Soluble Tumor Necrosis Factor Receptor Abrogates Myocardial Inflammation but Not Hypertrophy in Cytokine-Induced Cardiomyopathy

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Background—Transgenic mice with cardiac-specific overexpression of tumor necrosis factor (TNF)-α develop dilated cardiomyopathy. The present study was designed to evaluate therapeutic effects of adenovirus-mediated neutralization of TNF-α on this model.

Methods and Results—An adenovirus encoding the 55-kDa TNF receptor–IgG fusion protein (AdTNFRI) was injected intravenously into 6-week-old transgenic mice, which resulted in high levels of TNFRI in both plasma and myocardium. AdTNFRI did not reverse cardiomegaly but abrogated myocardial inflammation. Furthermore, AdTNFRI blocked the myocardial expression of intercellular adhesion molecule-1 and downstream cytokines, including interleukin-1β and monocyte chemotactic protein-1. Downregulation of α-myosin heavy chain was restored by the treatment, whereas upregulation of β-myosin heavy chain was not reversed. In contrast, the downregulation of sarcoplasmic reticulum Ca2+-ATPase and phospholamban was normalized by AdTNFRI. Echocardiographic measurements showed that left ventricular end-systolic diameter was significantly larger in transgenic mice than in control mice, and this increase was reversed by the AdTNFRI treatment. However, left ventricular wall thickening was not reversed.

Conclusions—These results suggest that anti-TNF therapy may hold promise in the treatment of end-stage heart failure. (Circulation. 2000;101:2518-2525.)

Key Words: viruses ■ genes ■ hormones

Tumor necrosis factor (TNF)-α is a proinflammatory cytokine with pleiotropic biological effects.1 Elevated plasma levels of TNF-α occur in a variety of cardiovascular diseases, including acute myocarditis, cardiac allograft rejection, myocardial infarction, and congestive heart failure (see review in Reference 2). Recent studies demonstrated that the heart itself can produce TNF-α in these disorders.3 To investigate the pathophysiologic importance of myocardial production of TNF-α, we created 2 lines of transgenic mice that overexpress TNF-α in the heart under the control of α-myosin heavy chain (MHC) promoter.4,5 When TNF-α was robustly overexpressed, all mice developed severe lymphohistiocytic myocarditis and died in the neonatal period.4 When TNF-α overexpression was more modest, most mice survived the neonatal period; however, this line displayed a 6-month mortality of nearly 25%.5 Transgenic mice developed ventricular hypertrophy and dilatation, interstitial infiltrates and fibrosis, attenuation of adrenergic responsiveness, reexpression of atrial natriuretic factor (ANF) in the ventricle, and overt congestive heart failure.

Suppression of TNF-α bioactivity ameliorates the severity of myocarditis induced by injection of myosin6 or encephalomyocarditis virus.7 Furthermore, soluble TNF-binding proteins reverse the negative inotropic effects of TNF-α in vitro.8 The present study was undertaken to assess the effects of TNF-α suppression in mice having myocardial inflammation secondary to TNF-α overexpression using a replication-deficient recombinant adenovirus encoding an extracellular domain of human 55-kDa TNF receptor coupled with a mouse IgG heavy chain (AdTNFRI).9

Methods

Animals

Previously characterized transgenic mice (TNF1.6)5 were used under protocols approved by the Institutional Animal Care and Use Committee, University of Pittsburgh.

Adenoviruses

A replication-deficient recombinant adenovirus (AdTNFRI)9 encoding the extracellular domain of the human 55-kDa TNF receptor coupled with a mouse IgG heavy chain9 was generously provided by Dr Bruce Beutler, University of Texas Southwestern Medical Center, Dallas. An adenovirus encoding LacZ (AdLacZ) served as a control. Viruses were propagated in 293 cells and purified as previously described.9,10
LVEDD and LVESD indicate left ventricular shortening (%) was calculated as LVFS (%) from 3 to 5 consecutive RR intervals. Left ventricular fractional thickness were made by use of the leading-edge convention of the largest left ventricular diameter.

M-mode imaging was obtained from the short-axis view at the level of the largest left ventricular diameter.

M-mode measurements of left ventricular end-diastolic and end-systolic diameters and left ventricular anterior and posterior wall thickness were made by use of the leading-edge convention of the American Society of Echocardiography. Three to 5 beats were averaged for each measurement. End diastole was determined at the maximal left ventricular diastolic dimension, and end systole was taken at the peak of posterior wall motion. Heart rate was determined from 3 to 5 consecutive RR intervals. Left ventricular fractional shortening (%) was calculated as LVFS (%)=(LVEDD−LVESD)/LVEDD)×100, where LVEDD and LVESD indicate left ventricular end-diastolic and end-systolic diameters, respectively.

### Quantification of Myocarditis

Myocardial infiltration was quantified in hematoxylin and eosin–stained sections by determination of nuclear density (nuclei/mm²). Because it is difficult to differentiate inflammatory cells from myocytes and/or fibroblasts, all nuclei were included. In each animal, 6 independent high-power fields (0.233×0.312 mm; 0.0729-mm² area) were analyzed and averaged by investigators blinded to the treatment group.

### RNase Protection

Total RNA was extracted from frozen tissues as described.\(^4,5\) RNase protection assays (RPAs) were performed according to the manufacturer’s protocol (RiboQuant, PharMingen; template sets mCK-1b, mCK-2b, mCK-3b, and mCK-5) with 5 μg of total RNA. After RNAase digestion, protected probes were resolved on denaturing polyacrylamide gels and quantified by PhosphoImager (ImageQuant software, Molecular Dynamics). The value of each hybridized probe was normalized to that of GAPDH included in each template set as an internal control.

### Slot Blot

Total RNA (2.5 μg each sample) denatured with formaldehyde was vacuum-blotted onto nitrocellulose. Hybridization probes were prepared from oligomers complementary to murine α- or β-MHC\(^11\) and other probes (18S ribosomal RNA, ANF, sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase [SERCA], and phospholamban) as previously described.\(^5,12\) MHC oligomer probes were hybridized (55°C in 4X SSC, 5× Denhardt’s, 0.1% SDS, 0.05% sodium pyrophosphate, rRNA 20 μg/mL, \(^32\)P-labeled oligomer 3 ng/mL), washed (2× SSC–0.1% SDS at 60°C), and exposed to a storage phosphor screen, and radioactive images were quantified as described above. Filters were rehybridized with the 18S oligonucleotide, reexposed, and quantified.\(^5,12\) Hybridization signals were normalized to that of the 18S probe and in turn normalized to the mean of the control samples.

### Reverse Transcription–Polymerase Chain Reaction

Reverse transcription–polymerase chain reaction (RT-PCR) reactions were performed as described.\(^4,5,12\) Oligonucleotide primer pairs included (1) intercellular adhesion molecule-1 (ICAM-1) sense primer corresponding to base pairs 1112 to 1135 and antisense primer complementary to murine α- and β-MHC\(^11\) and other probes (18S ribosomal RNA, ANF, sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\)-ATPase [SERCA], and phospholamban) as previously described.\(^5,12\) MHC oligomer probes were hybridized (55°C in 4X SSC, 5× Denhardt’s, 0.1% SDS, 0.05% sodium pyrophosphate, rRNA 20 μg/mL, \(^32\)P-labeled oligomer 3 ng/mL), washed (2× SSC–0.1% SDS at 60°C), and exposed to a storage phosphor screen, and radioactive images were quantified as described above. Filters were rehybridized with the 18S oligonucleotide, reexposed, and quantified.\(^5,12\) Hybridization signals were normalized to that of the 18S probe and in turn normalized to the mean of the control samples.

### Table 1. Body and Ventricular Weights of Adenovirus-Treated Mice

<table>
<thead>
<tr>
<th>Age</th>
<th>WT</th>
<th>TG</th>
<th>TG+AdLacZ</th>
<th>TG+AdTNFRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 wk old/2 wk Adv</td>
<td>n=18</td>
<td>n=15</td>
<td>n=10</td>
<td>n=15</td>
</tr>
<tr>
<td>BW, g</td>
<td>20.6±1.4</td>
<td>22.4±1.5*</td>
<td>22.3±1.5*</td>
<td>23.8±2.0*</td>
</tr>
<tr>
<td>VW, mg</td>
<td>82.9±6.8</td>
<td>114.8±13.1*</td>
<td>112.7±9.4*</td>
<td>113.0±9.9*</td>
</tr>
<tr>
<td>VW/BW, mg/g</td>
<td>4.03±0.19</td>
<td>5.15±0.73*</td>
<td>5.06±0.30*</td>
<td>4.76±0.23*</td>
</tr>
<tr>
<td>12 wk old/6 wk Adv</td>
<td>n=6</td>
<td>n=5</td>
<td>ND</td>
<td>n=5</td>
</tr>
<tr>
<td>BW, g</td>
<td>21.0±1.7</td>
<td>21.7±2.4</td>
<td>25.0±1.0†</td>
<td></td>
</tr>
<tr>
<td>VW, mg</td>
<td>84.1±4.2</td>
<td>103.1±3.9*</td>
<td>113.1±6.3†</td>
<td></td>
</tr>
<tr>
<td>VW/BW, mg/g</td>
<td>4.01±0.18</td>
<td>4.77±0.42*</td>
<td>4.54±0.34*</td>
<td></td>
</tr>
</tbody>
</table>

WT indicates wild-type mice; TG, transgenic mice; TG+AdLacZ, transgenic mice 2 weeks after inoculation with AdLacZ; TG+AdTNFRI, transgenic mice 2 or 6 weeks after inoculation with AdTNFRI; AdV, adenovirus; BW, body weight; and VW, ventricular weight. Values are mean±SD.

*P<0.05 vs WT, †P<0.05 TG+AdTNFRI vs TG.
Enzyme-Linked Immunosorbent Assay

Cytokine protein levels were assessed by ELISA (mouse TNF-α, mouse interleukin [IL]-1β, mouse monocyte chemotactic protein [MCP]-1, and human TNFRI, Quantikine, R&D Systems). Plasma samples (assayed in duplicate) were measured at a dilution of $10^{-1}$ to $10^{-6}$. Cytokines in the myocardium were measured as previously reported, with 100 μg of protein for TNF-α, IL-1β, and MCP-1 and 1 μg for TNFRI. Standard reference cytokines were provided by the manufacturer. Values are reported as pg/mg or ng/mg protein. Because this study used a different vendor for the TNF-α ELISA kits, the values of TNF-α protein in the myocardium were substantially lower than those previously reported. This is attributable to differences in the standard reference cytokines as well as a substantially lower background of TNF-α in tissue homogenates.

Immunohistochemistry

Mouse hearts were fixed in ice-cold 2% paraformaldehyde, immersed in ice-cold 30% sucrose, and flash-frozen in OCT (Miles) medium with isopentane cooled by liquid nitrogen. Blocks were cut at 10 μm, and sections were mounted, immersion-fixed in acetone, rinsed in PBS, and treated for 30 minutes with 5% normal rabbit serum. Primary antibodies used were polyclonal goat anti-murine TNF-α (1:100, Santa Cruz Biotechnology), goat anti-murine IL-1β, and goat anti-murine MCP-1 (both 1:100, R&D Systems). Samples were treated with primary antibody (24 hours at 4°C), rinsed with PBS, treated (2 hours) with multilink biotinylated anti-goat secondary antibody (1:4 dilution, Biogenex Laboratories), rinsed in PBS, and treated with avidin-biotin complex (45 minutes; Vector Laboratories). Reactions were visualized with 3-amino-9-ethyl-carbazole in 0.1 mol/L acetate buffer, pH 5.2. Sections were weakly counterstained with Mayer’s hematoxylin.

Statistics

Results are presented as mean±SD. Statistical comparisons were performed by ANOVA with Student-Newman-Keuls post hoc test. Differences were considered significant at a value of $P<0.05$.

Results

Production of TNF Receptor Fusion Protein

Pilot studies in male wild-type mice determined that intravenous injections of $10^7$ and $10^8$ pfu of AdTNFRI produced a substantial amount of TNFRI in plasma after 1 week, whereas intraperitoneal injection did not (Figure 1). Plasma levels of TNFRI declined thereafter but remained in the μg/mL range for as long as 6 weeks. Attempts to reinoculate mice to boost TNFRI levels were unsuccessful (data not shown). Subsequent studies used a single intravenous inoculation with $10^9$ pfu of adenovirus.

Six-week-old female transgenic (TNF1.6) mice were injected with $10^9$ pfu of either AdTNFRI or AdLacZ and euthanized for analysis after an additional 2 or 6 weeks. Age-matched transgenic or wild-type mice were euthanized as untreated controls. As in wild-type mice, AdTNFRI produced substantial amounts of soluble TNFRI in plasma (96.2±61.0 μg/mL) as well as in myocardium (82.5±21.5 ng/mg protein) after 2 weeks. Because the amounts of TNF-α protein in the myocardium of AdTNFRI-treated transgenic mice was 419±137 pg/mg protein, there was a large excess (~200-fold) of TNFRI protein in the heart.

Cardiomegaly

Transgenic mice presented significant cardiomegaly, as indicated by the increased ventricular weight/body weight ratio, and 2 weeks of anti-TNF treatment was insufficient to ameliorate cardiomegaly (Table 1). Furthermore, cardiomegaly remained unchanged 6 weeks after injection of AdTNFRI, although plasma levels of TNFRI remained high (32.3±8.8 μg/mL).

Myocardial Inflammation

Eight-week-old untreated transgenic mice presented a diffuse interstitial infiltration in the myocardium consisting of mostly histiocytes with some lymphocytes (Figure 2). Two weeks
after treatment with AdTNFRI, interstitial infiltration was substantially reduced, as evidenced by nuclear density (Figure 3), an effect not observed by treatment with AdLacZ. Myocardial infiltrates remained suppressed for 6 weeks (data not shown), reflecting the persistent elevation in plasma levels of TNFRI.

To assess the potential mechanism by which TNFRI overexpression reduces myocardial infiltration, we analyzed expression of ICAM-1 by RT-PCR in wild-type mice, TNF1.6 mice, and TNF1.6 mice 2 weeks after inoculation with AdTNFRI. RT-PCR assessment of total (endothelial and transgene-driven) TNF-α expression (bottom) in same animals. M indicates DNA marker; size is in base pairs.

Cytokine Expression

Because TNF-α can induce the expression of other proinflammatory cytokines and chemokines that contribute to TNF-α–induced pathophysiology,1 we examined the expression of additional cytokines by use of multiprobe RPAs. Representative images of RPAs are shown in Figure 5, and quantitative results are summarized in Table 2. Although overexpression of TNF-α induced a large number of cytokines, the induction of IL-1β and MCP-1 was particularly robust. With the exception of transforming growth factor-β induction, all of the changes in cytokine and chemokine expression evident in the TNF-α transgenic mice were reversed after 2 weeks of treatment with soluble TNF receptor.

To confirm that changes in mRNA reflected alterations at the protein level, we selected 3 cytokines for ELISA: TNF-α, IL-1β, and MCP-1. IL-1β was chosen because of its synergistic effects with TNF-α, as well as its independent effects on cardiomyocyte function and gene expression.12,13 MCP-1, a prominent signal for the accumulation of monocytes, is expressed in response to proinflammatory cytokines14 and may be an important mediator of myocardial infiltration.15 All 3 cytokine proteins were not found in wild-type mice but were abundant in the TNF-α transgenic mice (Figure 6). Two weeks of anti-TNF treatment abrogated the induction of IL-1β and MCP-1 proteins, consistent with changes in the transcript levels. The induction of these downstream cytokines remained inhibited 6 weeks after treatment (data not shown). In contrast, despite a reversal of myocardial infiltration and a reduction in other cytokine expression, anti-TNF treatment actually increased the immunodetectable levels of TNF-α in myocardium 2-fold.

Immunohistochemical stains were performed to identify the cell source of these cytokines (Figure 7). Anti–TNF-α resulted in diffuse background staining with some positive-stained interstitial cells, suggesting that both cardiomyocytes and infiltrating cells produced TNF-α in the myocardium. In contrast, the staining for IL-1β and MCP-1 was confined to interstitial cells, including inflammatory infiltrates and fibroblasts.

Figure 4. RT-PCR assessment of ICAM-1 expression (top) in ventricular RNA isolated from wild-type (WT), TNF-α transgenic (TNF1.6), and TNF-α transgenic mice 2 weeks after inoculation with AdTNFRI (TNF1.6 + AdTNFRI). RT-PCR assessment of total (endothelial and transgene-driven) TNF-α expression (bottom) in same animals. M indicates DNA marker; size is in base pairs.

Figure 5. Representative images of multiprobe RPA: (A) mCK-3b, (B) mCK-2b, (C) mCK-1b, and (D) mCK-5. Animal group abbreviations as in Figure 3. LT indicates lymphotxin; IL, interleukin; IFN, interferon; TGF, transforming growth factor; MIF, macrophage inflammatory protein; IP, interferon-inducible protein; and TCA, T-cell activation gene.
Cardiac-Specific Gene Expression

Figure 8 summarizes changes in the expression of cardiac-specific genes in TNF1.6 mice. Relative to wild-type untreated controls, α-MHC, SERCA, and phospholamban transcripts were downregulated, whereas β-MHC and ANF were upregulated, in the ventricles of transgenic mice. Treatment with AdLacZ had no significant effects on the expression of these genes, whereas anti-TNF treatment with AdTNFRI reversed changes in α-MHC, SERCA, and phospholamban, partially reversed ANF, and had no effect on β-MHC expression. Gene transcript levels after the 6-week treatment were similar to those observed after the 2-week treatment. α-MHC, SERCA, and phospholamban transcripts were restored to wild-type levels (0.89±0.11, 0.95±0.25, and 0.96±0.20, n=4, respectively), whereas β-MHC and ANF transcripts remained elevated (4.21±0.80 and 4.00±1.68, n=4). The trend toward further reduction in ANF transcripts was not statistically significant.

Anti-TNF Therapy and Cardiac Function

Examples of M-mode echocardiographic measurements in TNF-α transgenic mice with or without AdTNFRI treatment and wild-type controls are presented in Figure 9 and quantitative results in Table 3. At this age, the left ventricular fractional shortening in transgenic mice was comparable to that in controls, whereas left ventricular end-systolic diameter

### Table 2. Expression of Cytokines in the Myocardium

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>TG</th>
<th>TG + AdLacZ</th>
<th>TG + AdTNFRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>0.0006±0.0009</td>
<td>0.3832±0.0379*</td>
<td>0.3874±0.0766*</td>
<td>0.4903±0.1916*</td>
</tr>
<tr>
<td>TNF-β</td>
<td>0.0001±0.0002</td>
<td>0.0006±0.0008</td>
<td>0.0006±0.0008</td>
<td>0.0004±0.0007</td>
</tr>
<tr>
<td>LT-β</td>
<td>0.0007±0.0003</td>
<td>0.0162±0.0087*</td>
<td>0.0183±0.0134*</td>
<td>0.0044±0.0043†</td>
</tr>
<tr>
<td>IL-1α</td>
<td>0.0001±0.0001</td>
<td>0.0040±0.0039*</td>
<td>0.0021±0.0003*</td>
<td>0.0001±0.0001†</td>
</tr>
<tr>
<td>IL-1β</td>
<td>0.0005±0.0001</td>
<td>0.0477±0.0499*</td>
<td>0.0217±0.0026*</td>
<td>0.0013±0.0010†</td>
</tr>
<tr>
<td>IL-1Ra</td>
<td>0.0002±0.0001</td>
<td>0.0294±0.0000*</td>
<td>0.0149±0.0002*</td>
<td>0.0003±0.0002†</td>
</tr>
<tr>
<td>IL-10</td>
<td>&lt;0.0001</td>
<td>0.0007±0.0003*</td>
<td>0.0006±0.0007</td>
<td>0.0001±0.0001†</td>
</tr>
<tr>
<td>IL-12p35</td>
<td>&lt;0.0001</td>
<td>0.0017±0.0018*</td>
<td>0.0007±0.0001*</td>
<td>&lt;0.0001†</td>
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<tr>
<td>IL-12p40</td>
<td>&lt;0.0001</td>
<td>0.0008±0.0006*</td>
<td>0.0006±0.0001*</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>IL-15</td>
<td>0.0120±0.0030</td>
<td>0.0066±0.0025*</td>
<td>0.0074±0.0015*</td>
<td>0.0106±0.0023†</td>
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<tr>
<td>IL-18</td>
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<td>0.0029±0.0023*</td>
<td>0.0025±0.0001*</td>
<td>0.0004±0.0002†</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>0.0018±0.0009</td>
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<td>0.0087±0.0023*</td>
<td>0.0061±0.0013*</td>
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<tr>
<td>TGF-β2</td>
<td>0.0015±0.0011</td>
<td>0.0315±0.0095*</td>
<td>0.0221±0.0172*</td>
<td>0.0360±0.0246*</td>
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<tr>
<td>TGF-β3</td>
<td>0.0029±0.0035</td>
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<td>0.0149±0.0060*</td>
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<tr>
<td>MIF</td>
<td>0.0520±0.0047</td>
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<td>0.1148±0.0045*</td>
<td>0.0504±0.0130†</td>
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<tr>
<td>RANTES</td>
<td>0.0013±0.0006</td>
<td>0.0384±0.0177*</td>
<td>0.1451±0.1109*</td>
<td>0.0077±0.0055†</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>0.0010±0.0007</td>
<td>0.0091±0.0021*</td>
<td>0.0101±0.0047*</td>
<td>0.0037±0.0023†</td>
</tr>
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<td>Lymphotactin</td>
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<td>0.0040±0.0028*</td>
<td>0.0012±0.0010†</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.0034±0.0015</td>
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<td>0.0595±0.0251*</td>
<td>0.0026±0.0028†</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>0.0007±0.0004</td>
<td>0.0068±0.0010*</td>
<td>0.0063±0.0017*</td>
<td>0.0030±0.0015†</td>
</tr>
<tr>
<td>MIP-1β</td>
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<td>0.0007±0.0002*</td>
<td>0.0031±0.0019*</td>
<td>0.0003±0.0002†</td>
</tr>
<tr>
<td>MIP-2</td>
<td>0.0004±0.0001</td>
<td>0.0125±0.0024*</td>
<td>0.0102±0.0046*</td>
<td>0.0004±0.0003†</td>
</tr>
</tbody>
</table>

*P<0.05 vs WT, †P<0.05 TG + AdTNFRI vs TG + AdLacZ.

**Figure 6.** Protein levels of cytokines in myocardium: (A) TNF-α, (B) IL-1β, and (C) MCP-1. Abbreviations as in Figure 3. Values are mean±SD (n=6). *P<0.05 vs WT, †P<0.05 TG + AdTNFRI vs TG + AdLacZ.
was significantly larger in transgenic mice than in control mice, suggesting a latent left ventricular dysfunction in young transgenic mice. This increase in the diameter was reversed by the AdTNFRI treatment. In contrast, significant left ventricular wall thickening in the transgenic mice was not reversed after the TNF neutralizing therapy.

**Discussion**

Soluble TNF receptor was achieved by intravenous injection with a recombinant adenovirus encoding a fusion protein containing the extracellular domain of human TNFRI (p55) coupled with a mouse IgG heavy chain. The virus transfects the liver, which then produces soluble TNFRI. A sustained secretion of TNFRI into the plasma permeates the extracellular space of multiple organs, including the myocardium. The principal limitation of using recombinant adenovirus is that endogenous immunological activity reduces AdTNFRI expression and significant levels of TNFRI cannot be found after several months. However, beneficial biological effects of TNFRI in mice overexpressing proinflammatory cytokines

**Figure 7.** Immunohistochemical staining of myocardium of transgenic mice for (A) TNF-α, (B) IL-1β, and (C) MCP-1. Arrows indicate positive-stained interstitial cells.

**Figure 8.** Transcript levels of cardiac-specific genes: (A) α-MHC, (B) β-MHC, (C) ANF, (D) SERCA, and (E) phospholamban. Values are mean±SD (n=6 to 12). *P<0.05 vs WT, †P<0.05 TG+AdTNFRI vs TG+AdLacZ.
can be clearly demonstrated within 2 weeks of therapy and persist through 6 weeks.

Transgenic mice with cardiac-specific overexpression of TNF-α present lymphohistiocytic myocarditis, cardiomyopathy, cardiac dysfunction, and congestive heart failure.5 Whereas some of the phenotypic changes that characterize the TNF-α transgenic mice could be attributed to TNF-α alone, the TNF-α induction of “downstream” cytokines and chemokines may contribute to TNF-α-induced pathophysiology.1 Indeed, overexpression of TNF-α induced the expression of a large number of cytokines and chemokines. Nearly all of the induced cytokines showed reversal of expression after AdTNFRI treatment, suggesting their activation downstream of TNF-α.

Both transcript and protein levels of TNF-α, IL-1β, and MCP-1 were increased in the myocardium of transgenic mice and reversed by anti-TNF treatment. Immunohistochemical analysis suggested that IL-1β and MCP-1 were produced predominantly by nonmyocytes. In contrast, TNF-α expression was found in both myocytes and nonmyocytes, and the protein level, elevated in transgenic animals, was further increased after the treatment with AdTNFRI. Because TNF-α transcripts were not significantly higher in AdTNFRI-treated mice, the increase in TNF-α protein is unlikely to arise from enhanced transcriptional activity of the TNF-α genes. More likely, although TNF-α loses its bioactivity when bound by TNFRI, it may also gain stability as soluble TNF receptors stabilize TNF-α protein despite blockade of its bioactivity.17 Because the ELISA measures both receptor-bound and free TNF-α, the major contribution to the increase might be due to soluble receptor-bound TNF-α. Regardless of increased TNF-α protein in AdTNFRI-treated mice, the biological effects of TNF-α were attenuated, as shown by the abrogation of myocarditis and expression of downstream cytokines. Although anti-TNF treatment with AdTNFRI abrogated the expression of inflammatory mediators and the development of interstitial infiltrates, treatment had no effect on ventricular hypertrophy. Furthermore, treatment had no effect on the increased ventricular expression of β-MHC in TNF-α transgenic mice and only a partial suppression of the enhanced ANF expression. Because upregulation of β-MHC expression is an integral part of the development of myocardial hypertrophy, the failure of soluble TNF receptor to attenuate either β-MHC expression or ventricular hypertrophy is internally consistent. Because α- and β-MHC are thought to be regulated reciprocally, it is of interest that anti-TNF treatment reversed the TNF-α–induced downregulation of α-MHC but not the upregulation of β-MHC. Gupta and Zak19 demonstrated, however, that isoform shifts in the pressure-overload–induced hypertrophy model were not temporally related; α-MHC expression was normalized within 2 weeks after the removal of the aortic band, whereas β-MHC expression required 7 weeks to be normalized.

Although we cannot exclude the possibility that a more prolonged exposure to anti-TNF therapy will reverse hypertrophy and normalize β-MHC upregulation, these results suggest that the hypertrophic process may persist regardless of apparent neutralization of myocardial TNF-α. Indeed, the hypertrophic program may be initiated early in the development of heart failure and be self-sustaining and thus unresponsive to anti-TNF intervention at 6 weeks of age. Alternatively, a very low level of biologically active TNF-α may persist despite therapy with TNFRI, allowing continued stimulation of a hypertrophic signaling pathway.

α-MHC, SERCA, and phospholamban transcripts are downregulated in end-stage heart failure in both human20,21 and animal23 models. IL-1β inhibits expression of these genes in cultured neonatal cardiomyocytes,12 and both IL-1β and TNF-α alter cardiomyocyte contractile activity.13 Thus, the observation that cardiac-specific overexpression of TNF-α and induction of IL-1β is associated with downregulation of these genes is consistent with in vitro studies.

The potential utility of anti-TNF therapy in the management of congestive heart failure is currently an area of great interest.33 Infusion of a dimeric soluble TNF receptor has been shown to antagonize the negative inotropic effects of chronic TNF-α infusion in rats.24 This model is not accompanied by myocardial infiltrates or cardiac-specific overexpression of TNF-α, suggesting a beneficial role of TNF-α blockade in noninflammatory models of heart failure. However, the complete role of TNF-α in heart failure that does not arise as a consequence of TNF-α administration remains to be fully elucidated. Because anti-TNF therapies reduce the severity of autoimmune6 or virus-induced myocarditis,7 one

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**TABLE 3. Echocardiographic Measurements**

<table>
<thead>
<tr>
<th></th>
<th>WT (n=5)</th>
<th>TG (n=5)</th>
<th>TG+TNFRI (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDD, mm</td>
<td>3.36±0.15</td>
<td>3.31±0.19</td>
<td>3.29±0.18</td>
</tr>
<tr>
<td>ESD, mm</td>
<td>1.76±0.12</td>
<td>2.01±0.12</td>
<td>1.53±0.31†</td>
</tr>
<tr>
<td>AW Th, mm</td>
<td>0.71±0.02</td>
<td>0.87±0.04*</td>
<td>0.88±0.15*</td>
</tr>
<tr>
<td>PW Th, mm</td>
<td>0.76±0.02</td>
<td>0.87±0.04*</td>
<td>0.82±0.08*</td>
</tr>
<tr>
<td>FS, %</td>
<td>46.11±4.89</td>
<td>40.06±4.83</td>
<td>46.95±6.98</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>395±29</td>
<td>376±52</td>
<td>408±24</td>
</tr>
</tbody>
</table>

WT indicates wild-type control mice; TG, transgenic mice; EDD, end-diastolic dimension; ESD, end-systolic dimension; AW, anterior wall; Th, thickness; PW, posterior wall; and FS, fractional shortening. Values are mean±SD.

*P<0.05 vs WT; †P<0.05 vs TG.
might hypothesize that anti-TNF therapies would prove to be of benefit in cardiac inflammatory diseases. However, anti-TNF-α antibodies exacerbate the pathology in murine models of Chagas cardiomyopathy; hence, the full utility of anti-TNF therapy in the regulation of inflammatory heart disease and heart failure remains promising yet incompletely explored. Additional information regarding the contribution of TNF-α to heart failure may come from ongoing investigations of the efficacy of soluble TNF receptor therapy in patients with chronic congestive heart failure.

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