$ groups compared with sham groups. The total collagen fractions were 2.3±1.2% in WT, 4.3±0.6% in TNF$^{-/-}$, and 6.6±2.1% in sham-operated mice. The infarcted WT myocardium at days 3 and 7 after MI had a collagen

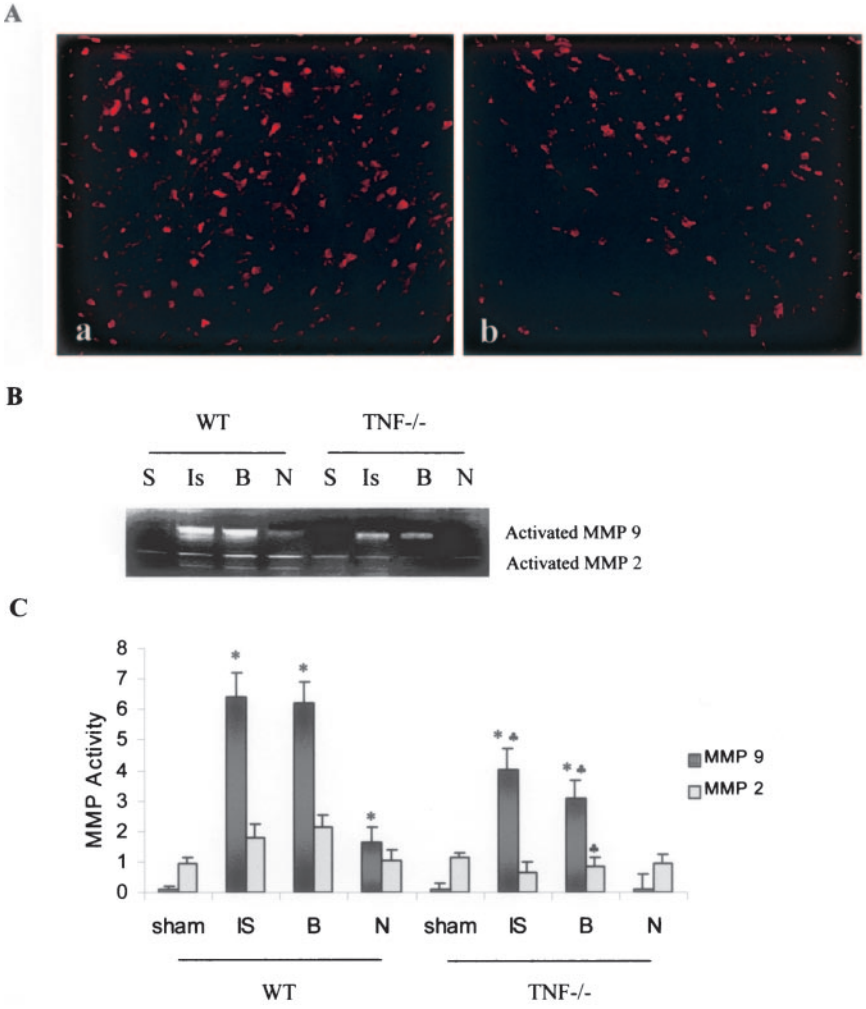

Figure 4. MMP expression and activation. A, MMP-9 expression in infarct border zone (magnification ×200) at day 3 in both groups (a, WT; b, $TNF^{-/-}$). B, Gelatin zymography of extracts from sham (S), infarct (Is), infarct border (B), and noninfarcted region (N) showed significantly increased MMP-9 and MMP-2 activity in WT compared with $TNF^{-/-}$ infarcts. C, Quantitative summary. *$P<0.001$ vs sham operated; ♣$P<0.005$ vs WT.

fraction that was significantly less than that in $TNF^{-/-}$ mice (Figure 5A, 5B).

Contribution of TNF in Chronic Phase After MI

LV Morphology

There were no apparent differences in the infarct size of both WT and $TNF^{-/-}$ groups at day 3 or 28 (Figure 6A). Furthermore, no apparent differences in infarct/circumferential fiber length were observed among the groups. Conversely, the LV circumferential length and diameter of WT mice tended to be increased compared with $TNF^{-/-}$ mice. The septal thickness in WT mice showed a significant decrease compared with $TNF^{-/-}$ mice (Figure 6B).

$TNF^{-/-}$ Improved Cardiac Function

On day 28 after MI, cardiac function, including LV peak pressure and $\pm dP/dt$, were assessed in the isolated heart (Figure 6C). In WT and $TNF^{-/-}$ animals after MI, there was a highly significant decrease in $\pm dP/dt_{max}$ (68.3%/65.3%) in WT mice compared with sham controls, but this was less apparent in $TNF^{-/-}$ mice (25.8%/28.8%). There was also a significant reduction in LV peak pressure (65.3%; $P<0.05$) in WT mice, with much less reduction (25.8%) in $TNF^{-/-}$ mice.

$TNF^{-/-}$ Prevented Collagen Deposit in Noninfarcted Region After MI

In both groups, collagen continued to accumulate at the site of infarction from weeks 1 to 4 after MI. In the infarct zone itself, the collagen deposition was not different between the 2 groups. However, increased collagen deposition was observed in WT myocardium remote from the site of infarction, including the septum, right ventricle, endocardium, and pericardium. In WT mice, multiple patchy foci of fibrosis were observed on microscopy in the remote zone, but this was conspicuously absent in $TNF^{-/-}$ mice (Figure 5A, b, d). Quantitative analysis also showed increased collagen volume in the noninfarcted region ($P=0.065$) in WT mice compared with $TNF^{-/-}$ mice (Figure 5B).

$TNF^{-/-}$ Reduced Apoptosis After MI

There was no difference in the percentage of TUNEL-positive apoptotic cells observed in the infarcted zone of $TNF^{-/-}$ mice compared with WT. However, in the noninfarcted zone, as shown in Figure 7, a significant decrease in the number of apoptotic cells was observed in $TNF^{-/-}$ mice at day 28 compared with WT animals. This is consistent with the improved ventricular function in the $TNF^{-/-}$ mice.

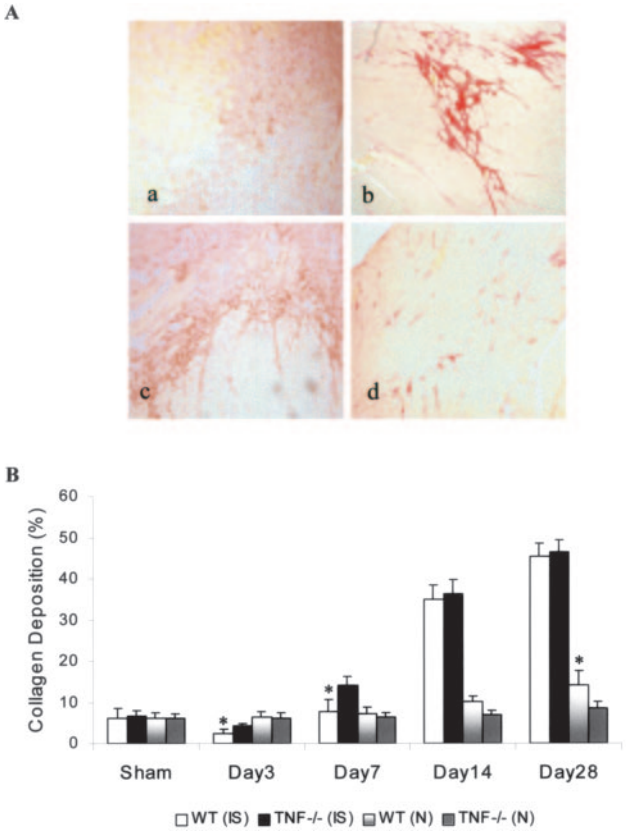

Figure 5. A, Picrosirus red staining for collagen in WT (a, b) and $TNF^{-/-}$ (c, d) hearts. Collagen deposition is seen in infarct region of both WT and $TNF^{-/-}$ mice (a, c) at day 7. At day 28, microscopic evidence of patchy fibrosis was observed in a remote region of WT (b) but not in $TNF^{-/-}$ mice (d). Magnification ×400. B, Quantitative collagen volume fraction in various groups. IS indicates infarcted; N, noninfarcted. *$P<0.05$ vs $TNF^{-/-}$.

Discussion

Cardiac rupture is an acute fatal complication in the early days after MI. Rupture may occur in up to 30.7% of non–hospital-based MI patients according to autopsy.[8] Hypertension, cardiac hypertrophy, and infarct expansion are predisposing factors. Previous basic studies suggested that inhibition of plasminogen activators, MMPs,[9] or cardiac overexpression of FrzA can prevent cardiac rupture,[10] whereas deletion of angiotensin AT_2 receptor can aggravate it.[11] However, the upstream regulators of the genes that trigger these responses have not been studied. TNF-α is a master cytokine that is produced in significant quantities within the infarcted myocardium very soon after MI.[12,13] TNF-α in turn actives other cytokines such as IL-1 and IL-6 and orchestrates the host tissue response to acute injury.[12,14,15]

In the present study we investigated whether TNF directly contributes to cardiac rupture and cardiac dysfunction after MI. In C57/BL WT mice, acute MI resulted in LV free wall rupture at a very high frequency (53.3%) and was associated with high levels of myocardial TNF-α. In contrast, in mice lacking the TNF-α gene with inability to produce tissue TNF-α, the cardiac rupture rate was markedly reduced to only 2.5%. These findings suggest that TNF-α is a potentially major contributor to acute cardiac rupture after infarction.

Cardiac repair after infarction is a highly complex process, involving diverse inflammatory and growth factor signaling pathways, extracellular matrix remodeling, and cell deaths and possibly progenitor cell mobilization and proliferation. In addition to intrinsic cardiac TNF-α production, inflammatory cells such as monocyte-derived macrophages release large quantities of cytokines.[4,15,16] Previous studies have demonstrated that cardiac-specific expression of TNF-α results in myocardial inflammation, cardiac hypertrophy, progressive dilatation, and increased apoptosis, which lead to heart failure and death.[17] Our study demonstrates that in a mouse model in which the TNF-α gene has been ablated, the extent of local leukocyte and macrophage infiltration in the infarcted myocardium is significantly reduced. This is consistent with a reduced expression of IL-6 in infarcted myocardium in $TNF^{-/-}$ mice, in which IL-6 mediates transendothelial migration and adhesion of neutrophils to infarct region.[9]

Inflammatory cell infiltration in turn facilitates the expression and activation of MMPs.[9] The latter is believed to play an important role in balancing extracellular matrix production and degradation in the myocardium. MMPs contribute to the degradation of the resident collagen network in the infarcted myocardium within days after MI. Intriguingly, our MMP-9 data indicate that it was localized mainly to leukocytes, supporting the notion that infiltrating inflammatory cells are an important source of MMPs in the infarcted myocardium. Other types of cells can also synthesize MMPs.[18] $TNF^{-/-}$ mice expressed much lower levels of MMPs before cardiac rupture, along with a lower expression of IL-1.[19]

Myocardial fibrillar collagen content depends on the balance between collagen synthesis and degradation. Type I collagen accounts for 85% of total collagen in the myocardium.[20] Type I collagen gene expression was activated on day 3, and collagen deposition was evident on day 7. Cardiac rupture occurs mainly on days 4 and 5 after MI and therefore is primarily associated with MMP-induced collagen degradation. This concept is supported by a recent study by Peuhkurinen et al,[21] who demonstrated that increased matrix degradation or a block in collagen synthesis was associated with an increased risk of cardiac rupture in patients. In our study there was no actual significant difference in collagen gene expression in the infarcted myocardium within the first week after MI between WT and $TNF^{-/-}$ mice. These findings suggest that overexpression and activities of MMPs induced by TNF at the site of MI may increase the degradation of existing collagen in the early stage of infarction, contributing to cardiac rupture. After week 1, newly formed collagen at the site of MI protects the heart from further cardiac rupture.

Early inflammatory response may have long-term consequences in ventricular function and remodeling. Our study indeed demonstrated that after MI, LV function in $TNF^{-/-}$ mice was significantly improved compared with WT mice. Morphometry also confirmed less LV chamber dilatation in the $TNF^{-/-}$ mice but no difference in infarct size. Potential mechanisms may include less apoptosis and fibrosis in the remote zone secondary to less inflammatory cytokine activation. We were surprised to see numerous patches of fibrosis in the noninfarcted myocardium at day 28 in the WT mice. Increased collagen deposition in the interstitium of the

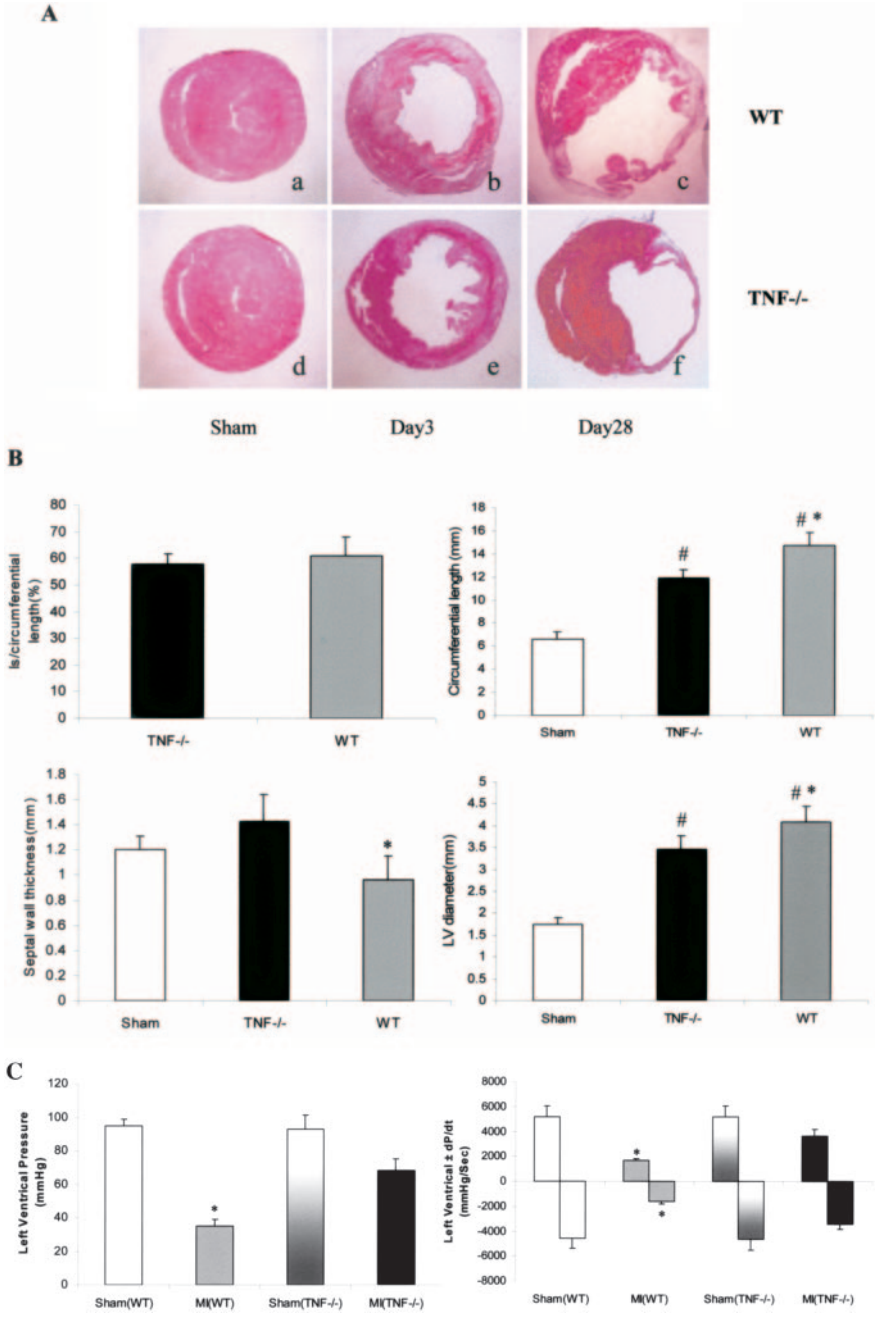

Figure 6. A, Heart morphology in WT (a, b, c) and TNF$^{-/-}$ (d, e, f) mice. Photomicrographs of sham (a, d), day 3 (b, e) and day 28 (c, f) transverse sections are shown. B, Morphometric quantification in percentage of infarcted (Is)/circumferential length, circumferential length, septal wall thickness, and LV diameter in WT and TNF$^{-/-}$ mice at day 28 after MI. #$P<0.05$ vs sham; *$P<0.05$ vs TNF$^{-/-}$. C, Quantitative analysis of LV peak pressure and dP/dt_{max}, dP/dt_{min} at day 28 after MI in both WT and TNF$^{-/-}$mice (n=15 per group). *$P<0.001$ vs TNF$^{-/-}$.

noninfarcted region may contribute to changes in ventricular compliance, resulting in increased stiffness and altered LV performance. These findings suggest that TNF-α–induced inflammatory responses stimulate diffuse myocardial fibrosis beyond the site of infarction, contributing to chronic ventricular dysfunction.

The aforementioned observation adds to currently existing understanding of the mechanisms by which TNF-α can directly depress cardiac function or indirectly depress it via nitric oxide pathways. Previous studies have demonstrated that TNF-α can directly decrease calcium release within the myocytes, possibly mediated by sphingomyelin pathways.[22,23] Indirect myocardial depression via upregulation of the inducible form of nitric oxide synthase[24,25] can in turn induce desensitization of myofilaments to intracellular calcium,[26] as well as modulate the contractile response to adrenergic stimulation.[27] These may be initially protective mechanisms attempting to decrease the amount of mechanical work output by the heart during acute MI. However, chronically this could increase wall stress, leading to further TNF-α production and secondary matrix and myocyte remodeling and aggravated mechanical dysfunction.[28]

A final contributing mechanism to the ventricular dysfunction may be the increased incidence of myocyte apoptosis in WT mice. In our study apoptotic myocytes were observed in the remote, noninfarcted myocardium but were seen very rarely in TNF$^{-/-}$ mice. The observation that TNF-$\alpha^{-/-}$ reduced the incidence of apoptosis in ventricular myocytes after infarction, despite evidence elsewhere,[29] was unexpected and counterintuitive. This suggests that TNF-α likely activates

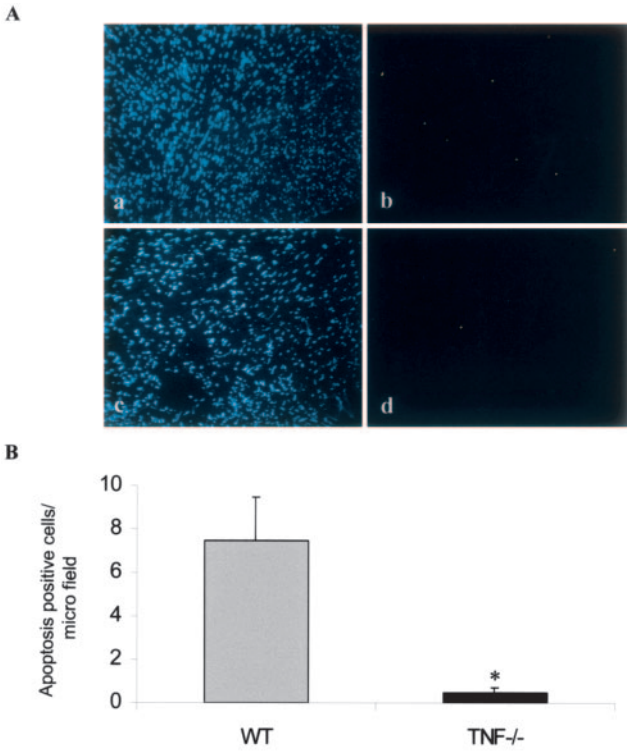

Figure 7. A, TUNEL analysis of myocardium from remote zone of WT (a, b) and TNF$^{-/-}$ mouse (c, d) heart. Nuclear staining is shown (a, c). Apoptotic nuclei of cardiomyocytes are indicated by green color (b, d) (magnification ×200). B, Number of apoptotic cardiomyocytes in remote zone of hearts from WT and TNF$^{-/-}$ mice at day 28 after MI. *P<0.001 vs WT.

dual signaling cascades in a cell-specific manner, with one pathway leading to apoptosis, whereas another pathway, mediated through NF-κB, suppresses apoptosis.[30] Our studies showed that NF-κB is activated in the myocardium after MI in both WT and TNF$^{-/-}$ groups, but much less in TNF$^{-/-}$ mice. However, the fact that TNF-α does not provoke apoptosis in the presence of a functional NF-κB signaling pathway, as seen here, suggests that TNF-α may have an alternative role as a stress response factor.[31] Moreover, because elevated TNF-α levels have been detected in end-stage heart failure[32] and viral myocarditis, it is tempting to speculate that NF-κB may modulate the inflammatory response of these conditions by suppressing or blunting the apoptotic response. This notion is supported by recent studies in which endothelial cells enriched with activated NF-κB were resistant to TNF-α, whereas endothelial cells defective for NF-κB activation readily underwent apoptosis provoked by TNF-α.[33] The mode by which NF-κB suppresses apoptosis is unknown but may be related to the activation of downstream genes that regulate the apoptotic process, such as cellular inhibitors of apoptosis (c-IAP) cIAP1, c-IAP2,[34] and IEX-1L.[35] Thus, whether NF-κB operates as a proapoptotic or antiapoptotic factor may depend on the context of cell type and the ensuing stimulus.

In summary, we found that, compared with WT mice, TNF$^{-/-}$ mice had a significantly reduced acute cardiac rupture rate and improved chronic LV function after MI. This was accompanied by a reduction of cardiac inflammatory cell infiltration, cytokine expression, and MMP-9 activity in the TNF$^{-/-}$ mice before risk of rupture. Chronically, the TNF$^{-/-}$ mice also showed less fibrosis and apoptosis in the remote myocardium away from the infarct zone, contributing to the improved ventricular function. However, despite these fundamental insights into TNF contributions to cardiac remodeling after MI, the best means of modulating the TNF effect clinically remains a challenge.[36]

Acknowledgments

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