

Elevated circulating levels of tumor necrosis factor in severe chronic heart failure

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N Engl J Med. 1990;323:236-241

Setting the stage for the cytokine hypothesis in heart failure, this study was the first one to describe elevated levels of proinflammatory cytokine, tumor necrosis factor alpha (TNF- α) in patients with advanced heart failure. TNF- α was initially named on the basis of its ability to kill tumor cells in vitro and cause hemorrhagic necrosis of transplantable tumors in mice. Concurrently, a factor known as "cachectin," a peptide involved in the loss of body fat in the course of wasting diseases, was isolated from mouse macrophages and was shown to be identical to TNF- α . Cachectin was identified as a catabolic hormone that suppressed the expression of lipoprotein lipase and other anabolic enzymes in fat. Still other studies demonstrated the powerful proinflammatory effects of TNF- α and revealed its role as a central mediator of endotoxic shock. Therefore, TNF- α was linked with wasting and was thought to be responsible for the cardiac cachexia seen in advanced heart failure patients.

To assess the potential role of TNF- α in the pathogenesis of cardiac cachexia, Levine and colleagues measured serum levels of the factor in 33 patients with chronic heart failure, 33 age-matched healthy controls, and 9 patients with chronic renal failure. Most of the patients with chronic heart failure had serum levels of TNF- α greater than 2 SD above the mean value for the control group. The patients with high levels of TNF- α were more cachectic than those with low levels and had more advanced heart failure, evidenced by their higher values for plasma renin activity. These findings indicated that circulating levels of TNF- α were increased in patients with chronic heart failure and that this elevation was associated with the marked activation of the renin-angiotensin system seen in patients with end-stage cardiac disease.

Even though this study was conducted to define the potential role of TNF- α in cardiac cachexia seen with heart failure, subsequently, other investigators noted that the TNF- α levels were elevated not only in cachectic, but also in non-cachectic advanced heart failure patients as well. TNF- α levels actually correlate with severity of heart failure rather than cachexia alone, and TNF- α can be associated with

many aspects of heart failure other than cachexia. TNF- α produces both immediate and delayed negative inotropic effect on myocardial contractility. When expressed at sufficiently high concentration, TNF- α can mimic some aspects of heart failure phenotype, including, but not limited to, progressive left ventricular dysfunction, pulmonary edema, left ventricular remodeling, fetal gene expression, and cardiomyopathy.

Following this original description by Levine and colleagues, numerous other studies have consistently identified elevated levels of TNF- α in patients with advanced heart failure. There is a progressive increase in TNF- α levels in relation to deteriorating New York Heart Association (NYHA) functional class, and, moreover, analysis of cytokine levels shows that there is a relation to increased mortality with increasing levels of TNF- α . Much like elevated neurohormones, TNF- α levels may be predictive of NYHA class and clinical outcome. Thus, the elaboration of cytokines may represent, much like neurohormones, a biological mechanism that is responsible for producing symptoms in patients with heart failure.

1990

At least 1400 Muslim pilgrims die
in a tunnel leading to the Kaaba shrine
inside Mecca's great mosque after the
air-conditioning fails in 43°C temperatures;
President Boris Yeltsin resigns from
the Communist party; and American cyclist
Greg Lemond wins his 3rd Tour de France



Increased circulating cytokines in patients with myocarditis and cardiomyopathy

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Br Heart J. 1994;72:561-566

In this paper, Matsumori and colleagues elucidate the potential role of cytokines in the pathogenesis of cardiomyopathy and myocarditis. The first recognition that tumor necrosis factor alpha (TNF- α) might participate in the development of congestive heart failure came in 1990 when Levine et al demonstrated that circulating levels of TNF- α were elevated in patients with end-stage heart failure and cachexia. Subsequent studies demonstrated comparable elevations in interleukin-6 (IL-6) and interleukin-1 β (IL-1 β), and a direct relationship between TNF- α levels and functional heart failure classification (New York Heart Association [NYHA]). Furthermore, direct relationships were identified between circulating levels of TNF- α and neurohumoral activation. However, there was no relationship between cytokine levels and the degree of cachexia. Further studies revealed that cytokine levels were elevated in a variety of other cardiac diseases including viral myocarditis, dilated cardiomyopathy, cardiac allograft rejection, myocardial infarction, and after cardiopulmonary bypass surgery.

In this study, the investigators measured plasma levels of IL-1 α , IL-1 β , IL-2, IL-6, TNF- α and TNF- β , granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage colony-stimulating factor, interferon- α , and interferon- γ in 13 patients with acute myocarditis, 23 patients with dilated cardiomyopathy, 51 patients with hypertrophic cardiomyopathy, 9 patients with acute myocardial infarction, 18 patients with angina pectoris, 12 patients with essential hypertension, and 17 healthy controls. Increased concentrations of IL-1 α , TNF- α , IL-2, macrophage colony-stimulating factor, and granulocyte colony-stimulating factor were detected in patients with acute myocarditis, dilated cardiomyopathy, acute myocardial infarction, hypertrophic cardiomyopathy, and angina pectoris. These findings suggested activation of macrophages and/or endothelial cells—not specific to these diseases, but perhaps to myocardial injury. Increased concentrations of cytokines were not detected in patients with essential hypertension or in controls. These results suggest that cytokines may play a part in the pathogenesis of myocardial injury in myocarditis and cardiomyopathies.

Despite repeated attempts to develop a unifying hypothesis that would explain the clinical syndrome of heart failure following different forms of cardiac injury, no single conceptual paradigm has withstood the test of time. After an initial cardiac injury such as myocardial injury or sustained hemodynamic loading, each of the initially adaptive stress responses, such as increased elaboration of neurohormones and cytokines, has the potential to become overtly maladaptive with sustained overexpression. Thus, the overexpression of cytokines in a variety of cardiac disorders may be considered as a common mechanism contributing to the progression of heart failure through direct depression in myocardial function or progression of left ventricular remodeling.

1994

Austria, Finland, and Sweden join the European Union, increasing the membership to 15 nations; Quentin Tarantino releases “Pulp Fiction” to great critical acclaim, resurrecting the film career of John Travolta; and the British actor and playwright John Osborne dies, aged 65

Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure

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Circulation. 1995;92:1479-1486

This is the first study to have identified the prognostic role of soluble tumor necrosis factor (TNF) in patients with severe congestive heart failure (CHF) and cardiac cachexia. TNF receptors are proteolytically cleaved from cell surfaces and exist in the circulation as soluble receptors. These two proteins, which are the extracellular domains of the TNF receptors (sTNFR1 and sTNFR2), shed from cell surfaces and can be detected in the urine and blood. They regulate TNF bioactivity either by inhibiting the binding of TNF trimers to the membrane receptors or by preventing TNF trimers from dissociation to inactive monomers. Therefore, measurements of circulating levels of sTNFRs provide more complete information on TNF activation in CHF.

This study analyzed the levels of bioactive TNF, antigenic TNF, and of the two soluble TNF receptors in 37 consecutive patients with various degrees of CHF vs 26 age-matched healthy subjects. In New York Heart Association (NYHA) class IV patients, both soluble TNF receptors were higher than in healthy subjects (3.8 times for sTNFR1 and 3.4 times for sTNFR2). In class II and III patients, the mean values of sTNFRs were not different from those of control subjects, but were significantly lower than those of class IV patients. Ten patients died within 1 month. These patients had significantly higher levels of antigenic TNF and soluble TNF receptors. There was a correlation between sTNFR2 values and duration of survival, but none with norepinephrine, atrial natriuretic peptide, renin activity, or aldosterone. Discriminant stepwise analysis showed that sTNFR2 was the most important single independent variable predicting death. The other parameters, including NYHA clinical classification, had a lower predictive value.

These data suggest that the TNF system is activated in preterminal CHF patients in the absence of "cardiac cachexia." sTNFRs, the naturally occurring inhibitors of TNF activity, can exert a counteraction that could be either advantageous or injurious for the organism. When present in the serum at physiological levels, they can protect trimeric TNF from monomerization and subsequent inactivation or can prolong the half-life of circulating TNF. Hypothetically,

sTNFRs could have exerted this protective action in healthy subjects or in patients with moderate CHF. At physiological concentrations, sTNFRs may act as a "slow-release reservoir" of bioactive TNF, thus increasing its half-life. When present at higher concentrations, as in the group of preterminal patients in class IV, sTNFRs could inhibit the pathological increase in TNF activity and act as anti-TNF molecules by forming complexes with high affinity to the cytokine. The shedding of these receptors and the resultant decrease in their concentration on the cell surface could also prevent cell damage. Administration of sTNFRs to experimental animals protects against shock and mortality induced by the TNF challenge. Alternatively, since TNF induces the shedding of its soluble receptors, it is also possible that increased sTNFRs simply reflect activation of the cytokine at a local level. In this latter case, sTNFRs could be sensitive "serum markers" of local TNF activation.

It can be concluded that measurement of sTNFRs, in addition to that of antigenic and bioactive TNF, is essential for evaluation of the TNF system in CHF. Both sTNFR1 and sTNFR2 are increased in preterminal CHF patients and might modulate the *in vitro* cytotoxicity of TNF. The increase in sTNFRs, particularly sTNFR2, correlates with poor prognosis. It is not clear whether the elevation of sTNFRs in terminal failure is due to an actual increase or to a reduced breakdown or elimination of these receptors. Further explorations are needed to more precisely define the meaning, molecular basis, and interaction of sTNFRs and TNF in CHF.

1995

Sterling Morrison, the former guitarist with the Velvet Underground, dies, aged 53;
the European golf team wins the Ryder cup against the USA; and rumors spread around the world that statues of Ganesh, the Hindu God of wisdom and success, is drinking milk.
Skeptics point out the statues are absorbent



Tumor necrosis factor–alpha and tumor necrosis factor receptors in the failing human heart

G. Torre-Amione, S. Kapadia, J. Lee, J.B. Durand, R.D. Bies, J.B. Young, D.L. Mann

Circulation. 1996;93:704-711

It is thought that the effects of cytokines are initiated by their binding to specific receptors that exist on the membranes of most mammalian cell types, including the adult cardiac myocyte. Tumor necrosis factor (TNF) binds to one of two TNF receptors, a lower affinity 55-kD “type 1 receptor” (also called TNFR1) and a higher affinity 75-kD “type 2 receptor” (also called TNFR2). Intracellular signaling through TNF receptors occurs as a result of TNF-induced cross-linking, or oligomerization, of the receptors. Both TNFR1 and TNFR2 share homology in their extracellular domains. However, no significant homology exists between the intracellular domains of TNFR1 and TNFR2, suggesting that each receptor has distinct modes of signaling and cellular function. Cardiac myocytes express both types of TNF receptors, and it appears that the type 1 receptor is responsible for mediating the negative inotropic effects of TNF. Studies have also shown that both TNF receptors are proteolytically cleaved from the cell membrane, and that they exist in the circulation as circulating soluble receptors referred to as sTNFR1 and sTNFR2, respectively. Interestingly, both these receptors retain their ability to bind their ligand, as well as to inhibit the cytotoxic activities of TNF. It has been suggested that they may serve as “biological buffers,” which are capable of rapidly neutralizing the highly cytotoxic activities of TNF. While the definitive biological role for these soluble TNF binding proteins *in vivo* is not known, it has been postulated that they may serve as “biological buffers,” which are capable of rapidly neutralizing the highly cytotoxic activities of TNF. Recent experiments have shown that sTNFRs are sufficient to both block as well as reverse the negative inotropic effects of TNF. It has also been hypothesized that soluble TNF receptors may stabilize TNF as a homotrimer, and hence increase TNF bioactivity relative to unstabilized TNF, which will dissociate into inactive monomers. Indeed, elevated levels of sTNFR2 have been shown to correlate with an adverse clinical outcome in patients hospitalized for heart failure.

This important study by Torre-Amione and colleagues examined messenger RNA (mRNA) and protein levels for TNFR1, TNFR2, and TNF- α in explanted hearts from organ

donors as well as in patients with end-stage dilated cardiomyopathy (DCM) and ischemic heart disease (IHD). They identified the presence of both types of TNF receptors in the nonfailing control and failing human myocardium. mRNA for TNFR1 and TNFR2 were present in failing hearts both with DCM or IHD. Interestingly, TNFR1 and TNFR2 receptor protein levels, as measured by enzyme-linked immunoassay (ELISA), were decreased 60% in the failing hearts compared with the nonfailing hearts. To determine a potential mechanism for the decrease in TNF receptor expression, the investigators measured levels of circulating sTNFRs in the failing hearts. This analysis showed that there was a significant one-and-a-half to threefold increase in sTNFRs in DCM and IHD patients compared with nonfailing control hearts. Another important finding was that TNF- α mRNA and TNF- α protein were present in the explanted hearts from DCM and IHD patients, but not in nonfailing hearts.

In summary, the results of this study constitute the initial demonstration that TNF receptor proteins are dynamically regulated in patients with advanced congestive heart failure. Moreover, the observation that failing hearts express elevated levels of TNF suggests that overexpression of this cytokine may be one of several different maladaptive mechanisms responsible for the progressive cardiac decompensation that occurs in advanced heart failure.

1996

IBM's Deep Blue defeats world chess champion Gary Kasparov; the British golfer Nick Faldo wins his third Masters title, overturning a six shot deficit at the beginning of the final round; and Gene Kelly, the dancing star of “Singing in the Rain” and “An American in Paris,” dies, aged 83

Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD)

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J Am Coll Cardiol. 1996;27:1201-1206

This was the first study to document a correlation between proinflammatory cytokine levels and the severity of the disease process in patients with heart failure. Subsequently, several papers confirmed this initial finding, showing that there is increasing cytokine elaboration in direct relation to the severity of the disease process.

Prior to this study, elevated levels of tumor necrosis factor α (TNF- α) had been identified in 30% to 40% of patients with heart failure. However, it was unclear which subsets of patients with heart failure elaborated TNF- α . It was also unclear what the mechanism for the increased expression of proinflammatory cytokines was.

Torre-Amione and colleagues sought to assess proinflammatory cytokine levels in patients in the Studies of Left Ventricular Dysfunction trial (SOLVD) in relation to both their New York Heart Association (NYHA) functional classification and their neurohormonal status before randomization. TNF- α and interleukin-6 (IL-6) levels were analyzed by enzyme-linked immunoassay using randomly selected plasma samples from patients in NYHA functional classes I to III who were enrolled in neurohormonal substudies of the SOLVD trial. Age-matched healthy subjects served as the control group. Plasma levels of TNF- α were elevated in patients in NYHA functional classes I to III, with values of 1.95 ± 0.54 , 2.63 ± 0.48 , and 6.4 ± 1.9 pg/mL, respectively compared with age-matched control subjects (0.75 ± 0.05 pg/mL), and were progressively elevated in relation to decreasing functional status of the patient. Plasma levels of IL-6 were elevated in patients in NYHA functional classes I to III (3.3 ± 0.55 , 6.2 ± 1.1 , and 5.22 ± 0.9 pg/mL, respectively) compared with age-matched control subjects (1.8 ± 0.5 pg/mL), and were progressively elevated in relation to decreasing functional status of the patient. Cox proportional-hazards analysis showed that there was a trend toward significance between plasma TNF- α and survival, whereas there was no significant relation for plasma IL-6. Except for atrial natriuretic factor, which correlated weakly with circulating TNF- α levels, there was no significant correlation between neurohormonal and proinflam-

matory cytokine levels. The investigators concluded that circulating levels of proinflammatory cytokines increased in patients as their functional heart failure classification deteriorated. Moreover, activation of the neurohumoral axis was unlikely to completely explain the elaboration of proinflammatory cytokines in heart failure, and there was a trend toward increasing mortality with increasing levels of TNF- α .

Thus, analogous to elevated levels of neurohormones, TNF- α levels may be predictive of NYHA class and clinical outcome in patients with heart failure, and may represent a biochemical mechanism that is responsible for producing symptoms in patients with heart failure. In a manner similar to the benefits seen in heart failure patients with agents that antagonize the neurohormonal system, it is reasonable to ask whether antagonizing cytokines may lead to clinical improvements in patients with heart failure

1996

US Commerce secretary Ronald H. Brown is killed in an air crash near Dubrovnik, Croatia; the Duke and Duchess of York are granted a divorce; and Theodore John Kaczynski, 53, a Harvard graduate and former professor of mathematics, is arrested on suspicion of being the long-hunted Unabomber



Cytokines and cardiac contractile function

R.A. Kelly, T.W. Smith

Circulation. 1997;95:778-781

For quite a while, investigators have been noticing that proinflammatory cytokines such as tumor necrosis factor (TNF) play a role in the cardiac dysfunction that accompanies systemic sepsis, viral myocarditis, and cardiac allograft rejection, but also in advanced heart failure (HF) syndromes resulting from diverse pathogenic insults. This superb review on the role of cytokines in cardiac contractile function gives a chronological account of these intriguing observations.

Inflammatory cytokines such as TNF and interleukin-1 β (IL-1 β) are locally acting autocrine (acting on the cell of origin), paracrine (acting on neighboring cells), or juxtacrine (acting on adjacent cells) agents whose biological activity is determined not only by the specific target cell type, but also by the intracellular milieu or biological context in which a cytokine acts. The first of such observations was by Lefer and Rovetto, who more than 25 years ago, in 1970, reported that the sera of septic patients and experimental animals contained a "myocardial depressant factor." During the past decade, many investigators used intact animals and in vitro isolated heart cell preparations to systematically investigate the factors that contribute to myocardial depression in systemic sepsis, and concluded that TNF- α and IL-1 β were present in the serum of septic patients and were responsible for most, if not all, of the reversible cardiac depression often seen with this syndrome. Systemic infusions of one or more recombinant cytokines such as TNF and IL-1 β in intact animal preparations usually resulted in decline in ventricular systolic function. Subsequently, it was noted that elevation in these proinflammatory cytokines was not only observed in acute inflammatory or infection states such as sepsis but also in patients with advanced HF. The first observation was published in 1990 by Levine and colleagues who reported elevated circulating levels of TNF in severe chronic HF. A number of subsequent studies consistently found elevated levels of TNF, IL-1, and IL-6 in patients with HF. Interest in these findings has been amplified by reports of elevated circulating as well as intracardiac TNF levels and concomitant increase in plasma levels of soluble TNF receptors, which appear to bind and neutralize most, if not all, circulating

TNF in patients with advanced HF. Cytokines may contribute to cardiac myocyte contractile dysfunction through several mechanisms. Inflammatory mediators, including bacterial endotoxin, can induce generation of specific cytokines and expression of vascular inducible nitric oxide synthase (iNOS) in macrophages and endothelial cells. These cells subsequently generate additional cytokines, which induce contractile dysfunction in cardiac myocytes by both NO-dependent and NO-independent mechanisms.

Recent reports indicate that iNOS expression is increased in the myocardium of patients with advanced HF, whether caused by ischemic heart disease, idiopathic cardiomyopathy, or valvular disease. These data are consistent with a number of recent reports that increased iNOS expression in cardiac myocytes and in microvascular and endocardial endothelial cells, which combined with infiltrating inflammatory cells, account for most NO production after regional or global iNOS induction in the heart, markedly suppresses basal and β -adrenergic agonist-stimulated myocardial inotropic responsiveness. However, there are no data that firmly establish an important role for iNOS induction in the pathophysiology of clinical HF. Nevertheless, the documentation of high intramyocardial and plasma levels of TNF in humans with HF, in combination with catecholamines and peptide autacoids known to enhance iNOS expression and activity in cardiac myocytes, indicates that this is an important hypothesis to be tested.

1997

Deng Xiaoping, communist leader of the PRC
since 1976, dies, aged 92;

Fox cartoon series "The Simpsons" airs its
167th episode, becoming the longest-running
animated series in cartoon history;
and the comet Shoemaker-Holt 2 makes
its closest approach to the Earth

Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor- α

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Circ Res. 1997;81:627-635

This is the landmark paper describing the dilated cardiomyopathy phenotype in transgenic mice with cardiac specific overexpression of tumor necrosis factor- α (TNF- α). The failing human heart expresses TNF- α . Its cardiac genetic overexpression was long awaited to support the pathophysiological significance of TNF- α for the heart.

Kubota and his colleagues had previously reported that robust overexpression of TNF- α in the murine heart causes lethal myocarditis. In this earlier report, a transgene construct was made containing the murine α -myosin heavy chain promoter and the coding sequence of murine TNF- α . Injection of this construct into fertilized eggs yielded three transgenic mice, all of which died spontaneously before the completion of weaning. Gross pathologic analysis of these mice demonstrated a decrease in body weight with markedly increased heart weight. Histologic examination of the heart revealed a substantial, diffuse lymphohistiocytic inflammatory infiltrate, associated with interstitial edema. Enzyme-linked immunosorbent assay demonstrated a substantial amount of TNF- α protein in the transgenic heart.

In this study, Kubota et al modified the transgene to reduce the production of TNF- α by preserving the destabilizing sequence in TNF- α cDNA. Expression was driven by the murine α -myosin heavy chain promoter. Use of this modified construct allowed the establishment of a murine transgenic line with more modest TNF- α overexpression rather than lethally toxic high levels, and most mice survived the neonatal period. These mice with modest cardiac TNF- α overexpression showed a significantly higher heart weight-to-body weight ratio consistent with heart failure, and there was a mild, diffuse, lymphohistiocytic interstitial inflammatory infiltrate in the transgenic hearts. Cardio-myocyte necrosis and apoptosis were present, although not abundant.

To characterize functional significance of the TNF- α overexpression, the investigators performed magnetic resonance imaging, which revealed that the transgenic heart was significantly dilated, with reduced left ventricular ejec-

tion fraction. In addition, its responsiveness to isoproterenol was significantly blunted, suggesting attenuation of adrenergic responsiveness. These functional defects were accompanied by expression of atrial natriuretic factor in the transgenic ventricle. A group of transgenic mice died spontaneously, and subsequent autopsies revealed exceptional dilation of the heart, increased lung weight, and pleural effusion, suggesting that they died of congestive heart failure. The cumulative mortality rate at 6 months was 23%.

In conclusion, the mice overexpressing TNF- α recapitulated the phenotype of congestive heart failure. This provides a novel model to elucidate the role of TNF- α in the development of congestive heart failure.

1997

South American guerilla

Ernesto "Che" Guevara is finally laid to rest in a mausoleum in Santa Clara, 30 years after his death in Bolivia; the International Committee to Ban Land Mines and its US coordinator, Jody Williams, wins the Nobel Peace prize; and the Guggenheim Museum of Art designed by Frank Gehry is inaugurated in the Basque city of Bilbao



Randomised investigation of effects of pentoxifylline on left-ventricular performance in idiopathic dilated cardiomyopathy

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Lancet. 1998;351:1091-1093

A number of studies attempting to suppress cytokine production in patients with heart failure have employed strategies that are designed to block tumor necrosis factor (TNF) expression at the transcriptional or translational levels. A potentially important pharmacological method for suppressing TNF production is through the use of agents that elevate cAMP levels, such as phosphodiesterase inhibitors. Recently, encouraging results with respect to modulating TNF levels through alterations in intracellular cAMP levels in heart failure have been reported by Sliwa and colleagues. They studied the effects of pentoxifylline in patients with dilated cardiomyopathy and New York Heart Association (NYHA) class II to III heart failure. Pentoxifylline is a methylxanthine phosphodiesterase inhibitor that prevents the synthesis of proinflammatory cytokines such as TNF, and had been formerly reported as being an effective drug in inhibiting TNF- α responses during septic shock. The inhibition of TNF- α production seems to be correlated with increased intracellular cAMP levels.

This novel study by Sliwa and colleagues aimed to assess the effects of pentoxifylline on left-ventricular function and functional NYHA class in patients with idiopathic dilated cardiomyopathy. They conducted a single-center, prospective, double-blind, randomized, placebo-controlled trial, in which 28 patients with idiopathic dilated cardiomyopathy were assigned pentoxifylline 400 mg three times daily or matching placebo. Clinical, echocardiographic, and radionuclide assessments were done at baseline and after 6 months of treatment. The primary end points of the 6-month study were NYHA functional class and left ventricular function. All patients were receiving concurrent therapy with digitalis, diuretics, and angiotensin-converting enzyme (ACE) inhibitors for 4 months. A total of 14 patients received pentoxifylline at a dose of 400 mg three times daily, and an equal number received placebo. Four patients died as a result of progressive pump dysfunction during the 6-month study period, all in the placebo group. At the end of 6 months, there was an improvement in functional NYHA class in the pentoxifylline group, whereas there was functional deterioration in the placebo group. There

was also a significant increase in the ejection fraction (from 22.3 ± 9.0 to 38.7 ± 15.0) in the pentoxifylline group, vs no significant change in the placebo group. There was, however, no change in left ventricular end-diastolic dimension in either group. An important observation was that TNF levels fell significantly from 6.5 ± 5.0 pg/mL to 2.1 ± 1.0 pg/mL in the pentoxifylline group, whereas there was no significant change in the TNF levels in the placebo group.

Thus, it appears that modulation of TNF levels via agents that alter intracellular cAMP levels, thus blocking transcriptional activation of TNF, may be a useful strategy for altering cytokine levels in heart failure. However, it is unclear whether the levels of intracellular cAMP levels that are necessary to suppress cytokine production will also be proarrhythmic in patients with heart failure.

1998

Large tracts of the Great Barrier reef are reported to have died due to increased water temperatures ascribed to global warming;
 Octavio Paz, Mexican Nobel prize winning poet and philosopher, dies, aged 84;
 and Birmaryan Chaudhary Majhi dies at Khanar, in the Sunsari district of east Nepal, at the alleged age of 141

Safety and efficacy of a soluble P75 tumor necrosis factor receptor (Enbrel, etanercept) in patients with advanced heart failure

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Circulation. 1999;99:3224-3226

Since the original reports of elevated levels of tumor necrosis factor (TNF) in patients with heart failure and the recognition that TNF may contribute to the progression of heart failure, there has been increasing speculation that anticytokine therapy targeting TNF may be beneficial in patients with heart failure. This is the seminal paper describing the important role of targeted anticytokine therapy in patients with heart failure.

Circulating soluble TNF receptors act as “decoys” to bind TNF, thus preventing it from binding to its cognate TNF receptors on cell surface membranes. Prior to this paper, experimental studies from the same laboratory had shown that the soluble dimeric p75 chimeric fusion protein, (Enbrel, etanercept), consisting of two of the extracellular p75 TNF receptors fused in duplicate to the Fc portion of the IgG₁ molecule (TNFR:Fc), was sufficient to reverse some of the deleterious cardiovascular effects of TNF in vitro and in vivo. In this study, Deswal et al examined the safety and efficacy of etanercept in patients with advanced heart failure. They studied 18 New York Heart Association (NYHA) class III heart failure patients who had an initial screening left ventricular ejection fraction (LVEF) <35% and elevated circulating plasma levels of TNF >3.0 pg/mL, which is >2 SD above the mean TNF level for normal subjects. The study was a randomized, double-blind, placebo-controlled, dose-escalation trial. The primary objectives were to evaluate the safety of etanercept and to assess clinical and laboratory indices for preliminary evidence of improvement in LVEF, patient functional status, and TNF bioactivity. The secondary objective was to evaluate the systemic pharmacokinetics of a single intravenous dose of etanercept.

There was no significant difference in age, cause of heart failure, LVEF, or peripheral TNF levels between the 3 groups (1, 4, and 10 mg/m²). All of the patients received ACE inhibitors, 94.4% received digoxin, 11% β-blockers, and 11% amlodipine; there was no significant difference between groups with respect to medication use. Circulating levels of biologically active TNF decreased by ≈50% in the patients who received etanercept; moreover, these levels remained significantly depressed at day 14. There was a significant

improvement in the quality-of-life score in the patients who received etanercept and a small but statistically significant increase in the LVEF vs no significant change in the placebo group. There was, however, no significant change in the 6-minute walk distance in the etanercept group. Because the 1-mg/m² dose was included in the study design as a “no-dose” effect, the above analyses were repeated after excluding the 4 patients who received 1 mg/m², and showed a significant overall improvement in the quality-of-life score, 6-minute walk distance, and LVEF for the patients who received 4 or 10 mg/m² of etanercept.

The results of this study support the concept that TNF is a potentially important therapeutic target in heart failure patients. A single intravenous infusion of etanercept was safe and well tolerated in patients with NYHA class III heart failure, was sufficient to lower levels of biologically active TNF, and led to improvements in the functional status of patients. However, the results of this phase I study must be regarded as provisional because of the relatively small numbers of patients and the relatively short duration of follow-up. Whether such beneficial effects observed can be sustained when etanercept is given repeatedly over longer periods of time and in larger patient populations is now being addressed in two ongoing multicenter clinical trials, The Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE), and the Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER).

1999

Nelson Mandela steps down as the first black president of South Africa; Indonesia holds its first democratic elections for 44 years; and human footprints believed to be 20 000-30 000 years old are discovered in a cave in the Ardèche, France



Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: role of tumor necrosis factor- α

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Circulation. 1999;100:1983-1991

This is a very original and important paper addressing the relationship of cytokine activation and endothelial dysfunction. Cytokine activation and endothelial dysfunction are typical phenomena of congestive heart failure. In normal vessels, acetylcholine induces nitric oxide synthesis by activating endothelial nitric oxide synthase (eNOS). Conversely, in patients with heart failure, acetylcholine results in a blunted vasodilating response, suggesting endothelial dysfunction. Nitric oxide-donor administration in heart failure patients, however, exerts a vasodilating response similar to that observed in normal controls, suggesting the integrity of the vascular muscle cell. One explanation for this apparent paradox is that eNOS expression is impaired in heart failure.

Because historically no significant correlation has been demonstrated between neurohormones and endothelial dysfunction in heart failure, the authors of this study tested the possible role of another system: the system of cytokines and, in particular, tumor necrosis factor (TNF). Cytokines, when used *in vitro*, are known to inhibit eNOS expression. This novel paper by Agnoletti et al demonstrated that incubating human umbilical vein endothelial cells (HUVECs) with serum from patients with heart failure downregulated constitutive eNOS and also induced apoptosis.

In the first part of the study, the incubation of HUVECs had no effect on eNOS in normal controls, whereas it resulted in a time-dependent downregulation of eNOS protein expression in patients with heart failure. TNF antibody partially counteracted the inhibitory effect of the serum from patients with heart failure. After stepwise selection, TNF levels showed a correlation with the reduction of eNOS expression, but this correlation was mild. It appeared that TNF did not completely account for the eNOS downregulation, because the addition of the anti-human TNF antibody only partially counteracted the effect of heart failure serum on eNOS. One explanation for this partial effect is that the effects of TNF on the expression and activity of eNOS *in vitro* are known to be enhanced by interferon- γ and interleukin- 1β , and it is possible that actually a cytokine

mixture—rather than one cytokine alone, present in the blood of patients with heart failure—may be responsible for eNOS downregulation. Thus, in the first part of the study, the authors demonstrated that serum from patients with heart failure downregulated the expression of eNOS, and that this was partially a TNF-mediated process.

In the second part of the study, the authors addressed whether serum from patients with heart failure induced apoptosis and whether this was a TNF-mediated process. Qualitatively, with optical microscopy, they demonstrated morphological aspects of nuclear apoptosis in all HUVECs treated with serum of patients with heart failure. With flow cytometry, incubation of HUVECs with serum from patients with heart failure resulted in a higher rate of apoptosis measured in comparison with normal controls. TNF antibody only partially counteracted this effect. The investigators found a strong correlation between eNOS downregulation and apoptosis, suggesting a link between the two phenomena. In addition, multiple linear regression analysis showed a significant correlation with apoptosis and cytokine expression. After stepwise selection, only TNF blood levels were significantly correlated with apoptosis. Conversely, no correlation was found among apoptosis, neurohormones, or any of the clinical parameters measured.

Although these findings cannot be fully extrapolated to *in vivo* clinical conditions, they do represent the important consequences of abnormal interaction between the bloodstream of patients with heart failure and human endothelium.

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