Toll-Like Receptor 4 Mediates Maladaptive Left Ventricular Remodeling and Impairs Cardiac Function After Myocardial Infarction

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Abstract—Left ventricular (LV) remodeling leads to congestive heart failure and is a main determinant of morbidity and mortality following myocardial infarction. Therapeutic options to prevent LV remodeling are limited, which necessitates the exploration of alternative therapeutic targets. Toll-like receptors (TLRs) serve as pattern recognition receptors within the innate immune system. Activation of TLR4 results in an inflammatory response and is involved in extracellular matrix degradation, both key processes of LV remodeling following myocardial infarction. To establish the role of TLR4 in postinfarct LV remodeling, myocardial infarction was induced in wild-type BALB/c mice and TLR4-defective C3H-$Tlr4^{LPS-d}$ mice. Without affecting infarct size, TLR4 defectiveness reduced the extent of LV remodeling (end-diastolic volume: 103.7±6.8 μL versus 128.5±5.7 μL; $P<0.01$) and preserved systolic function (ejection fraction: 28.2±3.1% versus 16.6±1.3%; $P<0.01$), as assessed by MRI. In the noninfarcted area, interstitial fibrosis, and myocardial hypertrophy were reduced in C3H-$Tlr4^{LPS-d}$ mice. In the infarcted area, however, collagen density was increased, which was accompanied by fewer macrophages, reduced inflammation regulating cytokine expression levels (interleukin [IL]-1α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, tumor necrosis factor-α, interferon-γ, granulocyte/macrophage colony-stimulating factor), and reduced matrix metalloproteinase-2 (4684±515 versus 7573±611; $P=0.002$) and matrix metalloproteinase-9 activity (76.0±14.3 versus 168.0±36.2; $P=0.027$). These data provide direct evidence for a causal role of TLR4 in postinfarct maladaptive LV remodeling, probably via inflammatory cytokine production and matrix degradation. TLR4 may therefore constitute a novel target in the treatment of ischemic heart failure. **(*Circ Res.* 2008;102:257-264.)**

Key Words: *myocardial infarction* ■ *remodeling* ■ Toll-like receptor 4

Myocardial infarction (MI) is a leading cause of morbidity and mortality in Western countries. The main determinants of patient outcome following MI are myocardial infarct size and left ventricular (LV) remodeling. Whereas infarct size is determined in the acute phase following MI, LV remodeling is a chronic maladaptive process, characterized by progressive ventricular dilatation, myocardial hypertrophy, fibrosis, and deterioration of cardiac performance over time, eventually leading to congestive heart failure. Despite the introduction of multiple treatments to counteract LV remodeling into the daily clinical practice (eg, β-blockers and angiotensin-converting enzyme inhibitors), the incidence of congestive heart failure continues to increase and remains associated with a more than 10-fold elevated risk of death.[1] A better understanding of the molecular mechanisms involved in this process and the search for alternative therapeutic targets to prevent LV remodeling are therefore of major importance.

Toll-like receptors (TLRs) serve as pattern recognition receptors within the innate immune system and recognize exogenous ligands in response to infection. Among these receptors, TLR4 is activated by bacterial lipopolysaccharide (LPS) and is therefore known as the LPS receptor.[2,3] During inflammation and oxidative stress, TLR4 is also activated in response to endogenous ligands, such as heat shock protein (HSP)60 and the alternatively spliced extra domain A (EDA) of fibronectin, resulting in the release of proinflammatory factors.[4,5] Besides its role in inflammation, TLR4 stimulation in monocytes induces the production of matrix metalloproteinase (MMP)9, which has been suggested to be a marker for

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extracellular matrix degradation.[6] This points to a regulatory role for TLR4 in inflammation and matrix turnover. This concept is supported by the finding that the endogenous TLR4 ligands HSP60 and EDA can be detected in arthritic and oncologic specimens, in which both inflammation and matrix turnover are important features.[7,8] In animal models, TLR4 has been shown to be involved in outward vascular remodeling, probably via activation by endogenous ligands and affecting collagen accumulation in the artery.[9,10] In heart tissue derived from patients with idiopathic dilated cardiomyopathy, focal areas of intense TLR4 staining have been observed.[11] Whether TLR4 plays a role in postinfarct LV remodeling, however, has not been investigated thus far.

Matrix turnover and inflammation are important features in LV remodeling. Therefore, the purpose of this study was to evaluate whether TLR4 is involved in the ventricular response to ischemic injury and mediates LV remodeling following MI.

Materials and Methods

Animals

All experiments on homozygous TLR4 defective mice with BALB/c background (34.2±0.5 g, 10 to 12 weeks old, C3H-*Tlr4*$^{LPS-d}$; The Jackson Laboratory, Bar Harbor, Me) and wild-type (WT) mice (26.2±0.5 g, 10 to 12 weeks old, BALB/c, Harlan, Indianapolis, Ind) were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and with prior approval by the Animal Experimentation Committee of the Faculty of Medicine, Utrecht University.

Surgical Protocol: MI

Under isoflurane anesthesia, MI was induced by permanent ligation of the left coronary artery. In sham-operated animals, the suture was placed under the artery and removed without ligating the artery. For a detailed description of the procedure, refer to the expanded Material and Methods section in the online data supplement, available at http://circres.ahajournals.org.

Magnetic Resonance Imaging

End-diastolic volume, end-systolic volume, ejection fraction, cardiac output, stroke volume, and LV mass were determined serially using high-resolution MRI (9.4 T). Infarct size was assessed in vivo 4 days after MI using late-enhancement MRI recordings 15 to 30 minutes following intravenous Gd-DTPA-BMA (gadolinium–diethylenetriaminepentaacetic acid bis-methylamide) infusion. For expanded details, refer to the online data supplement.

Systolic Blood Pressure

Systolic blood pressure was measured by external tail pulse detection using a tail cuff, as described previously, before MI, 14 days after MI, and 28 days after MI.[12] Mice were conditioned to restraint, warming chamber, and application of tail cuff pressure twice in the week before measurement.

Histology, Collagen Density, and Myocyte Cross-Sectional Area

Collagen density was assessed using picrosirius red staining as described previously.[13] Cardiomyocyte hypertrophy was determined by quantification of the myocyte cross sectional area from hematoxylin/eosin-stained sections. Macrophages (MAC-3), tumor necrosis factor (TNF)-α, and TLR4 were visualized using immunostaining. To assess apoptosis in the border area, a TUNEL assay was performed on the sections according to the instructions of the manufacturer (Roche). Detailed information is provided in the online data supplement.

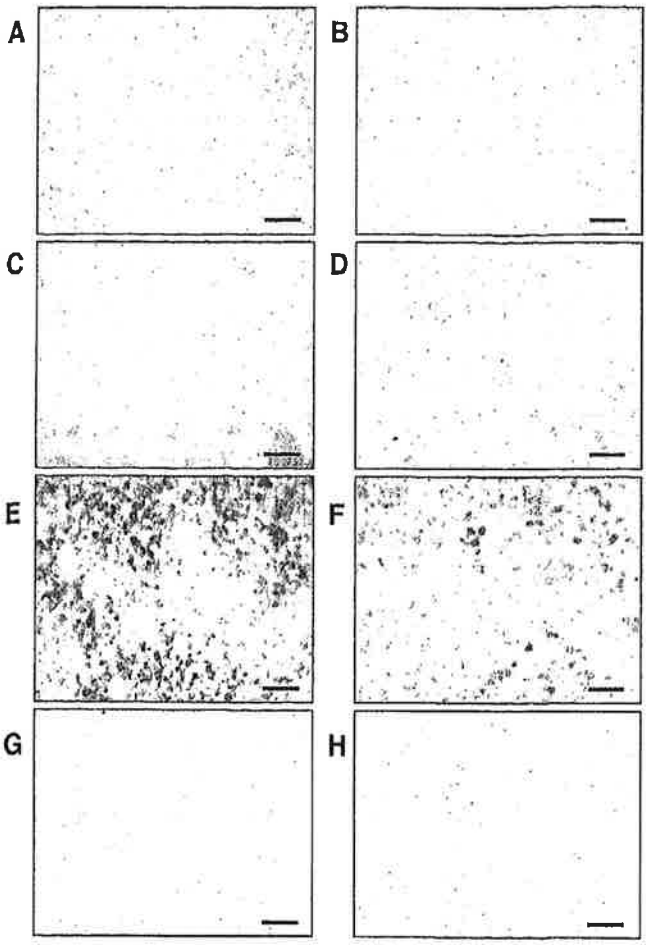

Figure 1. TLR4 expression profile. TLR4 is expressed in cardiomyocytes before (A), 4 days following (B), and 28 days following (C) MI. Four days after MI, TLR4 (D), MAC-3 (E), and TNF-α (F) are abundantly expressed in the infarct area. The colocalization indicates that TLR4 and TNF-α are both expressed by macrophages in infarct tissue. Twenty-eight days after MI, TLR4 (G) and MAC-3 (H) expression is largely absent in the infarct area. Magnification, ×400. Scale bars=500 μm.

MMP Activity Assay

MMP2 and MMP9 activity was determined using MMP activity assays according to the instructions of the manufacturer (Amersham, Munich, Germany). Detailed information can be found in the online data supplement.

Polymerase Chain Reaction

Gene expression levels of EDA, HSP60, transforming growth factor (TGF)-β_1, procollagen-I, TNF-α, intercellular adhesion molecular (ICAM)-1, and vascular cell adhesion molecule-1 were quantified using quantitative RT-PCR, as described previously.[14] Detailed information is provided in the online data supplement.

Flowcytomix

Inflammation-regulating cytokine expression (IL-1α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, TNF-α, interferon-γ, and granulocyte/macrophage colony-stimulating factor) was measured in Tripure (Roche) isolated protein samples using the Th1/Th2 10plex kit (Bender MedSystems, Vienna, Austria). The protein samples were diluted 1:1 in assay buffer, and the protocol was further followed according to the instructions of the manufacturer.

Data Analysis

All data were collected blindly. Data are presented as means±SE. Mortality between BALB/c mice and C3H-*Tlr4*$^{LPS-d}$ mice was compared using Fisher's exact test. Functional outcomes were compared using 2-way ANOVA for repeated measurements and post

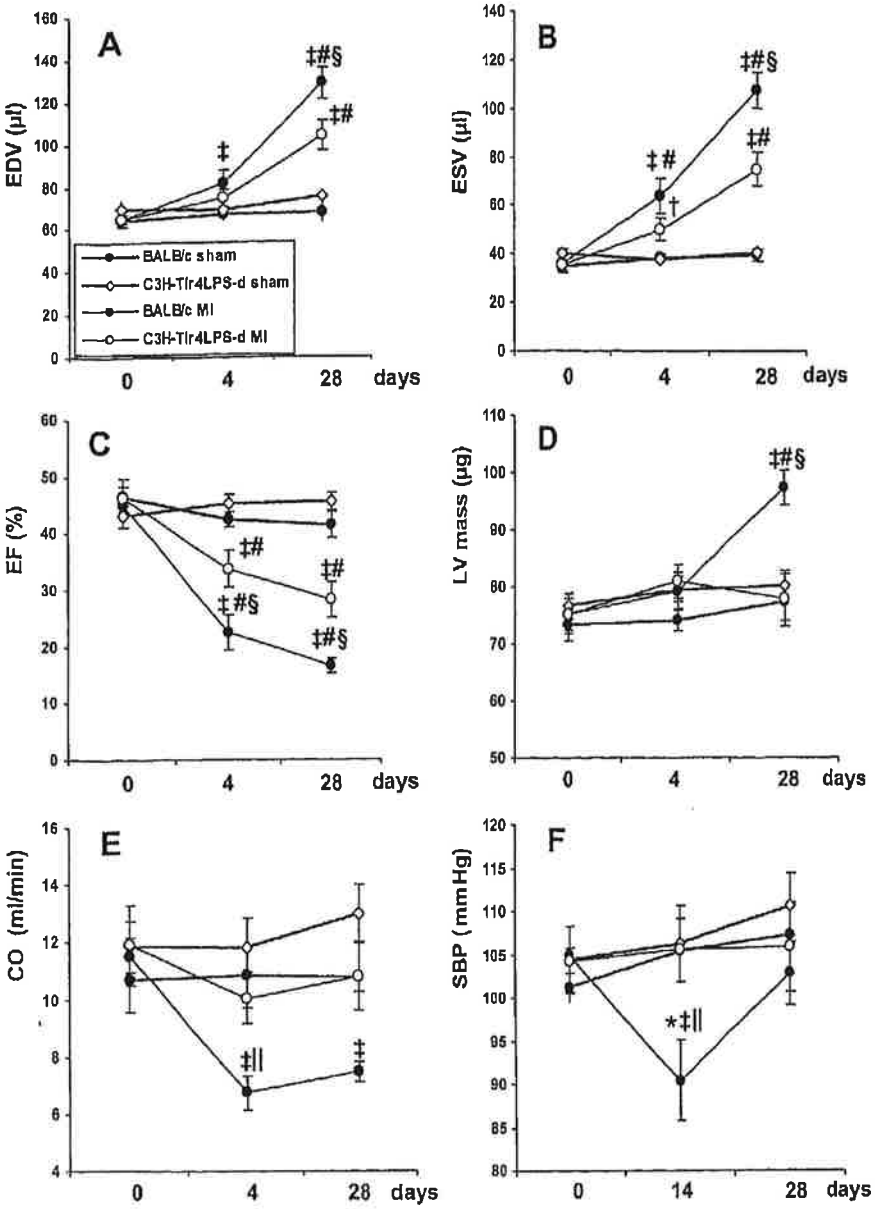

Figure 2. Remodeling and function. End-diastolic volume (EDV) (A), end-systolic volume (ESV) (B), ejection fraction (EF) (C), LV mass (D), and cardiac output (CO) (E) before and 4 and 28 days after surgery. Systolic blood pressure (SBP) (F) was measured before and 14 and 28 days after surgery (BALB/c sham, n=6; C3H-$Tlr4^{LPS-d}$ sham, n=6; BALB/c MI, n=8; C3H-$Tlr4^{LPS-d}$ MI, n=9). *P<0.05 compared with C3H-$Tlr4^{LPS-d}$ MI; §P<0.01 compared with C3H-$Tlr4^{LPS-d}$ MI; †P<0.05 compared with baseline value; ‡P<0.01 compared with baseline value; ‖P<0.05 compared with sham of the same genotype; #P<0.01 compared with sham of the same genotype.

hoc tests. Systolic blood pressures were log-transformed to obtain normality. A 2-way ANOVA with post hoc tests was used for comparison of expression levels between C3H-$Tlr4^{LPS-d}$ mice and BALB/c mice. TLR4 expression was compared using a 1-way ANOVA with post hoc tests, and infarct size was compared using Student's t test. Probability values <0.05 were considered significant.

Results

TLR4 Expression Profile

TLR4 expression was found in cardiomyocytes in noninfarcted hearts and in the remote area following MI (Figure 1A through 1C). Following MI, the TLR4 staining was also positive in the infarct area and colocated with positive MAC-3 staining and TNF-α staining (Figure 1D through 1F). Quantification of TLR4 protein expression revealed that TLR4 expression 4 days after MI was not increased in the remote area and infarct area (8.26±1.05 arbitrary values [baseline]; 10.11±1.60 arbitrary values [remote; P=0.937 versus baseline]; 8.82±1.03 arbitrary values [infarct; P=1.000 versus baseline]). Twenty eight days following MI, the inflammatory phase of myocardial infarct healing had past, and no more TLR4 expressing macrophages were present in the infarct area (Figure 1G and 1H).

Mortality

To establish the role of TLR4 in postinfarct LV remodeling, MI was induced in WT BALB/c mice and TLR4-defective C3H-$Tlr4^{LPS-d}$ mice. Ten (of 54) BALB/c mice and 10 (of 60) C3H-$Tlr4^{LPS-d}$ mice died before the planned date of termination, all within 2 days after coronary ligation, and were therefore excluded from the study. In these cases, no evidence was found for LV rupture during dissection, and the causes of death were therefore most likely surgery related, acute congestive heart failure, or arrhythmias. There was no difference in mortality between C3H-$Tlr4^{LPS-d}$ mice and BALB/C mice (18.5% versus 16.7% respectively; P=1.00).

Left Ventricular Remodeling, Systolic Function, and Infarct Size

Before coronary artery ligation, LV volumes, mass, and function were similar in C3H-$Tlr4^{LPS-d}$ mice and BALB/C

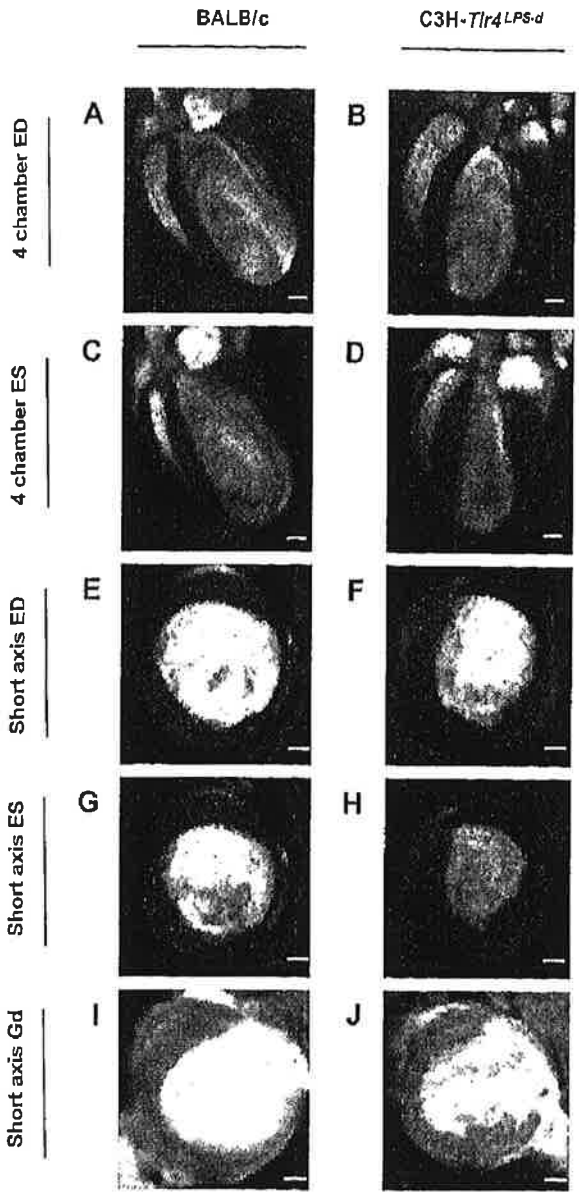

Figure 3. MRI. A through H, Representative 4-chamber and short-axis MRI Images of BALB/c mice and C3H-$Tlr4^{LPS-d}$ mice at end-diastole (ED) and end-systole (ES) 28 days following MI. I and J, Late-enhancement recordings, 15 to 30 minutes following Gd-DTPA-BMA Infusion and 4 days following MI. Gadolinium (Gd) infusion results in delayed signal enhancing in areas of MI. Scale bars=1.0 mm.

mice (Figure 2A through 2D). Following MI, end-diastolic and end-systolic volumes increased in both mouse genotypes, and cardiac function was decreased as observed by a reduction of the ejection fraction. In the C3H-$Tlr4^{LPS-d}$ mice, however, the extent of LV remodeling was significantly reduced (shown in Figure 2A and 2B). Also functional impairment was greatly reduced, which was demonstrated by higher ejection fractions (Figure 2C). In BALB/c mice, the LV mass increased following MI. The increase in LV mass was prevented in C3H-$Tlr4^{LPS-d}$ mice, which points toward reduced remodeling of the remote area (Figure 2D). Total heart weight did not differ between C3H-$Tlr4^{LPS-d}$ and BALB/c mice at baseline and following sham operation. Heart weight did not change in C3H-$Tlr4^{LPS-d}$ mice (101±2 mg [before MI], 107±2 mg [after sham], and 106±5 mg [after MI]), however, increased in BALB/c mice following MI (100±2 mg [before MI], 105±4 mg [after sham], and 133±4 mg [after MI; $P<0.01$ versus baseline and versus C3H-$Tlr4^{LPS-d}$ mice]). The perimeter of the remote area changed from 8.94±0.43 (4 days post-MI) to 10.92±0.46 mm (28 days post-MI) (22.7±4.3% increase) in WT mice and from 9.16±0.32 (4 days post-MI) to 10.02±0.34 mm (28 days post-MI) (10.0±3.9% increase) in TLR4-defective mice (relative increase WT versus TLR4 defective: $P=0.050$). The perimeter of the infarct area changed from 3.99±0.24 to 6.26±0.38 mm (60.5±15.5% increase) in WT mice and from 4.47±0.36 to 5.53±0.39 mm (25.3±5.7% increase) in TLR4-defective mice (relative increase WT versus TLR4 defective: $P=0.028$). This indicates that remodeling occurred in remote and infarct areas and that remodeling in both the remote and infarct areas were reduced in TLR4-defective mice compared with WT mice.

Loss of cardiac function in BALB/c mice also translated into unfavorable systemic hemodynamics, although systolic blood pressure had recovered after 28 days (Figure 2E and 2F). Cardiac output and blood pressure were preserved at all time points in C3H-$Tlr4^{LPS-d}$ mice. Heart rates were comparable in all groups at all time points (BALB/c sham: 356±19 [0 days], 375±17 [4 days], 395±27 [28 days]; C3H-$Tlr4^{LPS-d}$ sham: 391±10 [0 days], 375±29 [4 days], 380±60 [28 days]; BALB/c MI: 394±17 [0 days], 380±12 [4 days], 362±5 [28 days]; C3H-$Tlr4^{LPS-d}$ MI: 386±24 [0 days], 410±17 [4 days], 376±10 [28 days]). In sham-operated mice, functional parameters did not change over time.

Infarct size was assessed in vivo 4 days after MI using late-enhancement MRI recordings, 15 to 30 minutes following intravenous Gd-DTPA-BMA infusion. There was no difference in infarct size between C3H-$Tlr4^{LPS-d}$ mice and BALB/c mice (43.6±4.9 versus 40.6±3.2% of the LV; $P=0.682$). Illustrative MRI images are presented in Figure 3. Infarct size was also assessed with using Evans blue and 5-triphenyl tetrazolium chloride staining 24 hours following coronary artery ligation. These measurements confirmed that there were no differences in infarct size (40.4±3.8 versus 44.1±5.2% of the LV; $P=0.602$; 96.5±1.2 versus 96.9±0.5 of the area at risk; $P=0.808$). Using the hematoxylin/eosin staining, infarct size at 28 days following MI also appeared to be similar.

Collagen and Hypertrophy

Interstitial fibrosis in the remote noninfarcted myocardium is commonly observed in failing hearts and contributes to functional impairment. Collagen density 28 days following MI was increased in the remote (ie, noninfarcted) area of the BALB/c mice but not of the C3H-$Tlr4^{LPS-d}$ mice (Figure 4E). In contrast, collagen density in the infarct area was much higher in the C3H-$Tlr4^{LPS-d}$ mice compared with the BALB/c mice (Figure 4A through 4E).

In addition to interstitial fibrosis, myocardial hypertrophy is frequently observed in ischemic heart failure, which reflects the existence of compensatory mechanisms in response to impaired pump function. The myocyte cross-sectional area, a measure of cardiomyocyte hypertrophy, was increased in the remote areas of both mouse genotypes; however, the increase in myocyte cross-sectional area was less in the C3H-$Tlr4^{LPS-d}$ mice compared with the BALB/c

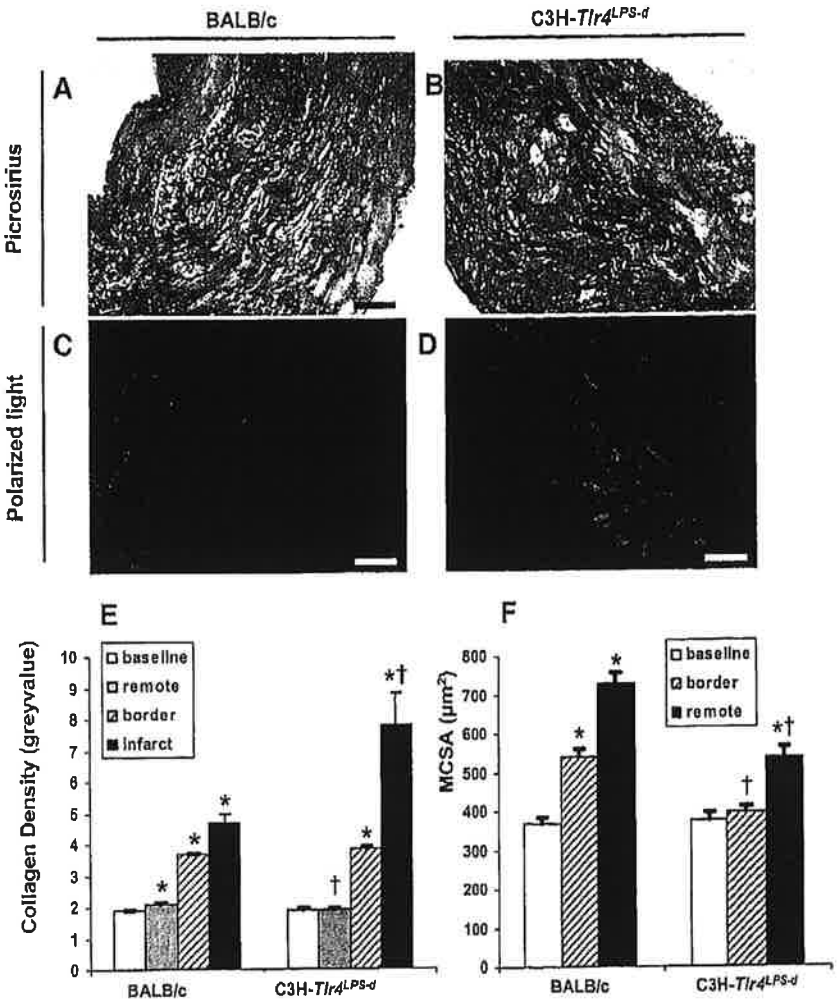

Figure 4. Collagen and hypertrophy. A through D, Representative infarct sections of BALB/c mice and C3H-$Tlr4^{LPS-d}$ mice, 28 days after MI, after picrosirius red staining under white light and polarized light (magnification, ×400; scale bars=500 μm). E, Collagen quantifications of baseline hearts (no MI) and remote areas, border areas, and infarct areas of MI hearts (28 days following MI) (BALB/c, n=9; C3H-$Tlr4^{LPS-d}$, n=10). F, Myocyte cross-sectional area (MCSA) quantifications of baseline hearts and border areas and remote areas of MI hearts (also 28 days following MI) (BALB/c, n=9; C3H-$Tlr4^{LPS-d}$, n=10). *P<0.05 compared with baseline; †P<0.05 compared with BALB/c.

mice (Figure 4F). In BALB/c mice, hypertrophy also occurred in the border zone. Despite LV dilatation and myocardial hypertrophy, LV mass did not increase in C3H-$Tlr4^{LPS-d}$ mice. Probably, myocardial cell loss during acute MI and subsequent apoptosis is balanced by cardiomyocyte hypertrophy in the remote and border areas. In BALB/c mice, excessive maladaptive myocardial hypertrophy and dilatation has led to increased LV weight and total cardiac weight.

Extracellular Matrix Turnover

Extracellular matrix turnover is a complicated process, in which the synthesis and degradation of matrix molecules play important roles. The increased infarct collagen density in the C3H-$Tlr4^{LPS-d}$ mice could be explained by increased collagen synthesis, decreased collagen degradation, or a combination. To evaluate collagen synthesis, procollagen-I and TGF-β_1 mRNA levels were measured. Both procollagen-I and TGF-β_1 appeared to be increased in the infarct areas of both mouse genotypes compared with the remote areas (Table). However, the levels did not differ between C3H-$Tlr4^{LPS-d}$ and BALB/c mice. MMP2 and MMP9 activity assays were performed to explore whether the higher collagen density in the infarcted area of the C3H-$Tlr4^{LPS-d}$ mice could be explained by decreased matrix degradation. In doing so, lower MMP2 and MMP9 activity was observed in the infarct area of C3H-$Tlr4^{LPS-d}$ mice compared with BALB/c mice (Figure 5A and 5B). Similar results were found using zymography with a decreased gelatin degradation in C3H-$Tlr4^{LPS-d}$ mice compared with BALB/c mice by MMP2 (3.39-fold decrease; P=0.037) and MMP9 (2.45-fold decrease; P=0.003).

Endogenous Ligands, Inflammation, and Apoptosis

Expression of EDA mRNA was increased in the infarct areas of BALB/c mice and C3H-$Tlr4^{LPS-d}$ mice compared with the remote areas (Table). HSP60 mRNA levels differed neither between infarct and remote areas nor between BALB/c and C3H-$Tlr4^{LPS-d}$ mice.

Inflammation was determined by counting the number of macrophages in the myocardial infarct and border area and by quantification of TNF-α mRNA levels and IL-1α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, TNF-α, interferon-γ, and granulocyte/macrophage colony-stimulating factor protein expression in the infarct area and remote area. The border areas and infarct areas of C3H-$Tlr4^{LPS-d}$ mice appeared to contain significantly fewer macrophages compared with BALB/c mice (3.5±0.7 versus 9.0±1.3 macrophages/mm² [P=0.001] and 13.0±3.2 versus 25.1±3.9 macrophages/mm² [P=0.029], respectively). Accordingly, TNF-α mRNA, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-10, IL-17, TNF-α, interferon-γ, and granulocyte/macrophage colony-stimulating factor expression were all lower in the infarct area of C3H-$Tlr4^{LPS-d}$ mice compared with BALB/c mice (Table). In BALB/c mice, the cytokine

Table. Expression Levels of Cytokines, Adhesion Molecules, and Endogenous Ligands in Sham Hearts and Four Days After MI in Infarct and Remote Areas of C3H-*Tlr4*$^{LPS-d}$ and BALB/c Mice

Product	BALB/c Sham	BALB/c Remote	BALB/c Infarct	C3H-*Tlr4*$^{LPS-d}$ Sham	C3H-*Tlr4*$^{LPS-d}$ Remote	C3H-*Tlr4*$^{LPS-d}$ Infarct
mRNA						
EDA	0.020±0.003	0.84±0.25	7.34±1.79*	0.016±0.002	1.62±0.37	8.01±1.33*
HSP60	0.14±0.001	1.28±0.39	3.55±1.70	0.22±0.014	0.74±0.16	0.88±0.40
TNF-α	0.002±0.0002	0.14±0.07	0.66±0.22*	0.002±0.0001	0.05±0.03	0.11±0.04†
ICAM-1	0.023±0.001	1.58±0.39	8.02±2.66*	0.025±0.001	0.90±0.21	1.69±0.28†
VCAM-1	0.014±0.0002	2.78±0.69	12.24±3.19*	0.014±0.0005	2.17±0.60	3.82±0.37†
TGF-β	0.046±0.002	0.22±0.07*	0.54±0.08*	0.035±0.001	0.23±0.06*	0.50±0.09*
Procollagen	0.56±0.04	9.82±2.79	48.94±11.65*	0.32±0.02	13.36±1.60	57.5±7.01*
Protein						
TNF-α	91±6	385±136*	1052±204*	100±21	165±85	142±67†
IL-1a	55±3	126±26*	286±59*	66±4	68±15	56±16†
IL-2	123±8	303±61*	481±132*	123±19	125±39	101±38†
IL-4	64±5	104±24	298±71*	57±12	54±11	49±15†
IL-5	31±3	68±18	142±35*	33±6	23±6	25±10†
IL-6	42±11	113±28	895±285*	46±18	43±10	207±94†
IL-10	91±8	206±62	337±122*	88±13	79±34	61±28†
IL-17	9±3	3±3	118±52*	4±3	4±3	5±4†
IFN-γ	32±4	57±13	193±46*	27±6	27±6	27±8†
GM-CSF	143±21	226±40	381±84*	130±23	103±19	92±23†

mRNA levels are expressed as a ratio to calnexin mRNA expression (BALB/c, n=13; C3H-*Tlr4*$^{LPS-d}$, n=12). Protein levels are expressed as picograms per milliliter of protein. *$P<0.05$ compared with sham; †$P<0.05$ compared with BALB/c. VCAM indicates vascular cell adhesion molecule; IFN, interferon; GM-CSF, granulocyte/macrophage colony-stimulating factor.

expression levels were higher in myocardial infarct tissue than in sham-operated hearts. In addition, the expression of IL-1α, IL-2, and TNF-α was increased in the remote areas compared with sham hearts. In C3H-*Tlr4*$^{LPS-d}$ mice, however, cytokine expression levels in the infarct area and remote area were not increased compared with sham hearts. The adhesion molecules ICAM-1 and vascular cell adhesion molecule-1 were also upregulated in myocardial infarct tissue. The levels were significantly higher in BALB/c mice compared with C3H-*Tlr4*$^{LPS-d}$ mice (Table). No differences were observed in apoptosis in the border areas between BALB/c mice and C3H-*Tlr4*$^{LPS-d}$ mice, as assessed by TUNEL assay at 4 days following MI (data not shown).

Discussion

Here we demonstrate that TLR 4 plays an important role in myocardial infarct healing and contributes to LV remodeling and functional impairment following MI. TLR4 plays a pivotal role within the innate immune system and contributes to the host defense against exogenous microbial pathogens. Besides a role in detecting exogenous ligands, such as microbial LPS, TLR4 has been shown to be stimulated by endogenous ligands during inflammation and oxidative stress.[4,5] In the present study, the endogenous TLR4 ligands HSP-60 and EDA were both observed in the myocardial infarct samples. The expression levels of the endogenous ligand EDA in the infarct area were much higher compared with expression levels in sham hearts, which makes EDA a putative candidate ligand responsible for TLR4 signaling during myocardial infarct healing. This novel finding is also in accordance with TGF-β_1 upregulation, which stimulates EDA production.[15]

Two essential prognostic factors following MI are (1) the size of the myocardial infarct and (2) maladaptive LV remodeling. Antiapoptotic effects of both TLR4 defectiveness and pharmaceutical inhibition of TLR4 have been described in animal models of ischemia/reperfusion injury.[16,17] This indicates that TLR4 antagonists can reduce infarct size and could be useful to change the fate of endangered cardiomyocytes in the acute phase following MI. Remodeling, however, is a completely different clinical problem. Although remodeling is markedly influenced by myocardial infarct size (large MI usually leads to more remodeling), it is also influenced by other parameters such as location of the infarct, wall stress, and biological processes including matrix turnover. Remodeling is a chronic process, which influences cardiac function and patient outcome weeks, months, and even years after MI occurred. The proportion of elderly people in the population, who have the highest risk of coronary artery disease and hypertension, is rising rapidly, and survival in patients with coronary artery disease is improving. For these reasons, the incidence of chronic ischemic heart failure is likely to increase even more in the coming years.[18] The exploration of new potential molecular targets to counteract remodeling and the progression of heart failure is essential. In the present study, an animal model of permanent coronary artery ligation was used to investigate the effect of TLR4 on LV remodeling. Using this model, infarct size was similar in C3H-*Tlr4*$^{LPS-d}$ mice and BALB/c mice, which can be explained by the permanent

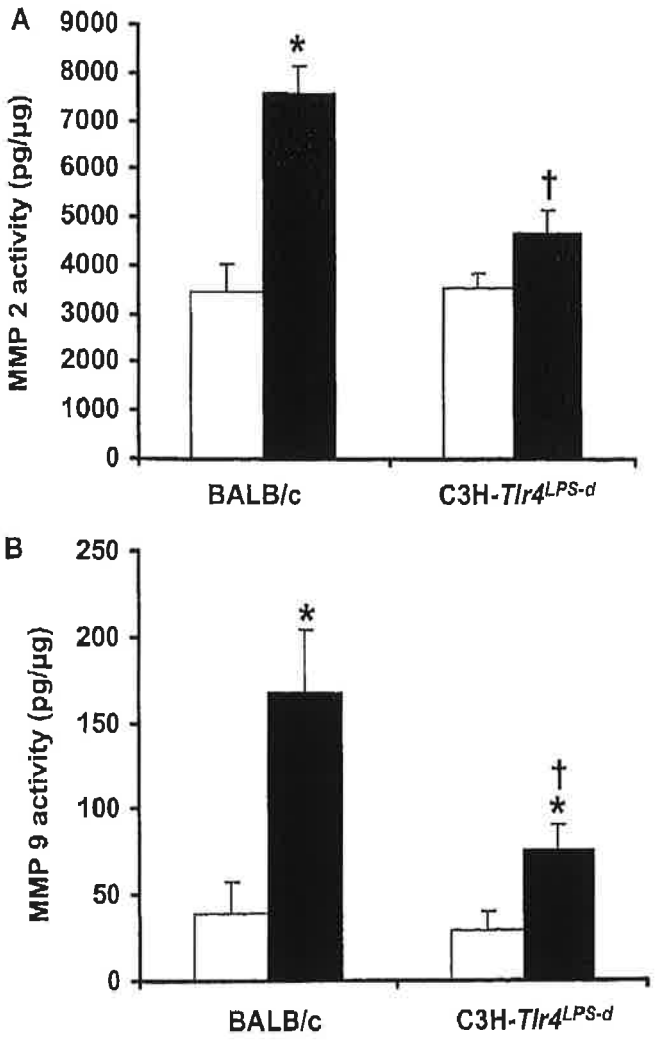

Figure 5. Matrix degradation. MMP2 activity (A) and MMP9 activity (B) as assessed by immunoactivity assays (BALB/c, n=8; C3H-$Tlr4^{LPS-d}$, n=9) in remote area (white bars) and infarct area (black bars) 4 days following MI. *$P<0.05$ compared with remote area; †$P<0.05$ compared with BALB/c.

nature of the coronary artery ligation. Because infarct size was similar in both genotypes, the differences in functional outcome were independent of infarct size.

The number of macrophages in the infarct and border area was reduced in C3H-$Tlr4^{LPS-d}$ mice compared with WT BALB/c mice. This is likely mediated by reduced expression of the cell adhesion molecules ICAM-1 and vascular cell adhesion molecule-1, which mediate monocyte homing to the myocardial infarct. As a consequence of reduced monocyte homing, the expression of a whole battery of inflammation-regulating cytokines was diminished, which likely contributed to preservation of infarct geometry and function. The production of inflammatory cytokines in the acute phase of myocardial infarct healing leads to enhanced local oxidative stress. Although TNF-α has been described to exert also cardioprotective properties,[19] the sustained presence of cytokines leads to myocyte phenotype transition and activation of MMPs, augmenting the remodeling process.[20]

Extracellular matrix turnover, a complicated process with collagen synthesis being balanced against collagen degradation, has been described to be important in LV remodeling.[21] Several studies have demonstrated that various members of the MMP family modulate postinfarct remodeling.[22–24] Specifically, MMP9 seems to play a pivotal role. Ducharme et al demonstrated that targeted deletion of MMP9 attenuated LV enlargement following MI.[25] MMP9 deletion was associated with decreased collagen density in the infarct because of compensatory overexpression of other members of the MMP family, like MMP2. We, however, did not observe such a compensatory mechanism. The significance of MMP2 is less clear. A study conducted in mice confirmed a role of MMP2 in late LV remodeling.[26] However, Matsumura et al did not observe differences in LV dimensions among MMP2 knockout mice, WT mice that were treated with a selective MMP2 inhibitor, and untreated WT mice.[27] In our present study, we observed a higher collagen density in the infarcts of the TLR4-defective mice compared with the WT mice. We found no evidence for increased collagen synthesis (procollagen-1 and TGF-β_1 mRNA levels were comparable between both mouse genotypes). Gelatinase activity by MMP2 and MMP9, however, was reduced, suggesting that extracellular matrix degradation was diminished.

Besides alterations in the infarct and border area, structural changes, such as hypertrophy, fibrosis, and expansion have been observed in the remote area following MI, and all of these changes were reduced in C3H-$Tlr4^{LPS-d}$ mice compared with WT mice. Several mechanisms may be responsible for this. First, it may have been an indirect effect of infarct remodeling. LV volumes increased to a greater extent in BALB/c mice compared with C3H-$Tlr4^{LPS-d}$ mice, and this was mainly attributable to remodeling of the infarct as became evident from separate MRI analysis of remote and infarct area. Remodeling of the infarct area caused by inflammation and matrix degradation probably resulted in increased volumes and subsequent increased wall stress to the remote area. Second, TLR4 may have induced cardiac hypertrophy in the remote area in response to heart failure with reduced cardiac output and systolic blood pressure. In a mouse model of aortic banding, Ha et al showed that TLR4 is also an important receptor that mediates signaling pathways that contribute to the development of cardiac hypertrophy.[28] Third, the induction of inflammatory cytokine expression was prevented in the remote area of C3H-$Tlr4^{LPS-d}$ mice, which may be responsible for reduced remodeling[20] and fibrosis.[29,30] Also cardiomyocytes have been reported to express TNF-α.[31]

Although the C3H-$Tlr4^{LPS-d}$ mice appeared to undergo less cardiac remodeling and dysfunction, this did not translate into improved survival. TLR4 is also an important mediator of the immune response. The fact that mortality rates are similar might be explained by the compromised immune system of TLR4-defective mice following highly invasive surgery.

Twenty-eight days after surgery, LV volumes of C3H-$Tlr4^{LPS-d}$ mice were greatly reduced and accompanied by a relative increase of the ejection fraction of 75% compared with BALB/c mice. It is therefore likely that TLR4 has a long-lasting modulating effect on remodeling and function following MI, which makes it an attractive candidate target for therapeutic purposes in patients with ischemic heart failure.

In conclusion, TLR4 mediates maladaptive LV remodeling and functional deterioration following MI, likely by inducing

macrophage homing, inflammatory cytokine production, matrix degradation, and cardiomyocyte hypertrophy. These data provide the first evidence for a causal role of TLR4 in post–myocardial infarct LV remodeling. TLR4 inhibition may therefore constitute a novel therapeutic option to counteract maladaptive LV remodeling in patients with ischemic heart failure.

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Disclosures

None.

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