Left ventricular hypertrophy in mice with a cardiac-specific overexpression of interleukin-1

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Nishikawa, Kenichiro, Mikoto Yoshida, Masatoshi Kusuhara, Norio Ishigami, Kikuo Isoda, Kohji Miyazaki, and Fumitaka Ohsuzu. Left ventricular hypertrophy in mice with a cardiac-specific overexpression of interleukin-1. Am J Physiol Heart Circ Physiol 291: H176–H183, 2006. First published February 10, 2006; doi:10.1152/ajpheart.00269.2005.—Recent studies have identified the importance of proinflammatory cytokines in the development of left ventricular (LV) hypertrophy. However, the precise role of interleukin-1 (IL-1), one of the major proinflammatory cytokines, in the myocardium is not fully understood. In this study, we investigated the pathophysiological consequences of cardiac expression of IL-1 in vivo. We generated mice with a cardiac-specific overexpression of human IL-1α. We then analyzed their heart morphology and functions. Histological and echocardiographic analyses revealed concentric LV hypertrophy with preserved LV systolic function in the mice. Our results suggest that myocardial expression of IL-1 is sufficient to cause LV hypertrophy.

INTERLEUKIN-1 (IL-1), one of the proinflammatory cytokines, exerts a wide variety of effects, such as an immune response, cell proliferation, and cell death, on many different cell types (4). An elevated expression of IL-1 in hearts has been revealed in a variety of cardiovascular diseases, including myocarditis (22), myocardial infarction (18), and congestive heart failure (13, 18, 22). IL-1 is also highly expressed in the hypertrophied myocardium in vitro and in vivo (21). We previously reported that mice with a ubiquitous overexpression of human IL-1α (hIL-1α), which were originally generated as a mouse model of rheumatoid arthritis, unexpectedly showed prominent left ventricular (LV) hypertrophy (7). The study did not, however, define whether the specific expression of IL-1α in the myocardium caused the LV hypertrophy, because the systemic expressions might induce the LV hypertrophy. Therefore, to elucidate the role of myocardial expression of IL-1α in the myocardium, we developed transgenic (Tg) mice with a constitutive overexpression of hIL-1α restricted to cardiomyocytes under the control of α-myosin heavy chain (MHC) promoter. The Tg mice exhibited concentric LV hypertrophy with a preserved LV systolic function. Our data suggest that cardiac expression of IL-1 thus is sufficient to cause LV hypertrophy.

METHODS

Generation of the Tg construct. A mature type of hIL-1α cDNA (Immunex) was derived from a plasmid of the chicken β-actin promoter containing cytomegalovirus enhancer-hIL-1α. The plasmid had been used for generation of mice with a ubiquitous overexpression of hIL-1α (17). The fragment containing hIL-1α cDNA was excised by Bgl II/Xba I (770 bp). The terminal fragment was blunted by the Klenow fragment (Takara Bio). The plasmid containing the α-MHC promoter construct (pGL-α-MHC-SVpA) (11) was kindly donated by Dr. Arthur M. Feldman. The fragment containing hIL-1α cDNA was inserted into the plasmid that was digested by Hind III. Finally, we generated the Tg construct pGL-α-MHC-hIL-1α-SVpA (9.88 kbp). BamH I digestion produced a linear 6.96-kbp fragment used for microinjection into mouse embryos (Fig. 1).

All studies were carried out according to the protocols approved by the National Defense Medical College Board for Studies in Experimental Animals.

Generation and identification of Tg mice. C57BL/6N mice were used for the generation of Tg mice. The Tg mice were identified by PCR with primers as follows: 5′-CAC ATA GAA GCC TAG CCC AC-3′ (forward) and 5′-TTC CAC CAC TGC TCC CAT TC-3′ (reverse). The PCR conditions were as follows: 30 cycles of denaturation at 95°C for 10 s, annealing at 62°C for 15 s, and extension at 72°C for 90 s.

Quantitative PCR was performed to estimate the DNA copy number of transgene loci in each line. The primers specific for hIL-1α and an internal normalizer gene, RNase P1, were used in multiplex PCR on a PCR thermocycler (TaqMan 7700, Applied Biosystems, Foster City, CA) following the manufacturer’s recommended conditions. The following primers were used: hIL-1α 5′-TGT ATG TAT CGA CTC CCC AAG ATG AA-3′ (forward) and 5′-CCT GTG ATG GTT TCG GGT ATC TC-3′ (reverse) and RNase P1 5′-CAC TTG ATC GTC TGT GAG AAA TTC A-3′ (forward) and 5′-TGC TGC TTC ACC AGA AGA TTC-3′ (reverse). The data are shown as the change in cycle threshold (ΔCt), which can be converted to the relative transgene DNA copy number as follows: 2−ΔCt.

Northern blot analysis. Total RNA was purified from quick-frozen cardiac tissue specimens from 12-wk-old male mice using the RNeasy protocol (Qiagen). Total RNA (5–10 μg) was electrophoresed in 1.2% agarose-formaldehyde gel, blotted onto nylon membranes (Roche Molecular Biochemicals), and UV cross-linked. Hybridization was performed overnight at 42°C using a digoxigenin-labeled probe generated with a digoxigenin DNA labeling kit (Roche Molecular Biochemicals). The probe was a 660-bp Hind III/Hind II fragment from hIL-1α cDNA (Immunex). For visualization of digoxigenin-labeled IL-1α cDNA, the membranes were washed and incubated with alkaline phosphatase-labeled anti-digoxigenin Fab fragments and then in a solution of alkaline phosphatase substrate (4-nitro blue tetrazolium chloride and bromo-4-chloro-3-indolyl phosphate). The hybridization signals were detected using a scanner (Epson). The mRNA levels were quantified by 28S and 18S ribosomal RNA ethidium bromide staining.

Determination of cytokine levels. The myocardial cytokine levels were determined using a specific ELISA of hIL-1α (Japan Immuno-
Arterial blood Measurement of blood pressure and heart rate. M-mode studies were analyzed as reported previously (7).

Cardiography was performed using a Hewlett-Packard Sonos 7500 (2.5 mg/kg), and their chests were shaved. Next, transthoracic echocardiography (T-PER, Pierce Biotechnology) with protease inhibitors (Sigma-Aldrich). The extracted 50 μg of protein were separated by SDS-PAGE, and the proteins were electrophoretically transferred to polyvinylidene difluoride filters. The filters were washed and incubated with primary antibody: rabbit polyclonal anti-nuclear factor (NF)-κB p65 antibody (Abcam), anti-phosphorylated p65 antibody (Ser526) and anti-phosphorylated p38 antibody (Cell Signaling Technology), and anti-p38 MAPK antibody (Santa Cruz Biotechnology). The filters were washed and then incubated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Amersham Pharmacia Biotech). The signals were chased from Applied Biosystems. Amplification was performed with TaqMan RT reagent (Applied Biosystems) with random oligonucleotide primers. Quantitative PCR technique. RT was performed with TaqMan RT product was applied to 25 μl reaction volume according to the Taq DNA polymerase (Perkin-Elmer). PCR conditions for the various primers were as follows: 32 cycles of denaturation at 95°C for 15 s, annealing at 62°C for 15 s, and extension at 72°C for 60 s for atrial natriuretic factor [ANF, 5′-AAG AGG GCA GAT CTA TCG GA-3′ (forward) and 5′-TTG TCT TCG CCA TAA TG-3′ (reverse)]; 30 cycles of denaturation at 95°C for 15 s, annealing at 61°C for 15 s, and extension at 72°C for 60 s for B-type natriuretic peptide [BNP, 5′-CAG TTC TTG AAG GAC CAA GG-3′ (forward) and 5′-AAG AGA CCC AGG CAG AGT CA-3′ (reverse)]; 30 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 60 s for α-MHC [5′-AGA GGA GAA GCC CAA GAA GG-3′ (forward) and 5′-GCA GCC GCA TTA ATG TCT TC-3′ (reverse)]; 32 cycles of denaturation at 95°C for 15 s, annealing at 62°C for 15 s, and extension at 72°C for 60 s for β-MHC [5′-cag ctc tg aag gac cca gg-3′ (forward) and 5′-aag aag ccc agg cag agt ca-3′ (reverse)]; 30 cycles of denaturation at 95°C for 15 s, annealing at 61°C for 15 s, and extension at 72°C for 60 s for sarco/endo/plasmic reticulum Ca2+-ATPase [SERCA2a, 5′-AAG CTAT GAG GGT GTG TG-3′ (forward) and 5′-ACA ACC AAG GGT CTC CAC AG-3′ (reverse)]; 30 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 60 s for ryanodine Ca2+-release channels [CRC, 5′-AGG TGG TGC GTC ATG AGT TC-3′ (forward) and 5′-CAT CCA CAG CCT GAG TT-3′ (reverse)]; 30 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 15 s, and extension at 72°C for 120 s for GAPDH [5′-GCT CCA GGG TTT CCT ACT CC-3′ (forward) and 5′-AAG CCC ATC ACC ATC TTC CA-3′ (reverse)]. Densitometry was done using an image analysis software (Scion Image) on a computer. mRNA levels were corrected for the mrna level of GAPDH and are expressed as percentage of non-Tg mice.

Gene expressions of adhesion molecules were detected using a quantitative PCR technique. RT was performed with TaqMan RT (Applied Biosystems) with random oligonucleotide primers. The conditions were as follows: 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. TaqMan probes and primer sets for intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), platelet-endothelial cell adhesion molecule 1 (PECAM-1), and β-actin mRNAs are commercially available and were purchased from Applied Biosystems. Amplification was performed with a sequence detection system (Prism 7700, Applied Biosystems) under the following conditions: 50°C for 2 min, 95°C for 10 min, and 40 cycles at 95°C for 15 s and 60°C for 1 min. PCR results were analyzed with Sequence Detector version 1.6 (Applied Biosystems). To correct for variations in the total RNA content and unequal RT efficiency, ICAM-1, VCAM-1, and PECAM-1 quantities were normalized to the amount of β-actin mRNA.

Statistical analysis. Values are means ± SE. Two-tailed Student’s t-test and the χ2 test were used for statistical analysis. P < 0.05 was considered to be statistically significant.

RESULTS

Generation of Tg mice. After three founder lines (Tg17, Tg50, and Tg71) were obtained, quantitative PCR was used to identify the homozygous members of each line. Mean ΔCt values were −4.2, −3.0, and −5.1 for Tg17, Tg50, and Tg71, respectively. Because the most prominent increase in heart weight-to-body weight ratio was observed in the Tg71 line,
male mice of the Tg71 line were used in this study. The Tg mice show normal growth and normal gross appearance, and the 1-yr survival rate of the Tg mice is similar to that of the non-Tg mice (>95% for each group).

**mRNA expressions of hIL-1α.** A Northern blot analysis demonstrated a high level of hIL-1α mRNA in the ventricular tissue specimens of the Tg mice (Fig. 2A). Signals were either weak or absent in other organs, such as the lung and spleen (Fig. 2B).

**Cytokine protein expressions.** The hIL-1α levels of ventricular tissue specimens in Tg mice were 73 pg/100 μg protein, on average, as determined by a specific ELISA (n = 6 per group; Table 1). However, the serum level of hIL-1α was too low to be detected. There were no significant differences in mouse IL-1α (too low to be detected in both groups), IL-1β (7.6 ± 0.7 and 8.1 ± 0.7 pg/100 μg protein, respectively), and TNF-α (1.1 ± 0.1 and 1.3 ± 0.1 pg/100 μg protein, respectively) in ventricular tissue specimens between the Tg and non-Tg mice (n = 6 per group; Table 1). The protein expression of IL-6, a downstream cytokine of IL-1, in the ventricular tissue samples was greater in Tg than in non-Tg mice (20.6 ± 8.2 vs. 2.0 ± 1.1 pg/100 μg protein, P < 0.05), suggesting that the Tg-derived hIL-1α was active as a proinflammatory cytokine in the Tg mice.

**Signal activation.** IL-1 is known to activate the signaling pathways of p38 MAPK as well as NF-κB (15). We thus examined the signal activations of p38 MAPK and p65 NF-κB, which are important intracellular signaling molecules in inflammation, to estimate whether the transgene-derived hIL-1α is biologically active as a proinflammatory cytokine in mice, because the transgene was of human origin.

**Heart morphology.** Postmortem weight measurements demonstrated a significant increase in heart weight-to-body weight ratio in the Tg mice compared with the non-Tg mice at 6 and 12 wk of age (Table 2).

The tissue sections stained for hematoxylin-eosin revealed an increase in the thickness of the ventricular free wall from the Tg mice (Fig. 4A). A histological examination of hearts from the Tg mice revealed a normal linear arrangement of myofibrils. No obvious myofibrillar disarray or prominent interstitial infiltrating cells were observed in any of the sections from the Tg mice (Fig. 4B).

The cross-sectional area of the ventricular myocytes measured on histological sections significantly increased by 20% in the Tg mice compared with the non-Tg mice (280 ± 7 vs. 230 ± 6 μm², P < 0.05; Fig. 5).

**Heart ultrastructural analysis.** An ultrastructural TEM study revealed that mitochondria were rounded and greater in number and T tubules were enlarged in the Tg mice compared with the non-Tg mice. The sarcomere structure of the Tg mice appeared to be normal (Fig. 6).

**Cardiac functions and hemodynamic parameters.** An echocardiographic analysis confirmed a decrease in LV end-diastolic diameter in the Tg mice compared with the non-Tg mice (3.18 ± 0.06 vs. 3.40 ± 0.08 mm), although the difference was not significant (P = 0.07). The end-diastolic LV posterior wall thickness increased significantly in the Tg mice compared with the non-Tg mice (0.92 ± 0.06 vs. 0.70 ± 0.05 mm, P < 0.05), indicating concentric LV hypertrophy in the Tg mice. The percent fractional shortening of the Tg mice was comparable to that of the non-Tg mice (45 ± 3 and 46 ± 4%, P = NS). There was no significant difference in heart rate and systolic blood pressure between the non-Tg and Tg mice (Table 3).

**mRNA expressions of hypertrophic markers and cell adhesion molecules.** Semiquantitative RT-PCR analyses demonstrated an increase in ANF (175 ± 7%), BNP (190 ± 12%), and β-MHC (536 ± 40%) gene expressions and a decrease in α-MHC (66 ± 4%), SERCA2a (80 ± 2%), and CRC (88 ± 3%) gene expressions in the Tg mice compared with the non-Tg mice (n = 4 per group; Fig. 7).

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**Table 1. Cytokine levels of ventricular tissue**

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<thead>
<tr>
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<th>Non-Tg</th>
<th>Tg</th>
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<tbody>
<tr>
<td>hIL-1α</td>
<td>UD</td>
<td>73.0 ± 7.8*</td>
</tr>
<tr>
<td>mIL-1α</td>
<td>UD</td>
<td></td>
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<tr>
<td>mIL-1β</td>
<td>8.1 ± 0.7</td>
<td>7.6 ± 0.7</td>
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<tr>
<td>mTNF-α</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.1</td>
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<tr>
<td>mIL-6</td>
<td>2.0 ± 1.1</td>
<td>20.6 ± 8.2*</td>
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Values are means ± SE expressed as pg/100 ng protein (n = 6). Transgenic (Tg) mice showed a significant increase in human (h) IL-1α and mouse (m) IL-6 protein levels compared with non-Tg mice. UD, undetected. *P < 0.05 vs. non-Tg.
We further evaluated the mRNA expressions of cell adhesion molecules, because no inflammatory infiltrates were microscopically detected in the Tg myocardium. The ICAM-1-to-β-actin (4.73 ± 0.46 and 2.15 ± 0.32% for Tg and non-Tg, respectively, \( P < 0.05 \)) and VCAM-1-to-β-actin (15.8 ± 2.0 and 8.3 ± 0.5% for Tg and non-Tg, respectively, \( P < 0.05 \)) mRNA ratios were significantly elevated in the Tg myocardium compared with the non-Tg myocardium (4 per group). In contrast, the PECAM-1-to-β-actin mRNA ratio (89.7 ± 3.9 and 88.8 ± 6.3% for Tg and non-Tg, respectively, \( P = \text{NS} \)) did not increase (4 per group).

**DISCUSSION**

In this study, we demonstrated concentric LV hypertrophy with preserved LV systolic function in mice with a cardiac-specific overexpression of mature-type hIL-1α. An elevated expression of IL-1 in hearts has been revealed in a variety of cardiovascular diseases, such as myocarditis (22), myocardial ischemia and infarction (18), and congestive heart failure (13, 18, 22). IL-1 is also implicated in the pathogenesis of the hypertrophied myocardium in vitro and in vivo (21). We found that mice with a ubiquitous overexpression of hIL-1α, which were originally generated as a mouse model of rheumatoid arthritis, unexpectedly showed prominent LV hypertrophy and congestive heart failure to death within 2 wk after birth. However, the findings we reported did not address whether hIL-1α expressed in the myocardium caused the LV remodeling, because hIL-1α was ubiquitously expressed in the Tg mice. Thus we generated and analyzed Tg mice with a cardiac-specific overexpression of hIL-1α in this study. The Tg mice showed an increased heart weight-to-body weight ratio. A histological analysis revealed that the hypertrophy was the result of the increased size of the myocardium. TEM observations revealed rounded mitochondria that were increased in number. The overexpression of hIL-1α increased mRNA expressions of ANF, BNP, and β-MHC and decreased mRNA expressions of α-MHC, SERCA2a, and CRC in the myocardium. An echocardiographic study revealed that the end-diastolic LV wall was thicker in the Tg than in the non-Tg mice. These findings are compatible with LV hypertrophy (9). The responses in the Tg heart are further evidence that LV hypertrophy is the result of hIL-1α overexpression, because hypertrophy was observed without evidence of hemodynamic overload.

Cardiac hypertrophy is defined as an enlargement of the heart associated with an increase in cardiomyocyte cell volume that occurs in response to diverse pathophysiological stimuli such as hypertension, ischemic heart disease, valvular insufficiency, infectious agents, or mutations in sarcomeric genes (14). Hypertrophic growth of the myocardium is thought to

**Table 2. Heart and body weights**

<table>
<thead>
<tr>
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<th>6 wk (n = 10)</th>
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<th>12 wk (n = 20)</th>
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<tbody>
<tr>
<td></td>
<td>HW, mg</td>
<td>BW, g</td>
<td>HW/BW, mg/g</td>
</tr>
<tr>
<td>Non-Tg</td>
<td>72.7±3.3</td>
<td>15.2±0.8</td>
<td>4.78±0.10</td>
</tr>
<tr>
<td>Tg</td>
<td>86.0±5.0</td>
<td>15.3±1.0</td>
<td>5.62±0.16*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Heart weight (HW)-to-body weight (BW) ratio was significantly higher in Tg than in non-Tg mice at 6 and 12 wk of age. * \( P < 0.05 \) vs non-Tg.
preserve pump function, although prolongation of the hypertrophic state is a leading predictor for the development of sudden death and heart failure (6, 12). In our study, the cardiac-specific hIL-1α-overexpressing mice showed LV hypertrophy but a preserved systolic function and a normal 1-yr survival rate. However, we revealed that the pathological significance of the LV hypertrophy observed in this study was an increase in ANF, BNP, and β-MHC gene expression and a decrease in α-MHC, SERCA2a, and CRC gene expression in the myocardium. Therefore, these results suggest that the hypertrophy may be a consequence of compensatory mechanisms. The result is different from those observed in the ubiquitous hIL-1α-overexpressing mice, which demonstrated congestive heart failure to death within 2 wk after birth (7). We speculate that the phenotypic difference most likely is due to the different regulation of the hIL-1α expression by each promoter as well as the distinct degrees of hIL-1α expression. The gene expression controlled under the α-MHC promoter can affect the myocardium only after birth, whereas the expression under the CAG promoter can affect the myocardium even before birth (20). The IL-1 transgene regulated under the CAG promoter acts on fetal myocardial cells, which maintain the capacity to divide. On the other hand, the IL-1 transgene under the α-MHC promoter acts on mature myocardial cells, which have no capacity to divide. This could lead to an increase in myocyte number and volume and cause severe ventricular hypertrophy and congestive heart failure in CAG-IL-1 Tg mice. In addition, because of a ubiquitous overexpression of IL-1 in CAG-IL-1 Tg mice, mononuclear cells and macrophages overexpressing IL-1 appeared to gain a tissue-destructive phenotype (17), which was likely to cause robust inflammatory infiltrations in the hearts and, subsequently, an aggravation of myocardial injury.

The effects of TNF-α, which is also a proinflammatory cytokine, on the myocardium have been well investigated. A recent report demonstrated that a chronic overexpression of TNF-α (TNF-α Tg) in mice induced LV hypertrophy, eventual LV dilation, and congestive heart failure (8). In contrast, in our

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**Fig. 4. Heart morphology.**

**A:** representative hematoxylin-and-eosin (HE)-stained thin slices of cardiac ventricles. Heart mass was increased in Tg mice. Arrows indicate thickness of left ventricular free wall. Scale bar, 1.0 mm. **B:** representative hematoxylin-and-eosin- and Masson’s trichrome-stained sections from adult heart of non-Tg and Tg mice. Sections revealed a normal linear arrangement of myofibrils in Tg mice. Scale bars, 30 μm.
Fig. 5. Cross-sectional area of ventricular cardiomyocytes (n = 50 per mouse) was evaluated on Masson’s trichrome-stained sections of hearts in non-Tg and Tg mice (n = 4 per group). Cross-sectional area was increased in Tg mice. Scale bars, 20 μm.

Fig. 6. Heart ultrastructural analysis. High-magnification images of representative slices from a compact layer from left ventricle illustrate mitochondria (M) and T tubule (arrowhead) alterations in Tg heart. Z, Z bands; S, sarcomere. Scale bars, 2 μm.
LV hypertrophy and interleukin-1

Table 3. Cardiac functions and hemodynamic parameters

<table>
<thead>
<tr>
<th></th>
<th>LVEDd, mm</th>
<th>LVESd, mm</th>
<th>FS, %</th>
<th>PWT, mm</th>
<th>HR, beats/min</th>
<th>SBP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Tg</td>
<td>3.40 ± 0.08</td>
<td>1.83 ± 0.10</td>
<td>46 ± 4</td>
<td>0.70 ± 0.05</td>
<td>556 ± 34</td>
<td>98 ± 12</td>
</tr>
<tr>
<td>Tg</td>
<td>3.18 ± 0.06</td>
<td>1.74 ± 0.09</td>
<td>45 ± 3</td>
<td>0.92 ± 0.06*</td>
<td>544 ± 30</td>
<td>101 ± 14</td>
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</table>

Values are means ± SE (n = 6). Echocardiographic analysis revealed concentric left ventricular (LV) hypertrophy and preserved LV systolic functions in Tg mice. No significant difference in heart rate (HR) and systolic blood pressure (SBP) was observed between non-Tg and Tg mice. LVEDd and LVESd, LV end-diastolic and end-systolic diameter; PWT, posterior systolic wall thickness; FS, fractional shortening. *P < 0.05 vs. non-Tg.

mice, a persistent overexpression of IL-1 in the heart induced ventricular hypertrophy, but not ventricular dilatation or congestive heart failure. These phenotypic differences might be explained by the cytokines expressed in the heart. The TNF-α Tg mice showed a significant increase in the myocardial TNF-α and IL-1β levels (8). Kadokami et al. (8) reported that, in TNF-α Tg mice treated with a TNF-α-neutralizing antibody, LV systolic function was improved to the same level as in the non-Tg mice; however, these TNF-α Tg mice still showed a high expression of myocardial IL-1β without any attenuation of development of LV hypertrophy. In our study, IL-1 Tg mice showed a significant increase in the myocardial level of hIL-1β, but not TNF-α (7).

There has also been considerable interest in the relative roles of IL-1α and IL-1β in cardiac hypertrophy. Both isoforms have been demonstrated to be involved in cardiac hypertrophy (1, 10), and mRNA expressions of IL-1β in hypertrophied myocardium were reported to be higher than those of IL-1α (1, 10). Although the relative pathogenic contributions of IL-1α and IL-1β to myocardial hypertrophy remain to be fully elucidated, the roles of IL-1β in cardiac hypertrophy have been well investigated. The expression of IL-1β increased in hypertrophied hearts with pressure overload in vivo (21) and in hypertrophied myocardium in vitro (13). In addition, in vitro experiments have revealed that IL-1β can induce cardiomyocyte hypertrophy (19, 23). The two isoforms bind to the same receptor, IL-1 type 1 receptor, to elicit the biological effects of IL-1 (4). However, the precursor form of IL-1α, in contrast to IL-1β, is known to act as a membrane-associated IL-1 (4). We could not detect hIL-1α protein in the serum, suggesting that membrane-associated IL-1 on the cardiomyocytes contributes to cardiac hypertrophy through cell-to-cell interaction. Furthermore, by histological evaluation, we recognized a lack of inflammatory infiltrates in the Tg myocardium. Thus we measured the mRNA expressions of cell adhesion molecules. ICAM-1 and VCAM-1 were found to induce the firm adhesion of inflammatory cells on the vascular surface, whereas PECAM-1 plays a critical role in the extravasation of leukocytes into underlying tissue as previously reported (2). In our study, we demonstrated an increase in ICAM-1 and VACM-1, but not PECAM-1, gene expression in the Tg myocardium. The chronic cardiac-specific overexpression of IL-1α did not augment PECAM expression in the myocardium, which might result in a lack of leukocyte extravasation into the myocardial tissue.

Niki et al. (17) reported that the bone marrow macrophages derived from hIL-1α-overexpressing mice stimulated proliferation of murine cells and that the mitogenic activity was suppressed by the neutralizing antibody against hIL-1α. In our study, to confirm the transactivation of murine myocardium by hIL-1α, we demonstrated an increase in IL-6 protein levels, phosphorylated p38 MAPK, and phosphorylated p65 (Ser536) NF-κB in the Tg hearts. IL-6, a downstream cytokine activated by IL-1, was reported to be a cardioprotective and hypertrophic signal via gp130. Thus gp130 signals as an effector might be involved in the pathogenesis of the phenotypes observed in this study. Both p38 MAPK and NF-κB have been traditionally implicated as pivotal intracellular mediators of the inflammatory responses induced by IL-1. p38 MAPK is also associated with myocardial hypertrophy. However, it remains to be fully elucidated whether p38 MAPK itself plays a role in the onset and development of myocardial hypertrophy in mammalian adult hearts (3, 16, 24).

Fig. 7. Gene expressions analyzed using semiquantitative RT-PCR. RT-PCR bands are representative of 4 separate experiments. RT-PCR analyses demonstrated an increase in atrial natriuretic factor (ANF), B-type natriuretic protein (BNP), and β-myosin heavy chain (β-MHC) gene expressions and a decrease in α-myosin heavy chain (α-MHC), sarco/endoplasmic reticulum Ca2+-ATPase type 2 (SERCA2a), and CRC gene expressions in Tg mice compared with non-Tg mice (n = 4 per group).
In summary, we generated mice with a cardiac-specific overexpression of hIL-1α. The Tg mice showed concentric LV hypertrophy with a preserved LV systolic function. Our findings suggest that the cardiac expression of IL-1 may, therefore, cause LV hypertrophy in vivo.

REFERENCES