The Role of Tumor Necrosis Factor in the Pathophysiology of Heart Failure

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Recent studies have focused their attention on the role of the proinflammatory cytokine tumor necrosis factor (TNF) in the development of heart failure. First recognized as an endotoxin-induced serum factor that caused necrosis of tumors and cachexia, it is now recognized that TNF participates in the pathophysiology of a group of inflammatory diseases including rheumatoid arthritis and Crohn’s disease. The normal heart does not express TNF; however, the failing heart produces robust quantities. Furthermore, there is a direct relationship between the level of TNF expression and the severity of disease. In addition, both in vivo and in vitro studies demonstrate that TNF effects cellular and biochemical changes that mirror those seen in patients with congestive heart failure. Furthermore, in animal models, the development of the heart failure phenotype can be abrogated at least in part by anticytokine therapy. Based on information from experimental studies, investigators are now evaluating the clinical efficacy of novel anticytokine and anti-TNF strategies in patients with heart failure; one such strategy is the use of a recombinantly produced chimeric TNF alpha soluble receptor. Thus, in view of the emerging importance of proinflammatory cytokines in the pathogenesis of heart disease, we review the biology of TNF, its role in inflammatory diseases, the effects of TNF on the physiology of the heart and the development of clinical strategies that target the cytokine pathways. (J Am Coll Cardiol 2000;35:537–44) © 2000 by the American College of Cardiology

Dilated cardiomyopathy is a disease of epidemic proportion in the U.S. Although more prevalent in the elderly, it affects patients of all ages, races and genders. Heart failure is generally believed to begin with myocyte damage secondary to a variety of insults including ischemia, toxins such as alcohol or immune mechanisms. However, in many cases the etiology remains undefined. Initially the heart compensates by dilatation and cellular hypertrophy; however, over a period of time, the heart eventually decompensates and patients present with signs and symptoms of heart failure. It is the transition from compensation to decompensation that has been the focus of clinical and basic research over the past two decades and the role of the neurohormones, norepinephrine and angiotensin II, in the development of symptomatic heart failure. However, investigators have recently focused their attention on the proinflammatory cytokine, tumor necrosis factor (TNF). This manuscript reviews the investigational data supporting the role of TNF in the pathogenesis of heart failure and its usefulness as a therapeutic target.

The proinflammatory cytokine TNF. In 1975, Carswell et al. (1) first identified tumor necrosis factor (TNF), an endotoxin-induced serum factor that caused necrosis of tumors. A decade later, investigators isolated a protein from endotoxin-treated cells that was named cachectin because of its presumed role in the molecular basis of cachexia (2,3). The subsequent cloning of the genes encoding cachectin and TNF alpha confirmed that these two molecules were identical (4,5). Produced as a prohormone of 233 amino acids, TNF alpha is anchored in the cell membrane and then processed to a 157 residue mature protein by cleavage of a 76 residue signal peptide. Tumor necrosis factor regulates the expression of a variety of peptide regulatory factors including IL-1, IL-6, platelet derived growth factor, transforming growth factor-beta, as well as a group of eicosanoids and hormones including platelet-activating factor and adrenaline (7). In addition, TNF alpha (and IL-1beta) contains a 33 nucleotide 3'-untranslated sequence that shortens messenger RNA (mRNA) half-life and, in so
Abbreviations and Acronyms

- alpha-MHC = alpha myosin heavy chain
- AMP = adenosine monophosphate
- beta-MHC = beta myosin heavy chain
- CHF = congestive heart failure
- ELAMS = endothelial leukocyte adhesion molecules
- ICAMS = intracellular adhesion molecules
- IL-1 = interleukin-1
- LPS = lipopolysaccharide
- MCP = monocyte chemoattractant protein
- mRNA = messenger RNA
- NYHA = New York Heart Association
- SERA = sarcoplasmic reticulum Ca$^{2+}$ ATPase
- TNF = tumor necrosis factor
- TNFR = soluble tumor necrosis factor receptor

TNF receptors. Once released from the cell, TNF alpha interacts with one of two TNF alpha receptors—a 55 kd high affinity receptor, soluble tumor necrosis factor receptor 1 (TNFR-1), and a 75 kd low affinity receptor (TNFR-2) (8,9). Intra cellular signaling occurs as a result of TNF alpha-induced cross-linking of the receptors. The extracellular portions of these two proteins form a receptor family containing four characteristic domains with regularly spaced cystine residues; however, no significant homology exists between the intracytoplasmic portions of the two TNF alpha receptors (10). Thus, investigators have proposed that these receptors may be coupled to distinct signaling pathways (11). In the presence of noxious stimulants including TNF alpha, lipopolysaccharide (LPS), okadaic acid and phorbol esters, TNF alpha receptors are “shed” into the circulation (12–14). These TNFR receptor proteins appear to be truncated fragments of the extracellular regions of the type 1 and type 2 membrane-bound receptors for TNF alpha (13,15). Although TNF receptors do not possess intrinsic protein kinase activity, within minutes of exposure to TNF alpha, phosphorylation of several distinct proteins occurs, which is likely due to activation of cellular kinases (16). The soluble receptors are able to bind ligand and inhibit the cytotoxic activities of TNF alpha (15). Thus, the shedding of soluble binding proteins might serve as a “biological buffer” that can rapidly neutralize the activities of TNF alpha.

Pleiotropic effects of TNF alpha. The cellular effects of TNF alpha are highly pleiotropic. At low concentrations, TNF alpha effects paracrine or autocrine regulation of leukocytes and endothelial cells and, thus, serves as an important regulator of the inflammatory response. Indeed, mice lacking the p55 TNF alpha receptor are highly sensitive to some types of bacterial infections (17). Tumor necrosis factor alpha enhances chemotaxis of macrophages and neutrophils, increases their phagocytic and cytotoxic activity (18) and promotes leucostasis by inducing increased expression of intracellular adhesion molecules (ICAMs) and endothelial leukocyte adhesion molecules (ELAMs) at sites of inflammation. At higher concentrations, TNF alpha production exceeds the number of TNF alpha receptors located on the cell surface with excess TNF alpha being released into the circulation. Once released, TNF alpha exerts endocrine or exocrine effects including initiation of metabolic wasting, microvascular coagulation, hypotension and fever (19,20). Paradoxically, TNF alpha also appears to modulate both tissue destruction and rebuilding. Tumor necrosis factor alpha stimulates fibroblast and mesenchymal cell proliferation directly and induces the biosynthesis of other growth factors. By contrast, it is directly cytotoxic to endothelial cells and can induce the biosynthesis of collagen, proteases, reactive oxygen intermediates and arachidonic acid metabolites (7). Tumor necrosis factor alpha has also been shown to have an important role in cell death through a variety of mechanisms including second messenger pathways, arachidonate metabolism, protein kinases, oxygen free radicals, nitric oxide, transcription of a variety of cytotoxic genes, regulation of nuclear regulatory factors, ADP-ribosylation and, potentially, DNA fragmentation (21). Under normal situations, these cytotoxic effects are important in host defense by initiating antitumor activity (1,22) and modulating cell growth and differentiation. However, the role of TNF alpha in the pathogenesis of malignancies remains controversial. While therapy with TNF improved survival in tumor-bearing mice, it also promoted tumor cell adhesion, nodule development (23) and metastasis (24). In addition, a direct relationship exists between the level of TNF expression and tumor grade in ovarian cancer (25). By contrast, excessive activation of TNF alpha or nontissue specific expression leads to tissue necrosis and apoptosis. Indeed, recent studies have demonstrated a causative role for TNF alpha in a group of diseases including: septic shock, rheumatoid arthritis (26), pre eclampsia (27), hemolytic uremic syndrome (28), allograft rejection (29) and regional enteritis (30).

Proinflammatory cytokines and the heart. The first recognition that TNF alpha might participate in the development of congestive heart failure (CHF) came in 1990 when Levine et al. (31) demonstrated that circulating levels of TNF alpha were elevated in patients with end stage heart failure and cachexia. Subsequent studies demonstrated comparable elevations in IL-6 and IL-1beta (32) and a direct relationship between TNF alpha levels and functional heart failure classification (Fig. 1 [33]). Furthermore, direct relationships were identified between circulating levels of TNF alpha and neurohumoral activation and the degree of anemia; however, there was no relationship between cytokine levels and the degree of cachexia (34,35). Cytokine levels were also elevated in patients with heart failure due to myocarditis (36). That the observed increases were of physiologic significance was demonstrated by studies in...
which injections of endotoxin into humans resulted in TNF alpha elevations, depressed left ventricular function and decreases in mean arterial pressure (37). Parenthetically, it should be noted that the different assay techniques, for example bioassays or ELISA measurements, used by various investigators provide varying values, thus obfuscating the literature to some extent.

The increase in circulating TNF alpha in patients with CHF was associated with a substantial decrease in myocardial TNF alpha receptors and an increase in soluble TNF alpha receptors; however, the stoichiometry favored free TNF alpha (38). Perhaps of greatest importance was the demonstration by Mann and colleagues (38) in 1996 that the nonfailing human heart does not express TNF alpha, whereas the end stage failing human heart reexpresses robust amounts of protein (Fig. 2).

Biologic effects of TNF alpha on the myocardium. Beginning with the observation that TNF alpha could inhibit contractility of isolated hamster papillary muscles in a concentration-dependent and reversible manner (39), a series of in vivo and in vitro basic science studies were performed, which paralleled the clinical studies assessing TNF alpha levels in patients with CHF (Fig. 3). First, it was demonstrated that the negative inotropic effects of TNF alpha are virtually immediate (40,41) and appear to be completely reversible upon removal of the cytokine. However, not only does TNF alpha have immediate negative inotropic properties, but it can recapitulate the cellular and biochemical abnormalities that characterize the failing human heart. For example, IL-1beta induces a down regulation of the expression of sarcoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA) and phospholamban at both the mRNA and protein level in neonatal myocytes (42), which is associated with a depression and prolongation of the Ca\(^{2+}\) transient, effects that may be initiated by TNF alpha (40). In addition, TNF alpha effectively uncouples the beta-adrenergic receptors from adenyl cyclase via an effect on the G inhibitory protein (43,44). Furthermore, TNF alpha activates metalloproteinases and inhibits the expression of inhibitors of metalloproteinases in vivo—effects that would be expected to activate extracellular matrix remodeling (45). Although initial studies suggested that the negative inotropic properties of TNF alpha were attributable to the ability of cytokines to induce nitric oxide synthase, subsequent investigations demonstrated that the induction of inducible nitric oxide synthase could not in and of itself induce contractile dysfunction in cardiac myocytes and that alterations in calcium homeostasis played an important role (40). Tumor necrosis factor alpha also provokes a hypertrophic growth response in cardiac myocytes, which may be an adaptive response to hemodynamic or environmental stress (46). Furthermore, a recent study suggested that the immediate negative inotropic effects of TNF alpha may be mediated by...
sphingosine (47). Finally, chronic infusion of TNF alpha produces a reversible dilated cardiomyopathy in a rat model—without evidence of inflammatory infiltrate or myocyte necrosis (48).

**TNF alpha transgenic mice—a model of dilated cardiomyopathy.** To pursue further studies of the role of cytokines in the development of CHF and to provide additional support for the “cytokine hypothesis,” a series of mice that harbored a transgene effecting cardiac-specific overexpression of TNF alpha were generated using the cardiac-specific alpha myosin heavy chain (alpha-MHC) promoter. Robust overexpression proved lethal due to (49) fulminant myocarditis; however, lower levels of TNF alpha expression allowed the production of viable founders (50). These mice recapitulate heart failure in humans as they demonstrate: 1) four-chamber dilatation, 2) myocyte hypertrophy, 3) interstitial infiltrates, 4) extracellular matrix remodeling with fibrosis, 5) diminished beta-adrenergic responsiveness; and 6) premature death with a six-month mortality of 25% (Fig. 4). The development of a heart failure phenotype secondary to overexpression of TNF alpha has recently been confirmed by a second laboratory (51). Taken together, the existing TNF alpha transgenic studies suggest that there is a direct relationship between transgene copy number and the degree of inflammation (52). Recent studies suggest that mortality in these animals is due, in part, to an arrhythmogenic event since continuous holter monitoring of transgenic mice demonstrates high grade ventricular ectopy with frequent runs of nonsustained ventricular tachycardia (53). At the cellular and molecular level, the TNF alpha transgenics are characterized by: 1) reexpression of the fetal gene program (e.g., atrial natriuretic factor and beta-MHC), 2) reexpression of downstream TNF alpha responsive cytokines and chemokines including IL-1beta, monocyte chemoattractant protein (MCP), and regulated upon activation, normal T-cell expressed and secreted, 3) increased activity of the metalloproteinases and diminished expression of inhibitors of metalloproteinases (54), 4) activation of both pro- and antiapoptotic pathways (55), 5) down-regulation of phospholamban and SERCA expression, and 6) a decrease in the alpha- and beta-myosin heavy chain mRNA ratio (56,57). Furthermore, the transgenic mice demonstrated reduced ejection fractions and fractional shortening and diminished ventricular compliance. The TNF alpha transgenic also demonstrated a substantial amount of apoptosis; however, this was largely isolated to the interstitium, and myocyte apoptosis was rare (55). Thus, the TNF alpha overexpressing transgenic recapitulates both the pathologic, cellular and molecular phenotype of the human failing heart and, therefore, provides an ideal model in which to study the pathobiology of progressive CHF and to test therapeutic strategies.

**“Rescuing” the TNF alpha transgenic with TNFR.** That there was a direct relationship between TNF alpha expression and negative inotropic effects was first shown by Kapadia et al. (58) using soluble TNF alpha binding proteins. However, to “prove” that overexpression of TNF alpha is a critical step in the development of end stage CHF (52), transgenic and wild type mice were injected with an adenoviral vector encoding a chimeric soluble TNF alpha protein consisting of two moieties of the human 55-kDa TNF alpha receptor extracellular domain fused to a mouse IgG heavy chain (59). Inoculation of mice with 10⁹ plaques forming units resulted in inhibitor production and release by hepatocytes and marked elevations in serum and myocardial TNF alpha receptor levels for as long as six weeks (57). These levels of soluble receptor were several orders of magnitude greater than the levels of myocardial TNF alpha, suggesting a favorable stoichiometry for TNF alpha inhibition. After both two and six weeks of treatment, TNFR completely abrogated the presence of interstitial infiltrates and normalized the expression of alpha-MHC, SERCA, and phospholamban. In addition, the reexpression of downstream cytokines and chemokines including IL-1beta, MCP-1 and rantes was completely inhibited by TNFR
therapy. By contrast, there was continued activation of beta-MHC expression, which was consistent with the finding of persistent ventricular hypertrophy in these TNFR treated mice. Finally, there was normalization of matrix metalloproteinase and tissue inhibitor of metalloproteinase expression and stabilization of the degree of interstitial fibrosis, suggesting that extracellular matrix remodeling had abated. Thus, these studies suggest that some, although not all, of the phenotypic characteristics of CHF can be abated by anticytokine strategies. However, both hypertrophy and fibrosis persist despite therapeutic intervention, suggesting early, but not late, therapy using anticytokine approaches might benefit patients with CHF.

**The molecular genetics of TNF alpha.** Genetic polymorphisms in the TNF locus have been associated with higher levels of TNF alpha production (60,61) and are known to be related to several autoimmune, infectious and neoplastic diseases (62,63). These polymorphisms include a G to A transition at position −308 in the promoter region of the TNF alpha gene (TNF alpha2) (64) and a G to A transition at position +252 in the first intron of the TNFbeta gene (65). The frequency of the TNF alpha2 allele, the allele associated with increased TNF expression, is increased in patients with rheumatoid arthritis and systemic lupus erythematosus (66). Similarly, people homozygous for the TNF alpha2 allele have a higher risk for death due to cerebral malaria (67) while homozygous for the TNFbeta2 allele have a higher mortality with sepsis (61). To date, investigators have been unable to demonstrate an association between either the TNF alpha or TNFbeta polymorphisms and the presence of CHF (68); however, these studies had an inherent bias, i.e., that patients with a potentially unfavorable polymorphism might have died prior to receiving medical attention (69). Therefore, further evaluation of this possibility is warranted. An additionally important, but as yet unanswered, question is how myocardial cytokine expression was further supported by a recent study by Loh et al. (89) demonstrating improved survival in heart failure patients having a common mutation in at least one allele of the AMP deaminase gene. In the peripheral muscle, and presumably in the heart, patients with this mutation have diminished AMP deaminase activity resulting in decreased metabolism of AMP to inosine and enhanced production of adenosine, leading us to hypothesize that adenosine agonists might have therapeutic utility in the management of patients with CHF (69).

**Clinical strategies for anticytokine therapy.** Based on the basic science and clinical observations regarding the role of proinflammatory cytokines in the development of CHF, efforts have been directed towards developing anticytokine strategies that might be useful in the therapy of patients. Yoshimura et al. (74) suggested that beta-adrenergic agonists could inhibit the production of both TNF alpha and IL-1beta by elevating intracellular cyclic AMP levels. Similarly, pretreatment with isoproterenol blunted the ability of LPS to induce TNF alpha production in conscious mice (75), whereas short-term, but not long-term preexposure of mononuclear cells to adrenergic agonists inhibited LPS-induced production of TNF alpha (76). Phosphodiesterase inhibitors can also serve as potent inhibitors to TNF alpha production (77,78). Indeed, the phosphodiesterase inhibitor, pentoxifylline, demonstrates salutary effects on heart failure signs and symptoms while substantially lowering circulating TNF alpha levels (79,80). However, other studies have failed to show an effect of cyclic AMP, cyclic AMP derivatives or beta-adrenergic agonists on TNF alpha production (81). Other inhibitors of cytokine expression include: amiodarone (82), ouabain (83) and thalidomide (84). In addition, a recent report suggests that estrogen may constitutively down-regulate TNF alpha expression (85).

Recently, studies have demonstrated that adenosine is a potent inhibitor of TNF alpha expression by both neonatal myocytes, adult myocytes, rodent papillary muscle preparations and adult human heart via activation of the adenosine A2 receptor (86,87). This effect appears to be partially selective for the heart as adenosine’s anticytokine effects in white cells is mediated via the A3 adenosine receptor (88). That adenosine may have an important role in regulating myocardial cytokine expression was further supported by a recent study by Loh et al. (89) demonstrating improved survival in heart failure patients having a common mutation in at least one allele of the AMP deaminase gene. In the peripheral muscle, and presumably in the heart, patients with this mutation have diminished AMP deaminase activity resulting in decreased metabolism of AMP to inosine and enhanced production of adenosine, leading us to hypothesize that adenosine agonists might have therapeutic utility in the management of patients with CHF (69).

Effective blockade of TNF alpha activity has also been effected using passive immunization. Monoclonal antibodies against TNF alpha have proved successful in ameliorating the effects of sepsis (in animal models) (90,91), cancer (92), synovial inflammation (93), inflammatory bowel disease (94,95) and chronic bacterial infection (96,97). While immunotherapy is effective for investigation studies in animal models, it has limitations in long-term disease therapy as the antibodies serve as immunogens and, therefore, can only be used for a limited period of time. To abrogate the immunogenicity of the antibody, several groups have proposed using monoclonal antibodies in combination with an immunosuppressive such as methotrexate. However, the rationale for using a potent immunosuppressive in a patient with a chronic disease such as heart failure remains questionable.

Perhaps the most exciting strategy that has recently been developed for inhibiting the effects of myocardial TNF alpha production is the use of a recombinantly produced chimeric TNF alpha soluble receptor (Etanercept; Immunex, Seattle, Washington) consisting of the p75 receptor linked to the Fc portion of human IgG (TNFR:Fc). Unlike synthetic pharmacologic agents that can have multiple cellular effects, recombinant proteins have the theoretical benefit of being highly specific. First used in the treatment
of rheumatoid arthritis, Etanercept was associated with dose-related reductions in disease activity without dose-limiting toxicity or the development of antibodies to the recombinant receptor protein (98). Based on the salutatory effects of Etanercept in clinical trials of patients with rheumatoid arthritis, the agent was recently approved by the Food and Drug Administration. However, postmarketing surveillance has raised concerns regarding the use of the agent in patients with serious infections.

In a Phase I study assessing the safety of soluble TNF alpha receptor therapy in patients with CHF, a single intravenous dose of Etanercept was well tolerated, suppressed circulating levels of biologically active TNF alpha between 70% and 85% for at least 14 days and improved functional status and quality of life (99). More recently, 47 patients with New York Heart Association (NYHA) class III–IV heart failure symptoms were randomized to receive either biweekly subcutaneous Etanercept or placebo for three months. Etanercept effected dose-related improvements in NYHA classification and quality of life and decreased cytokine expression (100). However, to assess the long-term effects of Etanercept, a randomized, double-blind and placebo-controlled clinical trial, RENAISSANCE, is presently enrolling patients with NYHA class II–IV heart failure symptoms in the U.S. and a companion study is underway in Europe and Australia.

Conclusions. In summary, both basic and clinical studies strongly support the hypothesis that myocardial expression of TNF alpha is an important step in the pathophysiologic pathway leading to progressive cardiac dilatation and failure. However, important questions remain to be answered: 1) What is the signal responsible for activating cardiac TNF alpha expression? 2) Why does the heart express TNF alpha? 3) Can we selectively attenuate cardiac TNF alpha expression? 4) What are the clinical benefits of TNF alpha inhibition? 5) When during the transition from compensated to decompensated heart failure is TNF alpha expressed? 6) What role is played by other proinflammatory cytokines such as IL-1beta? By addressing these important questions, it is hoped that investigators will be able to better understand the pathobiology of CHF while at the same time develop new and exciting therapeutic strategies.

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