Neutralization of Interleukin-1β in the Acute Phase of Myocardial Infarction Promotes the Progression of Left Ventricular Remodeling

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OBJECTIVES
We sought to examine the role of the pro-inflammatory cytokine, interleukin-1-beta (IL-1β), in the process of left ventricular (LV) remodeling in the early phase after myocardial infarction (MI).

BACKGROUND
Studies have shown that pro-inflammatory cytokines are closely related to the progression of LV remodeling after MI.

METHODS
Mice underwent coronary artery ligation, and the time course of LV remodeling was followed up to 20 weeks. The gene expression level of IL-1β was examined. In a second set of experiments, the mice underwent coronary artery ligation followed by treatment with anti–IL-1β antibody (100 µg, intravenously), versus control immunoglobulin G (100 µg, intravenously) immediately after the operation.

RESULTS
Rapid hypertrophy of noninfarcted myocardium was observed by four weeks, and interstitial fibrosis progressed steadily up to 20 weeks. Anti–IL-1β treatment increased the occurrence of ventricular rupture and suppressed collagen accumulation in the infarct-related area. At four and eight weeks after the operation, total heart weight and LV end-diastolic dimension were significantly greater in the anti–IL-1β-treated mice than in the other groups. In the infarct-related area, collagen accumulation was suppressed, whereas in the noninfarcted area, pro-collagen gene expression levels, particularly type III, were decreased in the anti–IL-1β-treated mice.

CONCLUSIONS
Anti–IL-1β treatment suppressed pro-collagen gene expression and delayed wound healing mechanisms—properties that are likely to lead to progression of LV remodeling. In the acute phase of MI, IL-1β appears to play a protective role. (J Am Coll Cardiol 2001;38:1546–53)

Myocardial infarction (MI) is followed by distinct changes, including hypertrophy of the noninfarcted myocardium, interstitial fibrosis and increases in left ventricular (LV) chamber dimensions, known as LV remodeling. The course of LV remodeling appears to be dependent on the size of the infarct, as well as on the load placed on the myocardium (1–3). Excessive progression of LV remodeling alters the structure of the heart and impairs its pumping function and blood supply; it is also associated with an increased risk of congestive heart failure (1,2,4,5). Prevention of excessive LV remodeling is one of the most important issues today (6–8).

High blood levels of the pro-inflammatory cytokines, interleukin-1-beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), have been observed after MI (9–11), and these cytokines are suspected to play an important role in the pathophysiology of LV remodeling (3,12,13). Recent studies have demonstrated that IL-1β and TNF-α induce myocyte hypertrophy in vitro (14–16) and hypothesized that they promote tissue fibrosis in vivo (12,16,17). In the rat, upregulation gene expression levels of these cytokines persist up to the chronic phase of MI. Furthermore, levels of IL-1β are related to the degree of interstitial fibrosis (12). Therefore, we have hypothesized that pro-inflammatory cytokines, particularly IL-1β, are major promoting factors of LV remodeling and that its progression could be attenuated by anti–IL-1β treatment.

The present study was designed to examine the role of IL-1β and the effects of anti–IL-1β-neutralizing antibody treatment in the process of LV remodeling. The time course of LV remodeling was first examined in mice with large MIs.

METHODS
The investigation conforms with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH), Bethesda, Maryland (NIH Publication no. 85-23, revised 1996).

Experiment 1. MICE. The experiments were performed in eight- to nine-week-old male C57BL/6 mice, weighing between 24 and 27 g, obtained from the Shizuoka Agricul-
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**EXPERIMENTAL DESIGN.** The time course of LV remodeling was followed at 1 and 3 days and at 1, 2, 4, 8, 12 and 20 weeks after the operation. At each time point, three to five mice were superficially anesthetized with ether, and transthoracic echocardiography (TTE) was performed to measure the LV end-diastolic dimension (LVEDD). After TTE, the hearts were harvested. Mice without large MIs involving the entire free wall and apex of the LV, which was easily distinguishable by the changes of color of the myocardium or wall thinning in the infarct-related area, were excluded from the analysis due to technical failure. The hearts were excised, halved along the ventricular short axis and weighed. The cephalad halves were embedded in paraffin for histologic examination, and the caudal halves were snap-frozen at −70°C until ribonucleic acid (RNA) extraction. Cardiomyocyte diameter was histologically examined using a computerized morphometric analysis system (Polaroid PDMCD 1e, NIH Image 1.61), as described previously (12). Interstitial collagen deposition levels were quantified using a digital image analyzer (LUSEX 3, Nikon, Tokyo, Japan). In brief, collagen matrix was discriminated by Sirius red F3BA staining, and slide images were obtained of the noninfarcted interventricular septum at a ×400 magnification. Twenty fields per section were scanned and computerized with a LUSEX 3 digital image analyzer on the basis of the red staining of the collagen. Volume collagen fraction was calculated as the sum of all connective tissue areas divided by the total area of the image (12).

**GENE EXPRESSION LEVEL OF IL-1β.** Additional experimental groups—1-, 3-, 6-, and 12-h groups of three mice of each—were made to examine the IL-1β gene expression levels in the acute phase of MI. The infarct-related and noninfarcted areas of the caudal parts of the heart were divided; total RNA was extracted; and complementary deoxyribonucleic acid was synthesized as previously described (19). At each time point, gene expression levels of IL-1β were measured in three mice by the real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) method. The PCR primers and probe of IL-1β were purchased from PE Biosystems (Foster, California). The gene expression level of GAPDH was quantified using the following forward (F) and reverse (R) oligonucleotides and probes (P) with fluorescent dye and quencher—F: 5′-TTCCACCCACCATGGAGAAGGC-3′; R: 5′-GGCATGGACTGTGTGGTCATGA-3′; and P: 5′-Fam-TTCATCCCTGCACACACACTGTTTAG-Tamura-3′. The values were averaged and expressed relative to the increases in GAPDH.

**Experiment 2. ANTI-IL-1β TREATMENTS.** The mice were randomized into three treatment groups: anti–IL-1β antibody, control immunoglobulin G (IgG) or the vehicle (phosphate-buffered saline [PBS]). The sham-operated mice were treated with control IgG. Hamster anti-mouse IL-1β monoclonal antibody and control IgG, both from Genzyme (Cambridge, Massachusetts) (20), were diluted with PBS in a final volume of 200 μl and injected into the tail vein immediately after coronary ligation in doses of 100 μg of antibody per mouse. This anti–IL-1β antibody recognizes the IL-1β precursor and the mature secreted form of mouse IL-1β. No detectable cross reactivity was observed with mouse IL-1α, TNF-α or interferon-gamma. Treatment with the vehicle was administered intraperitoneally immediately after coronary artery ligation.

**BLOOD PRESSURE.** To estimate the effects of each treatment on hemodynamic loading, blood pressure was measured three days after the operation in three mice from each treatment group, as described previously (21). Immediately after the studies, the hearts were harvested and examined histologically, and RNA was extracted, as described earlier.
HEART DIMENSIONS AND WEIGHT MEASUREMENTS. Trans-thoracic echocardiography was performed at one, four and eight weeks after the operation. Immediately after the studies, the hearts were excised, halved along the ventricular short axis and weighed. The hearts were examined histologically, and RNA was extracted, as described earlier.

COLLAGEN ACCUMULATION IN THE INFARCT-RELATED AREA. Three days and one week after the operation, the hearts of four mice were harvested, stained with hematoxylin-eosin and Sirius red. The amount of collagen deposition in the infarct-related area was measured, as described earlier.

QUANTIFICATION OF GENE Expression LEVELS. Additional study groups—one-day groups—were made to assess the effect of the treatment in the early phase. We examined the gene expression levels of types I and III pro-collagen by real-time quantitative RT-PCR at one and three days and one week after the operation in the noninfarcted area of four mice from each group, using the following forward (F) and reverse (R) oligonucleotides and probes (P) with fluorescent dye and quencher—pro-α-2(I)-collagen: F: 5′-GAGGCACCCCCTTCTACGTTGTA-3′; R: 5′-CAGTC-CAACAAGCATCTCTGGT-3′; and P: 5′-Fam-CAAACTGGCTGCCACCATTGATAGTCTCTC-Tamura-3′; and pro-α-1(III)-collagen: F: 5′-TTACTCCTCCACAGGTCACTCTC-3′; R: 5′-TATGAATTAGGCAGCCGCT-3′; and P: 5′-Fam-AACCA-GCCCGGATGTCTCAGTGCTT-Tamura-3′. Each primer was designed to amplify the specific sequence of each target gene. The values were averaged and expressed relative to the increases in GAPDH.

STATISTICAL ANALYSIS. The variables of LV remodeling (Fig. 1) are reported as the mean value ± SD. Gene expression levels were examined in three mice of each group and expressed as the mean value ± SEM. Other data are expressed as the mean value ± SD. One-way analysis of variance with the Fisher protected least significant difference test was used for statistical comparisons. The interaction between four groups and the time course was tested for each measure and found to be significant at a level of 0.05.

RESULTS

Experiment 1. MI. Coronary artery ligation was performed in 197 male C57BL/6 mice. The mean operation time was ~30 min per mouse. The perioperative mortality rate, within 24 h after the operation, was 47% (92 mice), and 17 additional mice died of ventricular rupture between five and

Figure 1. Time course of left ventricular remodeling. See text for details. d = days; LVEDd = left ventricular end-diastolic dimension; MI = myocardial infarction; W = weeks; *p < 0.05; †p < 0.01; **p < 0.05 vs. sham-operated mice; ††p < 0.01 vs. sham-operated mice.

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seven days after coronary artery occlusion (Table 1). Five mice were sacrificed to measure infarct size, as described previously (18). The infarct involved the entire LV free wall and apex, representing 57% of the LV, and variability was minimal (SD 4.2%). All sham-operated mice survived through the study interval (Fig. 2, Table 1).

**TIME COURSE OF LV REMODELING.** The time course of LV remodeling was examined by the changes in LVEDD, total heart weight, cardiomyocyte diameter and interstitial collagen deposition level (Figs. 1 and 2). Twenty-four hours after the operation, the mean heart weight in the MI group was slightly lower than that in the sham-operated group and, by four weeks, had increased markedly (Fig. 1A). Twenty-four hours after the operation, LVEDD was observed in the mice with MI by four weeks after the operation (Figs. 1B, 2A and 2B). The changes in cardiomyocyte diameter paralleled those of total heart weight (Fig. 1C). Collagen deposition in the noninfarcted area of the hearts increased gradually from 0.64 ± 0.27% at baseline to 9.38 ± 0.63% at 20 weeks after coronary occlusion. In contrast, no increase in collagen deposition was observed in the sham-operated mice (Figs. 1D and 2C).

**GENE EXPRESSION LEVEL OF IL-1β.** A rapid increase in the gene expression level of IL-1β, peaking at 6 h after coronary artery occlusion, was observed in the infarct-related area, followed by a prompt decrease. In the noninfarcted area, persistent gene expression of IL-1β was observed as late as 12 weeks after the operation (Fig. 1C). Collagen deposition in the noninfarcted area of the hearts increased gradually from 0.64 ± 0.27% at baseline to 9.38 ± 0.63% at 20 weeks after coronary occlusion. In contrast, no increase in collagen deposition was observed in the sham-operated mice (Figs. 1D and 2C).

**Experiment 2. Effects of Anti-IL-1β Treatment on LV Remodeling.** Mice in the one-day and three-days groups were excluded from the count, because ventricular rupture occurred between five and seven days after the operation in this model. It is particularly noteworthy that nine mice treated with anti–IL-1β died of ventricular rupture, as compared with two mice in the control (IgG–treated) group and three mice in the vehicle–treated group. There was no significant difference in the systolic blood pressure levels measured among the various treatment groups at three days after the operation (Table 2).

**HEART WEIGHT AND LVEDD.** There were no significant differences in mean heart weight and LVEDD between the vehicle–treated and control (IgG–treated) groups. Mice treated with anti–IL-1β antibody had an increase in total heart weight and LVEDD, which continued up to eight weeks (Fig. 4). These results indicate that anti–IL-1β treatment in the acute phase of MI promoted LV dilation and hypertrophy of the noninfarcted myocardium.

**Collagen Accumulation in the Infarct-Related Area and Pro-collagen Gene Expression Levels.** Wound healing was evaluated by the degree of collagen accumulation in the infarct-related area. At four and eight weeks after the operation, the infarct-related area was almost completely replaced by fibrous scar (Fig. 2A), so the wound healing process was evaluated at three days and one week after the operation. One week after coronary ligation, treatment with anti–IL-1β antibody had significantly inhibited collagen accumulation. There was no significant difference between the control (IgG–treated) and vehicle–treated mice (Fig. 5). At the same time, anti–IL-1β treatment demonstrated an inhibitory effect on collagen gene expression in the noninfarcted area, particularly on type III, which persisted beyond one week after treatment. There was no significant difference between the vehicle–treated and control (IgG–treated) mice (Fig. 5).

**DISCUSSION**

**LV remodeling after MI in mice.** This study describes the time course of LV remodeling after MI in mice. In this model, LV dilation was prominent within one week, and cardiomyocyte hypertrophy was apparent just three days after coronary occlusion. The interstitial fibrotic changes continued for up to 20 weeks after the operation. Overall, the model demonstrated the typical LV remodeling characteristics that follow MI. These results prompted us to examine changes at one week, the time of early remodeling, at four weeks, the transition to chronic remodeling, and at eight weeks, during chronic remodeling.

**The role of cytokine in LV remodeling after MI.** Cytokines play pathogenic roles in a variety of inflammatory diseases (22–24). Interleukin-1β and TNF-α, in particular, are suspected to play an important role in LV remodeling (9,12,25). These pro-inflammatory cytokines promote myocyte hypertrophy in vitro, and persistent gene expression can be observed in the noninfarcted myocardium (12,26,27). Interleukin-1β, in particular, is closely associated with

### Table 1. Comparison of Surgical Outcomes and Survival Between Mice With MI and Sham-Operated Mice

<table>
<thead>
<tr>
<th></th>
<th>MI Group (n = 197)</th>
<th>Sham Group (n = 32)</th>
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</thead>
<tbody>
<tr>
<td>Mortality within 24 h</td>
<td>92 (46.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Causes of death within 24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Acute heart failure</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>Operative success</td>
<td>105 (53.3%)</td>
<td>32 (100%)</td>
</tr>
<tr>
<td>Causes of death or sacrifice beyond 24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular rupture</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>Heart failure</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Sacrifice</td>
<td>63*</td>
<td>32</td>
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*Twenty-nine mice were excluded. Operative success was judged 24 h after the operation. All sham-operated mice survived to the end of each study point. One hundred ninety-seven mice received coronary occlusion. After coronary occlusion, 12 mice died of pneumothorax immediately after the operation, and 80 mice died within 24 h. One hundred five mice survived beyond 24 h. Among them, 17 died of ventricular rupture between five and seven days after the operation (11 mice on day 5, 4 on day 6 and 2 on day 7). Twenty-five mice probably died of heart failure. Twenty-nine mice that did not have a large infarction involving the entire free wall and apex of the LV were excluded from experiment due to technical failure at the time of sacrifice.

MI = myocardial infarction.
Figure 2. Myocardial infarction (MI) in mice. (A) Evolution of representative serial sections of the hearts. Mice without extensive MI involving all of the free wall and apex of the left ventricular (LV) were excluded from the analysis. Note the remarkable dilation of the LV cavity and hypertrophy of the noninfarcted myocardium. Left ventricular dilation and hypertrophy were observed as early as four weeks after the operation. (B) The transthoracic echocardiography study. Note the marked increase in LV end-diastolic dimension, as compared with that in the sham-operated mice. (C) Collagen staining. Note the scar formation in the infarct-related area and the abundant collagen deposition in the interstitial space 12 weeks after coronary ligation, as compared with that in the sham-operated mice (magnification ×200).
fibrosis after MI (12). We therefore hypothesized that anti–IL-1β treatment would effectively inhibit the progression of LV remodeling. Interleukin-1β gene expression increased rapidly after MI. To obtain specific suppression of IL-1β bioactivity, a dose of monoclonal anti–IL-1β-neutralizing antibody was administered intravenously, immediately after coronary ligation (28).

The effect of anti–IL-1β antibody treatment on wound healing and LV remodeling. To our surprise, anti–IL-1β treatment in the acute phase of MI increased the occurrence of ventricular rupture, promoted LV dilation and increased total heart weight. The exact mechanism of this phenomenon remains to be clarified. However, it is clear that IL-1β plays a protective role in the acute phase of MI.

In the process of LV remodeling, wound healing responses that result in fibrous tissue formation develop in the affected ventricle, at the site of MI (1,2). Previous studies suggest that collagen deposition after MI is important to preserve ventricular architecture and function, and that a low infarct collagen content is associated with more pronounced remodeling and greater ventricular dilation (29–32). It seems reasonable to hypothesize that fibrosis taking place in the area with acute infarction is needed to prevent the development of remodeling. Anti–IL-1β treatment was associated with less collagen deposition in the infarct-related area, which means a delay in wound healing and suppression of pro-collagen gene expression in the noninfarcted area. Anti–IL-1β treatment is suspected to strongly interfere with this response, resulting in an increase in the incidence of ventricular rupture and in rapid LV dilation.

Figure 3. Gene expression levels of interleukin-1β (IL-1β) were examined in three mice at each point by real-time quantitative reverse transcription-polymerase chain reaction. A rapid increase in IL-1β gene expression level, peaking at 6 h, was observed in the infarct-related area, and decreased thereafter. In the noninfarcted area, gene expression levels remained upregulated at 12 weeks after the operation. GADPH = glyceraldehyde-3-phosphate dehydrogenase; h(s) = hour(s); ds = days; w(s) = week(s).

Table 2. Study Schedule, Mortality Distribution and Three-Day Blood Pressure Measurements of the Various Treatment Groups

<table>
<thead>
<tr>
<th>MI Group (n = 110)</th>
<th>Sham Group (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control IgG (G) (n = 32)</td>
<td>Anti–IL-1β (A) (n = 44)</td>
</tr>
<tr>
<td>Mortality within 24 h</td>
<td>11</td>
</tr>
<tr>
<td>Operative success</td>
<td>21 (65.6%)</td>
</tr>
<tr>
<td>Exclusion (technical failure)*</td>
<td>3</td>
</tr>
<tr>
<td>Causes of death</td>
<td></td>
</tr>
<tr>
<td>Ventricular rupture</td>
<td>2</td>
</tr>
<tr>
<td>Heart failure</td>
<td>4</td>
</tr>
<tr>
<td>Sacrifice</td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>4 (0)</td>
</tr>
<tr>
<td>4 weeks</td>
<td>5 (1)</td>
</tr>
<tr>
<td>8 weeks</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Systolic blood pressure (day 3, n = 3 of each)</td>
<td>89.3 ± 1.15</td>
</tr>
</tbody>
</table>

*Data are presented as the mean number of mice excluded from the experiment due to technical failure. Other data are presented as the number (%) of mice or mean value ± SD. †p < 0.05 vs. G. ‡p = NS vs. G. Operative success was judged 24 h after the operation. There was no significant difference in the operation success rate according to the antibody treatment. It is noteworthy that nine mice treated with anti–IL-1β antibody died of ventricular rupture during the follow-up period. In this experiment, no mouse died of pneumothorax. There was no significant difference in systolic blood pressure among the study groups.

IgG = immunoglobulin G; IL = interleukin; MI = myocardial infarction.
The pathophysiologic differences in the acute and chronic phases after MI. The role of anti-IL-1β treatment in late remodeling remains to be clarified. It has been observed that, in the chronic phase of MI in the rat, IL-1β is closely associated with interstitial fibrosis in the non-infarcted area (12). Continued remodeling of the noninfarcted area, with collagen deposition and fibrosis, has been shown in rats and humans (33,34), and a close correlation between myocardial stiffness and the amount of interstitial fibrosis has been reported (35). In this murine model, gene expres-

![Figure 4](image1.png)

**Figure 4.** Effect of anti-interleukin-1 (IL-1) treatment (monoclonal antibody) on the progression of left ventricular (LV) remodeling. Neutralization of IL-1β in the acute phase of myocardial infarction significantly increased total heart weight and LV end-diastolic dimension (LVEDD). There was no significant difference between the vehicle-treated and control (immunoglobulin G [IgG]-treated) groups (four mice in each). n.s. = not significant.

![Figure 5](image2.png)

**Figure 5.** Effect of anti-interleukin-1β (IL-1β) antibody treatment on infarct healing and pro-collagen gene expression. At three days and one week after the operation, collagen accumulation in the infarct-related myocardium was measured as described in the text. Anti-IL-1β treatment inhibited collagen accumulation in the infarct-related area, which caused a delay in wound healing. Gene expression of type III pro-collagen was suppressed by anti-IL-1β treatment by one and three days and one week after the operation. There was no significant difference between the vehicle-treated and control (immunoglobulin G [IgG]-treated) groups (four mice in each). Ab = antibody; Col I and III = collagen type I and III, respectively; GADPH = glyceraldehyde-3-phosphate dehydrogenase; n.s. = not significant.
ension of IL-1β and progression of interstitial fibrosis persist in the chronic phase, and anti–IL-1β treatment suppressed the gene expression of type III pro-collagen in the noninfarcted area. Persistent activation in the chronic phase is suspected to promote excessive interstitial fibrosis, leading to impairment of pumping function.

Conclusions. Distinct differences seem to exist between the acute and chronic phases of MI in the pathogenesis of LV remodeling. The treatment should be adjusted according to the characteristics of its evolution. In the acute phase, wound healing should be facilitated, whereas in the chronic phase, the inflammatory response should be suppressed. Interleukin-1β is an important therapeutic target in the process of LV remodeling.

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