Decreased Expression of Tumor Necrosis Factor-α in Failing Human Myocardium After Mechanical Circulatory Support
A Potential Mechanism for Cardiac Recovery

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Background—An increasing number of observations in patients with end-stage heart failure suggest that chronic ventricular unloading by mechanical circulatory support may lead to recovery of cardiac function. Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine capable of producing pulmonary edema, dilated cardiomyopathy, and death. TNF-α is produced in the myocardium in response to volume overload; however, the effects of normalizing ventricular loading conditions on myocardial TNF-α expression are not known. We hypothesize that chronic ventricular unloading by the placement of a left ventricular assist device (LVAD) may eliminate the stress responsible for persistent TNF-α expression in human failing myocardium.

Methods and Results—Myocardial tissue was obtained from normal hearts and from paired samples of 8 patients with nonischemic end-stage cardiomyopathy at the time of LVAD implantation and removal. Tissue sections were stained for TNF-α, and quantitative analysis of the stained area was performed. We found that TNF-α content decreased significantly after LVAD support. Furthermore, the magnitude of the changes did not correlate with the length of LVAD support, although greater reductions in myocardial TNF-α content were found in patients who were successfully weaned off the LVAD who did not require transplantation.

Conclusions—These data show for the first time that chronic mechanical circulatory assistance decreases TNF-α content in failing myocardium; furthermore, we suggest that the magnitude of the change may predict which patients will recover cardiac function. (Circulation. 1999;100:1189-1193.)

Key Words: heart assist device ■ heart failure ■ tumor necrosis factor-α

Evidence acquired from large randomized clinical trials with ACE inhibitors and more recently with β-blockers in patients with advanced heart failure indicates that the progression of heart failure may be attenuated, and perhaps in some cases, cardiac dysfunction may be reversed.1,2 In support of this hypothesis, there are an increasing number of reports in which patients with end-stage nonischemic cardiomyopathy who were treated for extended periods of time with mechanical circulatory support for progressive refractory heart failure recovered cardiac function to allow function without the assist device or a transplant.3-6 More interestingly, chronic ventricular unloading by mechanical circulatory assistance appears to restore cellular and genetic abnormalities in cardiac muscle even in patients who were presumed to have end-stage cardiomyopathies.7,8 The importance of these observations is 2-fold: (1) if failing myocardium recovers after prolonged mechanical support, it would be reasonable to use left ventricular assist device (LVAD) implantation more liberally, with the idea of providing mechanical support as a bridge to cardiac recovery; and (2) the discovery of the mechanisms responsible for recovery of cardiac function after prolonged mechanical circulatory support may result in new therapeutic strategies for patients with end-stage heart failure. Conceivably, novel pharmacological agents may allow the heart to “rest” by emulating a state of chronic ventricular unloading.

Tumor necrosis factor-α (TNF-α) is a myocardial protein that stimulates cardiac growth, produces cardiac enlargement, heart failure, and death in experimental animals.9 High circulating levels of this cytokine are found in patients with severe heart failure,10,11 and more interestingly, transgenic mice that chronically overexpress myocardial TNF-α develop cardiac hypertrophy, fibrosis, and subsequent dilated cardiomyopathy and die prematurely.12,13 Myocardial TNF-α is
preliminary experiments, myocardial samples were stained at various concentrations of anti–TNF-α antibody ranging from a 1/10 to a 1/100 dilution of antibody. Staining over a range of expression demonstrated that an antibody concentration of 1/300 was consistently part of the linear response curve. Therefore, this antibody concentration was used for all subsequent studies.

### Quantitative Analysis of Stained Areas

Stained sections were photographed with a Leaf MicroLumina digital camera mounted on a Zeiss microscope. Multiple digital images were taken and stored for each sample stained. Staining was analyzed by Zeiss image-analysis software with color-cube–based selection criteria for positive staining. Both intensity level (range) and area were analyzed according to the method of Matsuo et al. Results in the present report are based on area of positive staining within the color spectrum for DAB of all intensities greater than those found in control antibody (IgG)–stained sections, without correction for intensity. For TNF-α, 4 low-power fields were analyzed and the results expressed as mean ± SD. The intra-assay variability was 10%. However, because variation exists between the intensity of the staining from one experiment to the other, comparisons among groups were only performed within the same experiment. The analysis was done by observers who were blinded to the sample source.

### Statistical Analysis

To compare the magnitude of the change in intracardiac TNF-α content in patients supported with LVAD who underwent transplantation versus those who had the LVAD removed for cardiac recovery, we used the t test, assuming equal variances.

### Results

#### Demographics

The Table shows the characteristics of the population studied. All patients underwent placement of an LVAD system for progressive cardiac deterioration (despite maximal intravenous inotropic support) and impending end-organ failure. The cause of heart failure was nonischemic cardiomyopathy in all cases. Age range was from 23 to 49 years, and the length of support varied from 113 to 446 days. Four patients underwent orthotopic heart transplantation, and 4 were successfully weaned off the device. Among the patients who were weaned off the device, 3 remained off the transplant list; these patients were taking oral medical therapy and were in New York Heart Association (NYHA) functional class I or II. One weaned patient died of bleeding-related complications on the fourth day after surgery, with adequate ventricular function (LV ejection fraction 35% to 40%).

#### Source of Human Myocardium

Normal myocardium was obtained from hearts of patients who died of noncardiac causes. Failing myocardium was obtained from the left ventricular (LV) apex at the time of LVAD placement and removal. Histological analysis on all 8 patients failed to demonstrate evidence of active inflammatory infiltrates. Four patients with end-stage cardiomyopathies underwent placement of a Novacor LVAD (Baxter Healthcare Corporation), and 4 received the Heart Mate LVAD (Thermo Cardiosystems, Inc). These devices are both intracorporeal volume–sensitive devices capable of complete LV unloading. Four of these patients were eventually weaned off the LVAD because it was predicted that cardiac function had recovered based on echocardiographic criteria, whereas the other 4 required heart transplantation. Myocardial tissue samples were fixed in 2% paraformaldehyde for 45 minutes immediately on collection. Tissue samples were then dehydrated by graded alcohols, after which they were cleared in xylene and embedded in paraffin by use of standard protocols. Five-micrometer sections were cut, collected on slides, and rehydrated.

#### Myocardial TNF-α Levels

Myocardial TNF-α content was determined in samples from the LV apex unless specifically indicated. Therefore, all paired samples from LVAD-treated patients represent changes in TNF-α concentrations within the same region. TNF-α content was determined by a semiquantitative analysis of stained area. For immunostaining of TNF-α, we used a polyclonal anti–TNF-α antibody (R&D Systems, Inc/Genzyme) at a 1/300 dilution. Staining was performed with a kit (Vector Laboratories, Inc) with a peroxidase-conjugated avidin–biotin system and diaminobenzidine (DAB) as a substrate. For preliminary experiments, myocardial samples were stained at various times of LVAD implantation.

### Methods

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Increased Expression of TNF-α in Failing Myocardium

Because it was not known whether TNF-α was differentially expressed in the LV, we first determined TNF-α content in the apex, septum, and LV free wall from 4 patients with end-stage heart failure due to nonischemic cardiomyopathies. As shown in Figure 1A, we found higher levels of TNF-α in the apex, followed by the septum and free wall. For all subsequent experiments, TNF-α measurements were always performed in samples obtained from the LV apex. We next determined TNF-α levels in normal control hearts and in failing myocardium obtained at the time of LVAD implantation from 8 patients with nonischemic cardiomyopathy. As shown in Figure 1B, TNF-α was present in failing myocardium, and very low or undetectable levels were found in normal controls.

Effect of Chronic Ventricular Unloading on Myocardial TNF-α Expression

Figure 2 shows immunostaining for TNF-α in normal heart and in failing myocardium of a patient with end-stage heart failure who underwent LVAD placement, at the time of implantation and removal. Positive immunostaining for TNF-α was present predominantly in myocytes. The important findings shown in this figure are that normal myocardium does not contain TNF-α and that mechanical circulatory support decreases TNF-α content. To further characterize and quantify the changes that occurred in failing myocardium after mechanical circulatory support, we next determined TNF-α content in paired myocardial samples at the time of LVAD placement and removal. As shown in Figure 3, TNF-α content decreased by 10% to 95% of the initial value. In addition, while the LVAD was implanted, no patient had increased levels of intracardiac TNF-α. To be certain of the consistency of our assay, each patient sample was assayed on 2 separate occasions. The inset in Figure 3 demonstrates the consistency of the assay.

Myocardial TNF-α: A Marker for Recovery of Cardiac Function?

We next analyzed whether the extent of intracardiac TNF-α reduction was related to the length of support. As shown in Figure 4A, there was no correlation between the time on the LVAD and the reduction of TNF-α (r=0.2419). However, there were greater reductions in TNF-α content in LVAD-
treated patients who were weaned off the device for cardiac recovery than in those who underwent cardiac transplantation ($P=0.05$; Figure 4B).

**Discussion**

An increasing number of reports have demonstrated that prolonged mechanical circulatory support for patients with end-stage cardiomyopathy may lead to the recovery of cardiac function. We report for the first time that mechanical circulatory assistance results in significant reductions in intracardiac TNF-α content. First, we showed that failing myocardium expressed increased levels of TNF-α and that there was differential expression of TNF-α in the failing LV. Second, we showed that prolonged mechanical circulatory support results in decreased content of intracardiac TNF-α. Finally, we found greater reductions in myocardial TNF-α content in LVAD-treated patients who recovered cardiac function than in those who required cardiac transplantation.

The patient population studied included only patients with nonischemic cardiomyopathy, because the observations on recovery of cardiac function after LVAD implantation have been limited to this patient population. Although various degrees of inflammatory infiltrates have been reported in patients with nonischemic cardiomyopathies, histological analysis performed on myocardium samples obtained at the time of LVAD placement failed to demonstrate active inflammation. Thus, myocardial TNF-α content in failing myocardium at the time of LVAD placement was not related to inflammatory cells that can potentially increase intracardiac TNF-α concentrations.

For these studies, it was important to determine myocardial and not peripheral TNF-α concentrations. We have previously determined that there was no correlation between myocardial and serum TNF-α levels. Furthermore, in experiments conducted in transgenic mice that overexpress TNF-α in the myocardium, it was found that mice can develop a cardiomyopathy even in the absence of increased peripheral TNF-α levels. Therefore, it appears that in terms of the pathophysiology of heart failure, it is the local protein concentration that determines the deleterious effects of TNF-α in cardiac function. In heart failure patients, however, the amount of TNF-α found in the periphery most likely represents the contribution of cardiac and noncardiac production; thus, it was appropriate to develop a strategy to measure the local concentration of TNF-α rather than make indirect predictions based on peripheral levels. Accordingly, the data from the present report directly demonstrate a reduction in myocardial concentrations of TNF-α, which is presumably pathophysiologically relevant to disease progression.

The effect of long-term mechanical circulatory assistance on hemodynamics is to normalize or significantly decrease ventricular filling pressures. This results in elimination of volume forces that may regulate myocardial gene expression. Indeed, previous reports have documented that chronic ventricular unloading normalizes the expression genes involved in calcium handling in myocardial cells.
the present report, we expand the previous observations to demonstrate that the expression of TNF-α, a cytokine that appears to play a major role in the pathogenesis of heart failure, is significantly decreased after prolonged ventricular unloading. This finding is important because it demonstrates that the regulation of TNF-α expression in the heart is intimately linked to hemodynamic loading conditions.

Although it is unlikely that the clinical benefit of prolonged mechanical circulatory support is solely dependent on normalization of TNF-α content, the association of improved cardiac function and reduction in TNF-α expression supports the ongoing hypothesis that increased myocardial expression of TNF-α induces cardiac injury. It also suggests the interesting possibility that therapeutic strategies may be designed for heart failure patients or for patients supported with LVADs in whom specific anti–TNF-α therapy is used concomitantly. In this regard, a phase I study was recently completed of patients with heart failure and NYHA functional class IV who were treated with either a TNF receptor (p75) fusion soluble protein (which blocks the biological effects of TNF-α) or placebo. In that study, quality of life and functional class improved in the treated patients compared with controls, which suggests that anti–TNF-α therapy may be beneficial in heart failure patients. More conclusive data will come from the results of a large, randomized, multicenter study that is currently under way.

Another important implication of the findings of the present study is the possibility that cardiac levels of TNF-α may be used as a potential marker of cardiac recovery. Among the patients with end-stage nonischemic cardiomyopathy studied, greater reductions in TNF-α content occurred in those who had the LVAD removed and remained stable without mechanical circulatory support. Whether the observations from the present study can be extended to patients with ischemic cardiomyopathy is not known, but myocardial TNF-α content does not differ among patients with ischemic or nonischemic cardiomyopathies. Thus, we suggest that normalization of loading conditions in heart failure patients with ischemic cardiomyopathy would also result in reduced levels of intracardiac TNF-α.

The implications of our studies support the hypotheses that not only does TNF-α play a pathogenetic role in heart failure, but the regulation of TNF-α expression in the human heart is linked to hemodynamic loading conditions. The data from the present study strongly support the idea that chronic mechanical unloading favorably alters the “heart failure milieu,” including deactivation of potentially deleterious protein expression, such as TNF-α. These findings also suggest the design of therapeutic strategies to block TNF-α in heart failure patients with the hope of preventing or reversing disease progression.

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