

# Cytokines

## Lead Article

- Cytokines in ischemic heart disease and heart failure **135**  
*D.L. Mann, P. Knueferman, G. Baumgarten*

## Expert Answers to Three Key Questions

- Do cytokines and endothelial function have an impact on myocardial activity? - *L. Agnoletti, L. Comini* **149**  
 What are the implications of the interaction between the neurohumoral system and the cytokines? - *H. Drexler, B. Schieffer* **154**  
 Has the time arrived to use anticytokine therapy in chronic heart failure? - *S.D. Anker* **162**

## Fascinoma Cardiologica

- Surfing the Heart:** International Society for Heart Research – The Cochrane Library – Annals of Improbable Research – European Society of Cardiology - *C. Ceconi* **171**  
**Trails of Discovery:** The importance of chance and the prepared mind in the discovery of the  $\beta$ -blockers - *D. Fitzgerald* **172**

## Summaries of Ten Seminal Papers - *B. Bozkurt* **177**

- |  |  |
|--|--|
| Elevated circulating levels of tumor necrosis factor in severe chronic heart failure - <i>B. Levine and others</i>   | Cytokines and cardiac contractile function - <i>R.A. Kelly, T.W. Smith</i>   |
| Increased circulating cytokines in patients with myocarditis and cardiomyopathy - <i>A. Matsumori and others</i>   | Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor- $\alpha$ - <i>T. Kubota and others</i>                |
| Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure - <i>R. Ferrari and others</i>  | Randomised investigation of effects of pentoxifylline on left-ventricular performance in idiopathic dilated cardiomyopathy - <i>K. Sliwa and others</i>        |
| Tumor necrosis factor- $\alpha$ and tumor necrosis factor receptors in the failing human heart - <i>G. Torre-Amione and others</i>   | Safety and efficacy of a soluble P75 tumor necrosis factor receptor (Enbrel, etanercept) in patients with advanced heart failure - <i>A. Deswal and others</i> |
| Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD) - <i>G. Torre-Amione and others</i> | Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: role of tumor necrosis factor- $\alpha$ - <i>L. Agnoletti and others</i> |

## Bibliography of One Hundred Key Papers **189**

# The ISHR-ES/SERVIER Research Fellowship

The **European Section of the International Society for Heart Research** and **SERVIER** invite submissions for the first **ISHR-ES/SERVIER Research Fellowship**. The purpose of this Fellowship is to support the initiation and development of scientific collaborations between outstanding groups in the field of cardiovascular biology by providing a young investigator from a European laboratory with one-year postdoctoral support allowing him/her to carry on a research program in another European country. The term European refers not only to the countries of the European Community, but also to all countries belonging to the European Section of the ISHR.

#### Details of the competition are as follows:

1. Candidates must be members (or have applied for membership) of the ISHR-ES (membership application forms are available at the ISHR-ES web site [www.biomed.cas.cz/fgu/ishr\\_es/](http://www.biomed.cas.cz/fgu/ishr_es/) or can be obtained from Dr Frantisek Kolar, Secretary of the ISHR-ES, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4, Czech Republic; phone: 420 2 475 2559; fax: 420 2 475 2125; e-mail: kolar@biomed.cas.cz).
2. Candidates must have defended their PhD thesis not earlier than January 1, 1999, and be less than 35 years of age on July 1, 2001.
3. Applications must include the following:
  - Curriculum vitae (family name, first name, date of birth, current employment and position, summary of previous positions, degrees, special area of interest and expertise, other activities, publications);
  - Research program of a maximum of 10 pages (including one page for the summary and references) detailing the research program (title, aims, rationale, working hypothesis, scientific expertise of each group, preliminary results if any, plan of investigation detailing the scientific procedures, and role of each investigator of each group and the precise role of the candidate in the proposed program and funding);

- Letters of the candidate's current immediate supervisor and future immediate supervisor (Division Heads, Department Chairmen, or Institute Directors) detailing why the collaboration between the two research groups is essential for the success of the research program and why, among all other potential applicants, the applicant is the most appropriate candidate for the Fellowship, and offering a rationale for their opinion.

4. Eight copies of the application should be sent to Dr Jean-Jacques Mercadier, President of the ISHR-ES, Departments of Physiology and Cardiology and INSERM U460, Groupe Hospitalier Bichat – Claude Bernard, 46 rue Henri Huchard, 75877 Paris Cedex 18, France, **no later than March 30, 2001. Applications received after this deadline will not be considered.**
5. The two collaborating research groups can submit only one application.
6. The applications will be reviewed in Paris in May 2001 by a committee composed of six members of the ISHR-ES Council and one representative of SERVIER. The three best applications will be classified. The second and the third will receive a one-year free electronic subscription to the *Journal of Molecular and Cellular Cardiology*.
7. The winner of the Fellowship will receive a travel grant to cover economy airfare and other travel costs towards his/her attendance at the XVIIth World Congress of the ISHR in Winnipeg, July 6-11, 2001. At the Congress, the winner will present his/her research program to the Society. He/she will receive a plaque and check of €20 000 as a personal support. Any winner who, for any reason, cannot personally present his or her research program at the Congress must withdraw from the competition. Substitute presenters are not allowed.
8. It is expected that the results of the investigation will be presented by the recipient at the annual ISHR-ES Congress in Strasbourg, France, in 2003.
9. Applications will not be returned.

Jean-Jacques Mercadier, MD, PhD  
President, ISHR-ES



**DEADLINE FOR APPLICATIONS: MARCH 30, 2001**



# Cytokines in ischemic heart disease and heart failure

**Douglas L. Mann, MD; Pascal Knueferman, MD; Georg Baumgarten, MD**

Winters Center for Heart Failure Research - The Cardiology Section - Department of Medicine - Veterans Administration Medical Center - and Baylor College of Medicine - Houston - Texas - USA

Recent studies have identified the importance of biologically active molecules such as neurohormones in disease progression in heart failure. More recently, it has become apparent that, in addition to neurohormones, another portfolio of biologically active molecules, termed cytokines, is also expressed in the setting of heart failure. This article reviews recent clinical and experimental material that suggests that cytokines such as tumor necrosis factor  $\alpha$ , interleukin-1, and interleukin-6, analogous to the role of the classic neurohormones, may represent another class of biologically active molecules responsible for the development and progression of heart failure. This article also looks at the early results of clinical trials that have utilized various cytokine antagonists in patients with heart failure.

*“...but when the parenchyma of the heart has been harmed by various diseases its motion is necessarily much altered; for if the parenchyma of the heart is burdened with too much fat, labours under inflammation, abscess or wound, so it cannot vibrate or contract without great trouble or difficulty, it soon gives up its motion, whence the movement of the blood also to the same degree becomes weak and languid.”*

Richard Lower, *Tractus de Corde*, 1669

Although clinicians have recognized the potential importance of inflammatory mediators in the pathogenesis of heart disease for well over 200 years, it has taken nearly as many years for clinicians and scientists to focus on the basic biological mechanisms by which inflammatory mediators contribute to the pathogenesis of cardiac disease states. Nonetheless, despite the relatively delayed onset in interest in the mechanistic role that inflammatory mediators play in heart disease, there has been considerable interest over the past decade in the role that inflammatory mediators play in regulating cardiac structure and function. Accordingly, in the present chapter, we will summarize the recent growth of knowledge that has taken place in this field, with a particular emphasis on the potential role that proinflammatory (“stress-activated”) cytokines play as mediators of disease progression in the ischemic and failing human heart.

## SELECTED ABBREVIATIONS

<b>GM-CSF</b>	granulocyte/macrophage colony-stimulating factor
<b>IL-1, -6</b>	interleukin-1, interleukin-6
<b>IL-1RA, -6R</b>	soluble interleukin receptors 1RA, 6R
<b>INF-<math>\gamma</math></b>	interferon gamma
<b>SOLVD</b>	Studies Of Left Ventricular Dysfunction
<b>sTNFR1, R2</b>	soluble tumor necrosis factors R1, R2
<b>TNF-<math>\alpha</math></b>	tumor necrosis factor $\alpha$

**Keywords:** tumor necrosis factor; interleukin-1; interleukin-6; cytokine; heart failure

**Address for correspondence:** Douglas L. Mann, MD, Cardiology Research (151C) VA Medical Center, 2002 Holcombe Blvd, Houston, TX 77030, USA (e-mail: dmann@bcm.tmc.edu)

## CYTOKINES AND THE HEART

### Cytokines: definition and role

The term cytokine is applied to a group of relatively small molecular weight protein molecules (generally 15-30 kDa) that are secreted by cells in response to a variety of different inducing stimuli. Although “proin-

flammatory cytokines” have traditionally been thought to be produced by the immune system, one of the more recent intriguing observations is that virtually all nucleated cell types within the myocardium, including cardiac myocytes themselves, are capable of synthesizing a portfolio of proinflammatory cytokines, including tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin-1

(IL-1), and interleukin-6 (IL-6), in response to various forms of cardiac injury (Table I). Thus, from a conceptual standpoint, these molecules should be envisioned as proteins that are produced locally within the myocardium by “cardiocytes” (ie, cells that reside within the myocardium), in response to one or more different forms of environmental stress, including hemodynamic pressure overload and myocardial ischemia/infarction. An important corollary of this statement is that the expression of these “stress-activated” cytokines can occur in the complete absence of activation of the immune system.

• Acute viral myocarditis
• Cardiac allograft rejection
• Myocardial infarction
• Unstable angina
• Myocardial reperfusion injury
• Hypertrophic cardiomyopathy*
• Heart failure*
• Cardiopulmonary bypass*
• Magnesium deficiency*
• Pressure overload*

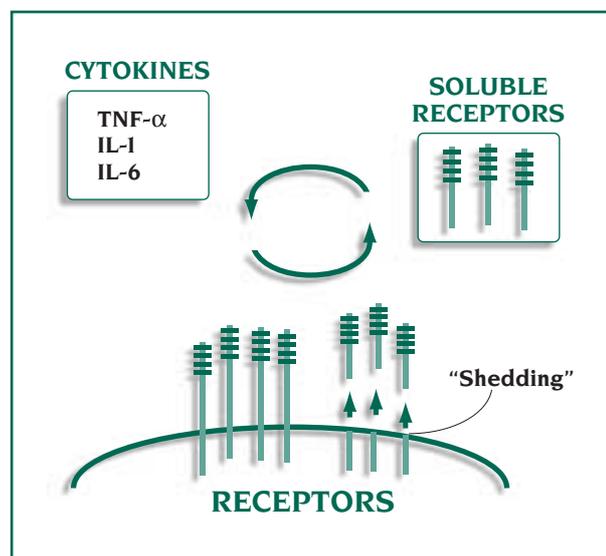
\* Indicates conditions not traditionally associated with immunologically mediated inflammation

**Table I.** Pathophysiological conditions associated with activation of “proinflammatory cytokines.”

• Produces left ventricular dysfunction in humans
• Produces pulmonary edema in humans
• Produces cardiomyopathy in humans
• Associated with reduced skeletal muscle blood flow in humans
• Promotes left ventricular remodeling experimentally
• Promotes thromboembolism experimentally
• Produces abnormalities in myocardial metabolism experimentally
• Produces anorexia and cachexia experimentally
• Produces $\beta$ -receptor uncoupling from adenylate cyclase experimentally
• Abnormalities of mitochondrial energetics experimentally
• Activation of the fetal gene program experimentally
• Produces cardiac myocyte apoptosis experimentally
• Produces endothelial cell apoptosis experimentally

**Table II.** The potential untoward effects of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in heart failure.

Modified from ref 1: Bozkurt B, Shan K, Seta Y, Oral H, Mann DL. Tumor necrosis factor  $\alpha$  and tumor necrosis factor receptors in human heart failure. Heart Fail Rev. 1996;1:211-219. Copyright © 1996, Kluwer Academic Publishers Group.



**Figure 1.** Cytokine bioactivity. When interpreting the biological activity of any cytokine, it is important to know the concentration of the cytokine that one is measuring, the concentration of the receptors on which the cytokine is acting, as well as the presence and/or absence of any circulating antagonists or agonists for the particular cytokine of interest. TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; IL-1, -6, interleukin-1, -6.

Modified from ref 1: Bozkurt B, Shan K, Seta Y, Oral H, Mann DL. Tumor necrosis factor  $\alpha$  and tumor necrosis factor receptors in human heart failure. Heart Fail Rev. 1996;1:211-219. Copyright © 1996, Kluwer Academic Publishers Group.

The current interest in understanding the role of “stress-activated” cytokines in heart failure relates to the observation that many aspects of congestive heart failure can be explained by the *known* biological effects of these molecules (Table II).<sup>1</sup> Simply stated, when expressed at sufficiently high concentrations, cytokines are sufficient to mimic some aspects of the so-called heart failure phenotype, including (but not limited to) progressive left ventricular (LV) dysfunction, pulmonary edema, LV remodeling, fetal gene expression, endothelial dysfunction and cardiomyopathy.<sup>2-5</sup> Thus, the “cytokine hypothesis”<sup>6</sup> of heart failure holds that heart failure progresses, at least in part, as a result of the



direct toxic effects that cytokines exert on the heart and circulation. It bears emphasis that the cytokine hypothesis does not imply that cytokines cause "heart failure" per se, but rather that the overexpression of cytokines contributes to the progression of heart failure once LV dysfunction ensues. Accordingly, the elaboration of cytokines, much like the elaboration of neurohormones, may represent a biological mechanism that is responsible for producing symptoms in patients with heart failure.

However, in order to understand how cytokines play a role in heart failure, it is important to digress for a moment to delineate the concept of cytokine bioactivity. As shown in *Figure 1*,<sup>1</sup> when interpreting the biological activity of any cytokine, it is critically important to know the concentration of the cytokine that

one is measuring, the concentration of the receptors on which the cytokine is acting, as well as the presence and/or absence of any circulating antagonists or agonists for the particular cytokine of interest.

### Which proinflammatory cytokines are elevated in human heart failure?

*Table III* provides a summary of the studies that have examined circulating cytokine levels in patients with symptomatic heart failure secondary to ischemic and/or dilated cardiomyopathy.<sup>7-27</sup> Although large-scale comparisons of cytokine levels in ischemic and dilated cardiomyopathy have not been performed, the extant literature suggests that there is no difference in the degree of activation of cytokines in patients with ischemic and/or dilated cardiomyopathy.

	Cytokines					Cytokine receptors			
	TNF- $\alpha$	IL-1	IL-2	IL-6	IFN- $\gamma$	sTNFR1	sTNFR2	IL-1RA	IL-6R
Levine, 1990 <sup>8</sup>	+	nd	nd	nd	nd	nd	nd	nd	nd
McMurray, 1991 <sup>9</sup>	+	nd	nd	nd	nd	nd	nd	nd	nd
Dutka, 1993 <sup>10</sup>	+	nd	nd	nd	nd	nd	nd	nd	nd
Wiederman, 1993 <sup>11</sup>	+	-	nd	+	-	nd	nd	nd	nd
Katz, 1994 <sup>12</sup>	+	-	+	nd	nd	nd	nd	nd	nd
Matsumori, 1994 <sup>13</sup>	+	-†	-†	-†	-†	nd	nd	nd	nd
Ferrari, 1995 <sup>14</sup>	+	nd	nd	nd	nd	+	+	nd	nd
Pritchett, 1995 <sup>15</sup>	-	nd	nd	nd	nd	nd	nd	nd	nd
Torre-Amione, 1996 <sup>16</sup>	+	nd	nd	nd	nd	+	+	nd	nd
Torre-Amione, 1996 <sup>17</sup>	+	nd	nd	+	nd	nd	nd	nd	nd
Milani, 1996 <sup>18</sup>	+	nd	nd	nd	nd	+	nd	+	nd
Munger, 1996 <sup>19</sup>	-	-	nd	+	nd	nd	nd	nd	nd
Testa, 1996 <sup>20††</sup>	+	+	-†	+	nd	nd	+	+	nd
Anker, 1997 <sup>21</sup>	+	nd	nd	nd	nd	nd	nd	nd	nd
MacGowan, 1997 <sup>22</sup>	+	nd	nd	+	nd	nd	nd	nd	nd
Mohler, 1997 <sup>23</sup>	+	nd	nd	+	nd	nd	nd	nd	nd
Nishigaki, 1997 <sup>24</sup>	+	nd	nd	+	nd	nd	nd	nd	nd
Anker, 1997 <sup>25</sup>	+	nd	nd	nd	nd	+	+	+	nd
Tsutamoto, 1998 <sup>26</sup>	+	nd	nd	+	nd	nd	nd	nd	nd
Dibbs, 1999 <sup>27</sup>	+	nd	nd	+	nd	+	+	nd	+

+ Elevated levels present  
 - Mean levels not different from control values or not detectable with assays used  
 nd Not done  
 † Elevated cytokine level detected in <5% of patients  
 †† Also detected elevated levels of circulating IL-6R

**Table III.** Cytokines and cytokine receptors in human heart failure. Expanded names of cytokines and cytokine receptors, see box on page 135.

Modified from ref 7: Kapadia S, Dibbs Z, Kurrelmeyer K, et al. The role of cytokines in the failing human heart. In: Crawford M, ed. *Cardiology Clinics*. Philadelphia, Pa: W.B. Saunders, 1998:645-656. Copyright © 1998, W.B. Saunders.

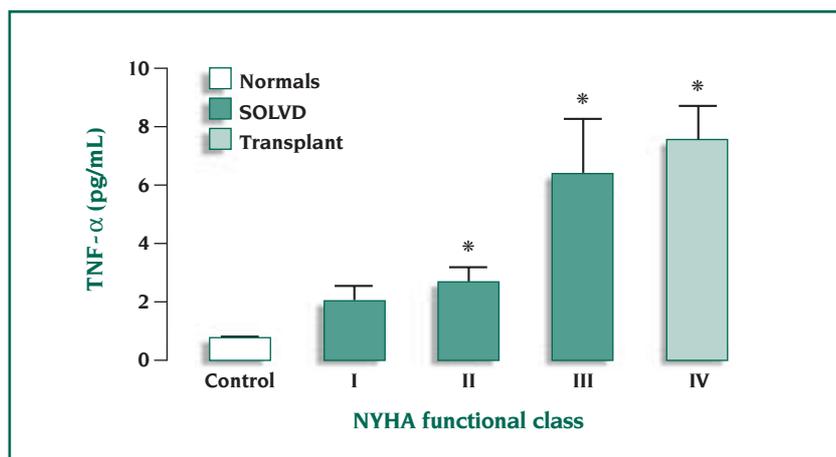
**Inspection of Table III discloses several important clinical themes:**

• First, whereas a number of studies have examined the role of TNF- $\alpha$  in the setting of chronic heart failure, comparatively less is known about circulating levels of IL-1, IL-2, IL-6, and interferon-gamma (IFN- $\gamma$ ) in this clinical setting. Nonetheless, despite this limitation, several insights are apparent. For example, save for two studies that used assay systems that were perhaps not sensitive enough to measure circulating levels of TNF- $\alpha$  observed in heart failure patients,<sup>15,19</sup> elevated levels of TNF- $\alpha$  have been consistently identified in patients with advanced heart failure. Moreover, several studies suggest that there is increasing cytokine elaboration in direct relation to the severity of the disease process.<sup>14,17,20,22</sup> As shown in *Figure 2*, there is a progressive increase in TNF- $\alpha$  levels in direct relation to deteriorating New York Heart Association (NYHA) functional class.<sup>6</sup> Thus, much like elevated levels of

neurohormones, TNF- $\alpha$  levels may be predictive of NYHA class and the clinical severity of disease.

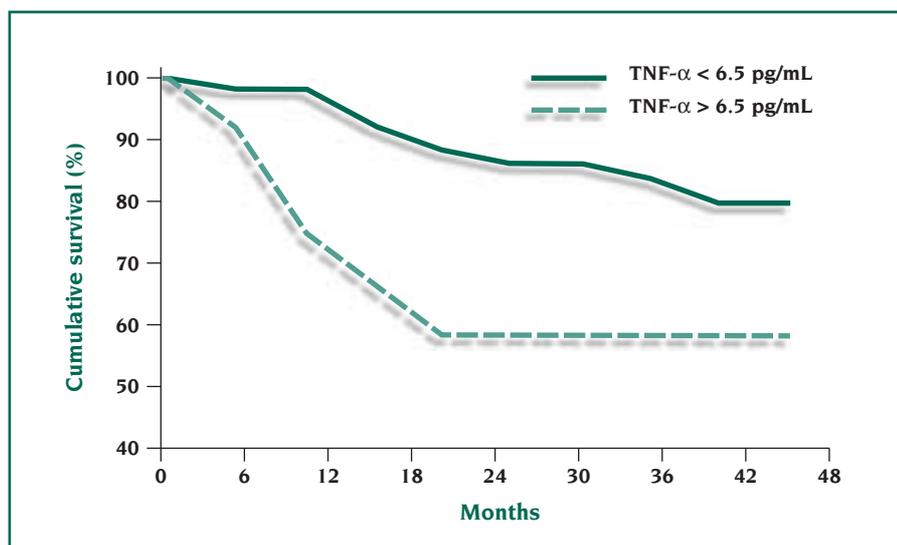
• Second, most studies have consistently found elevated levels of IL-6 in the setting of congestive heart failure, although this has not been true for all studies.<sup>13</sup> Although the mechanism for increased elaboration of IL-6 in heart failure is not known, it is interesting to note that TNF- $\alpha$  is sufficient to induce IL-6 gene and protein expression in a variety of cell types,<sup>28</sup> suggesting that there may be a “cytokine cascade” in the setting of heart failure. Indeed, two studies have identified a significant correlation between elevated levels of TNF- $\alpha$  and elevated levels of IL-6.<sup>17,22</sup> In the report by MacGowan and colleagues, there was a statistically significant correlation between elevated levels of IL-6 and elevated right heart pressures.<sup>22</sup>

• Third, at the time of this writing, there is limited clinical evidence that supports an important role for IL-1, IL-2, or IFN- $\gamma$  in heart failure. However, this state-



**Figure 2.** TNF- $\alpha$  levels in patients with New York Heart Association (NYHA) class I to IV heart failure. In comparison with age-matched control subjects (open bar), there was a progressive increase in serum tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) levels in direct relation to decreasing functional heart failure classification. The solid bars denote values for patients enrolled in the Studies of Left Ventricular Dysfunction (SOLVD)<sup>17</sup>; the shaded bar denotes values for NYHA class IV patients who were undergoing cardiac transplantation.<sup>16</sup>  $P < 0.05$ .

Reproduced from ref 6: Seta Y, Shan K, Bozkurt B, Oral H, Mann DL. Basic mechanisms in heart failure: the cytokine hypothesis. *J Card Fail.* 1996; 2:243-249. Copyright © 1996, W.B. Saunders.



**Figure 3.** Prognostic values of tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) levels. A Kaplan-Meier analysis was performed for Studies of Left Ventricular Dysfunction (SOLVD) patients with TNF- $\alpha$  levels less than or greater than 6.5 pg/mL (90th percentile). As shown, there was a significant increase in overall patient mortality for patients with TNF- $\alpha$  levels greater than 6.5 pg/mL when compared with patients with TNF- $\alpha$  levels less than 6.5 pg/mL.

Reproduced from ref 6: Seta Y, Shan K, Bozkurt B, Oral H, Mann DL. Basic mechanisms in heart failure: the cytokine hypothesis. *J Card Fail.* 1996;2:243-249. Copyright © 1996, W.B. Saunders.



ment must be regarded as provisional, given that relatively few studies have measured circulating levels of these cytokines in this clinical setting. Moreover, as noted above for IL-1, this cytokine may largely subserve autocrine, paracrine, and intracrine functions in the heart, and may therefore not “spill over” into the peripheral circulation.

Thus, in summary, on the basis of the clinical material extant at this time, the preponderance of clinical data suggest that TNF- $\alpha$  and IL-6 are at least two of the major clinically relevant stress-activated cytokines that are elaborated in the setting of heart failure.

### Which cytokine receptors are elevated in human heart failure?

Another important finding shown by *Table III* is that there are elevated circulating levels of cytokine receptors and cytokine receptor antagonists in heart failure, including soluble tumor necrosis factors sTNFR1, sTNFR2, and soluble interleukin receptors IL-1RA and IL-6R. Thus far, four reports have demonstrated that elevated circulating levels of sTNFR1 and sTNFR2 are present in patients with heart failure.<sup>14,16,25-27</sup> In the report by Ferrari et al,<sup>14</sup> circulating levels of sTNFR2 correlated independently with worse short-term prognosis. Although the clinical significance of this finding is uncertain, it has been suggested that sTNFRs may act as a biological reservoir for TNF- $\alpha$ , which stabilizes the molecule and slowly releases this cytokine into the circulation. Alternatively, as discussed above, elevated levels of sTNFRs may be protective in that they bind to TNF- $\alpha$  and inactivate it. *Table III* further shows that there are elevated levels of circulating IL-1 receptor antagonists in heart failure. However, given that there is relatively little evidence that IL-1 levels are elevated in heart failure, the clinical significance of this finding remains uncertain at present. Recent studies have suggested that the soluble IL-6 receptor is not elevated in patients with heart failure.<sup>27</sup>

### Do elevated proinflammatory cytokine levels have prognostic importance?

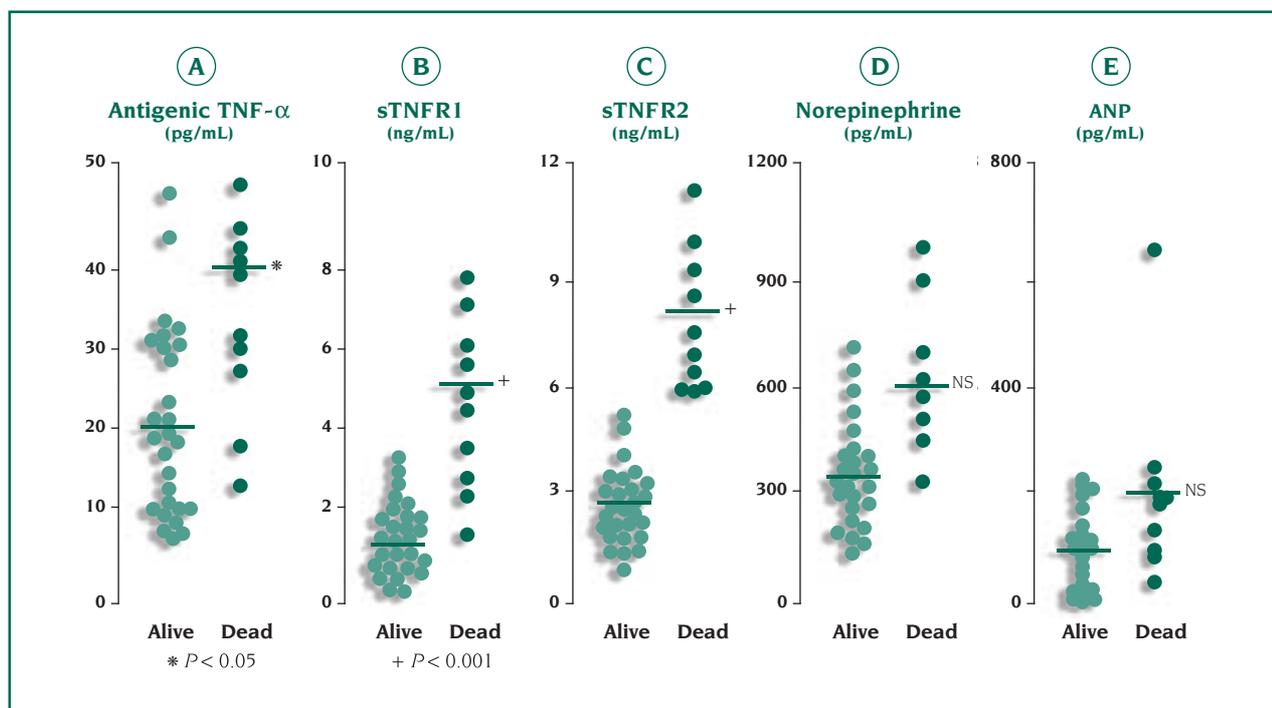
The question as to whether proinflammatory cytokine levels correlate with patient prognosis has been addressed in several studies. To determine whether there was a relationship between patient survival and TNF- $\alpha$  levels, a Kaplan-Meier analysis was performed for Studies Of Left Ventricular Dysfunction (SOLVD) patients in whom cytokine levels had been determined.<sup>17</sup> At the end of the 48-month follow-up period

for the SOLVD patients, 14 patients (22.2%) had died and 49 (77.8%) patients with NYHA class I to III remained alive. As shown in *Figure 3*,<sup>6</sup> when patients with TNF- $\alpha$  levels >6.5 pg/mL (90th percentile) were examined, there was a significant increase in overall patient mortality when compared with patients with TNF- $\alpha$  levels <6.5 pg/mL. However, these data must be regarded as provisional, since the overall patient cohort was relatively small and select.

Nonetheless, these data suggest that vasodepressor cytokines such as TNF- $\alpha$  may have prognostic significance in heart failure. Ferrari and colleagues<sup>14</sup> observed that levels of TNF- $\alpha$  and sTNFR2 were significantly higher in hospitalized patients with the worst clinical outcomes (*Figure 4, next page*),<sup>14</sup> and that elevated levels of sTNFR2 were the single most important variable predicting patient death in a stepwise discriminant analysis. More recently, Tsutamoto et al<sup>26</sup> reported that elevated levels of IL-6 were a significant independent predictor of mortality in patients with NYHA class II to IV heart failure, and that IL-6 levels were independently predictive of high plasma norepinephrine levels, elevated levels of atrial natriuretic factor, and a depressed LV ejection fraction. Interestingly, an elevated TNF- $\alpha$  level was not predictive of mortality in this study. Thus, although there is increasing evidence that elevated levels of proinflammatory cytokines may correlate with patient prognosis, at the time of this writing, it is not clear exactly which cytokines will serve as the best markers for patient outcomes in heart failure.

### What is the site and source of stress-activated cytokines in heart failure?

Following the original descriptions of elevated cytokine levels in cachectic patients with heart failure, one of the more intriguing challenges that arose was to identify the mechanism(s) responsible for the production of inflammatory mediators in heart failure. As shown in *Table IV (next page)*, there are at least five hypotheses with respect to the source of production of proinflammatory cytokines in heart failure. The original suggestion with regard to the mechanism for cytokine overproduction was that these molecules were produced secondary to the “immune activation” that occurred in response to tissue injury.<sup>13</sup> This concept was subsequently challenged by the observation from several laboratories that TNF- $\alpha$  and nitric oxide synthase were expressed by the failing human heart in the absence of a demonstrable inflammatory infiltrate, suggesting that the heart was a potential source of



**Figure 4.** Serum levels of antigenic tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) (A), soluble tumor necrosis factor receptors 1 and 2 (sTNFR1 [B], sTNFR2 [C]), norepinephrine (D), and atrial natriuretic peptide (ANP [E]) in patients with heart failure (alive) and those who died during hospitalization (dead). A stepwise discriminant analysis showed that the levels of sTNFR2 were the best predictor of adverse outcome in the hospitalized patients in this study.

Reproduced from ref 14: Ferrari R, Bachetti T, Confortini R, et al. Tumor necrosis factor soluble receptors in patients with various degrees of congestive failure. *Circulation*. 1995;92:1479-1486. Copyright © 1995, The American Heart Association.

production of inflammatory mediators.<sup>29</sup> A third hypothesis suggests that the elaboration of cytokines is the result of underperfusion of systemic tissues.<sup>26,30</sup> While this may be true for IL-6 production, there is no evidence that the increase in TNF- $\alpha$  production is the result of increased peripheral arteriovenous production.<sup>26</sup> More recently, it has been suggested that increased bowel wall edema in patients with heart failure leads to translocation of bacterial endotoxin from the gut, with resultant activation of the immune system.<sup>31</sup> While the “endotoxin hypothesis” remains as an attractive explanation for the elaboration of cytokines in edematous patients with advanced heart failure, this mechanism does not account for the elaboration of

cytokines in patients with milder forms of heart failure, who are edema-free.<sup>17,27</sup> Finally, it has been shown in cell culture models that increased levels of cyclic adenosine monophosphate (cAMP) will serve to stabilize the messenger RNA (mRNA) for several inflammatory mediators, most notably nitric oxide synthase.<sup>32</sup> This, in turn, has given rise to the suggestion that the adrenergic nervous system may serve to augment cytokine production in the setting of heart failure. However, given that agents that increase cAMP are known to block cytokine production, it is unclear at the time of this writing whether this explanation will prove to be an important mechanism for the increased elaboration in the setting of heart failure. Given that no single mechanism described above is sufficient to explain the production of cytokines in the setting of heart failure, it is unlikely that we will ever identify a single site or source of cytokine production in a disease state that is as complex as heart failure. Indeed, it is becoming increasingly likely that there will be multiple sites and sources of cytokine production as heart failure advances, analogous to the situation with activation of the renin-angiotensin system and the adrenergic system, both of which are activated in an extremely complex manner as heart failure progresses.

- Immune activation
- Myocardial biosynthesis
- Hypoperfusion of metabolic tissue
- Endotoxin absorption from the gut
- Activation of the adrenergic nervous system

**Table IV.** Potential sites and sources for cytokine elaboration in heart failure.



## CYTOKINES AS POTENTIAL THERAPEUTIC TARGETS IN HEART FAILURE

Given that excessive elaboration of stress-activated cytokines appears to mimic a number of aspects of the heart failure phenotype, it is reasonable to ask whether antagonizing cytokines may lead to clinical improvements in patients with heart failure. Insofar as the majority of extant literature has focused on the regulation of TNF- $\alpha$  biosynthesis in the heart, we will restrict the present discussion to the clinical studies that have attempted to modulate this cytokine. However, before doing so, it is instructive to digress for a moment to review the regulation of TNF- $\alpha$  biosynthesis in the heart.

### TNF- $\alpha$ gene expression in the heart

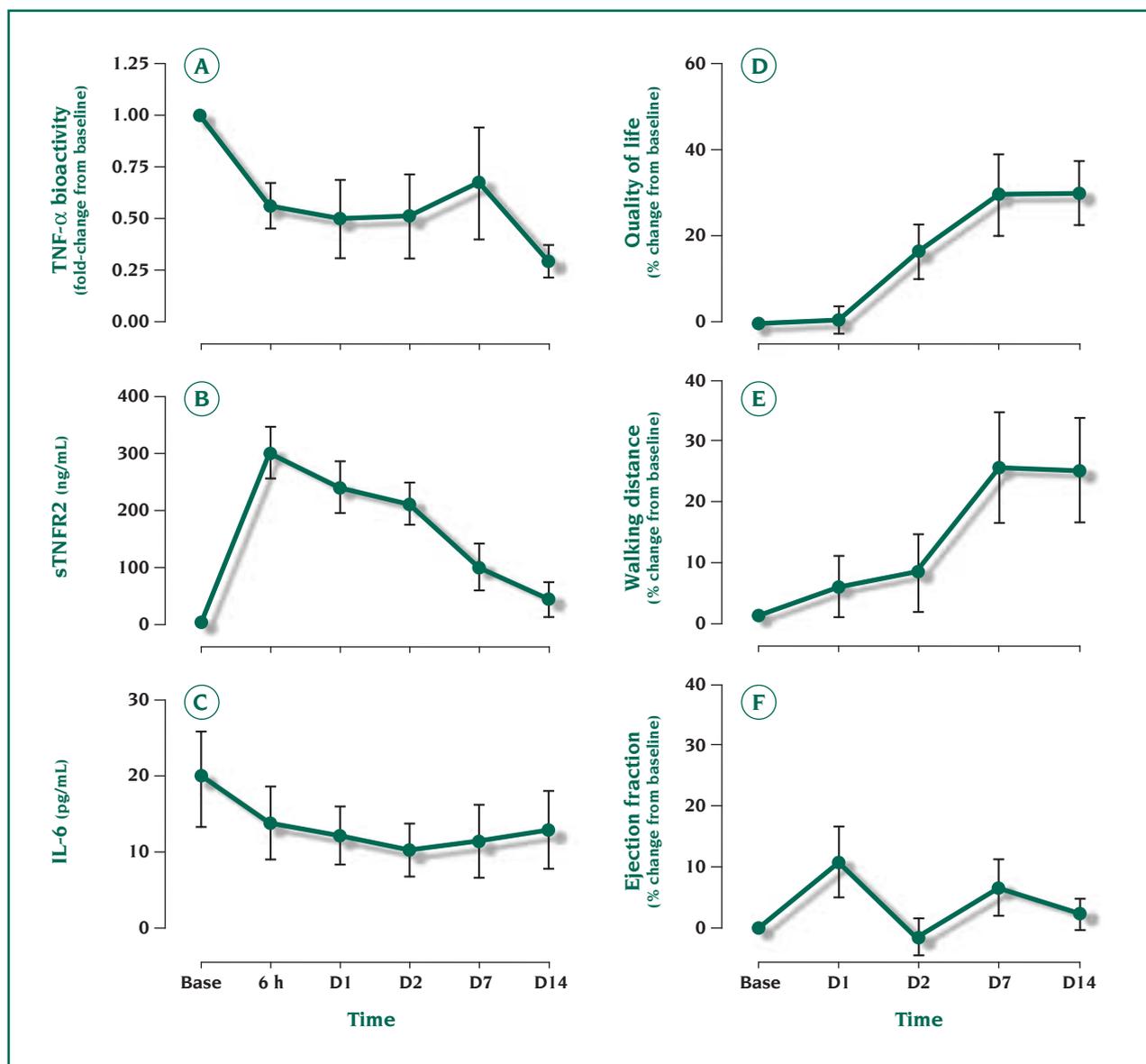
Although the literature with respect to TNF- $\alpha$  gene regulation in the adult heart is limited at present, at least three important themes have emerged thus far. First, neither TNF- $\alpha$  mRNA nor TNF- $\alpha$  protein appear to be constitutively expressed in the unstressed adult mammalian heart.<sup>33</sup> Second, both TNF- $\alpha$  mRNA and protein are rapidly synthesized by the heart in response to an appropriate stressful stimulus.<sup>33</sup> Third, once TNF- $\alpha$  mRNA biosynthesis is initiated, myocardial TNF- $\alpha$  mRNA levels return rapidly toward baseline following removal of the inciting stress. Taken together, the above experimental studies suggest that in the normal adult heart, TNF- $\alpha$  gene and protein expression are self-limited and occur only in relation to a superimposed environmental stress. Thus, these observations suggest that strategies designed to either block TNF- $\alpha$  gene expression or hasten degradation of TNF- $\alpha$  mRNA might be effective in terms of modulating TNF- $\alpha$  expression in the failing heart.

### Pharmacological modulation of TNF- $\alpha$ expression

Relevant to the above discussion is the observation that agents that raise cAMP levels, such as pentoxifylline, amrinone, and milrinone, prevent TNF- $\alpha$  mRNA accumulation, largely by blocking the transcriptional activation of TNF- $\alpha$ .<sup>34,35</sup> Dexamethasone is thought to suppress TNF- $\alpha$  biosynthesis primarily at the translational level, but may also block TNF- $\alpha$  biosynthesis at the transcriptional level as well.<sup>36</sup> When initially translated, TNF- $\alpha$  exists as a 26-kDa propeptide with 76 extra amino acids appended to the amino terminus. This propeptide is efficiently cleaved by a matrix metalloproteinase that exists in cell membranes,<sup>37</sup> to form

the mature 17-kDa TNF- $\alpha$  peptide. Recently, specific inhibitors of matrix metalloproteinases have been shown to prevent the proteolytic cleavage of the 26-kDa form of TNF- $\alpha$  from the membrane,<sup>37</sup> thus preventing the 17-kDa form of TNF- $\alpha$  from being released into the peripheral circulation. Thalidomide ( $\alpha$ -*N*-phthalimido-glutarimide) represents another class of drug that may be useful in suppressing TNF- $\alpha$  production. Prior studies have shown that thalidomide selectively inhibits TNF- $\alpha$  production in monocytes,<sup>38</sup> but has no effect on the production of IL-1 $\beta$ , IL-6, or granulocyte/macrophage colony-stimulating factor (GM-CSF). Thalidomide appears to reduce TNF- $\alpha$  levels by enhancing mRNA degradation.<sup>39</sup> While the clinical utility of thalidomide may be limited by its teratogenic properties as well as the associated sedative properties of the compound, thalidomide analogs are now being developed that have more potent TNF- $\alpha$ -lowering properties, while at the same time appearing to be nonteratogenic.

On the basis of the above observations, it is not surprising that the great majority of strategies that have attempted to suppress cytokine production in patients with heart failure have been designed to block TNF- $\alpha$  expression at the transcriptional or translational levels. As one example of this, Parrillo and colleagues<sup>40</sup> randomly assigned 102 patients to treatment with either prednisone (60 mg per day) or placebo. Following 3 months of therapy, they observed an increase in ejection fraction of  $\geq 5\%$  in 53% of the patients receiving prednisone, whereas only 27% of the controls had a significant improvement in ejection fraction ( $P=0.005$ ). Overall, the mean ejection fraction increased  $4.3\% \pm 1.5\%$  in the prednisone group, as compared with  $2.1\% \pm 0.8\%$  in the control group ( $P=0.054$ ). The patients were then further categorized prospectively in two separately randomized subgroups: "reactive" patients, who had fibroblastic or lymphocytic infiltration or immunoglobulin deposition on endomyocardial biopsy, a positive gallium scan, or an elevated erythrocyte sedimentation rate; or "nonreactive" patients, who had none of these features. At 3 months, 67% of the reactive patients who received prednisone had improvement in LV function, as compared with 28% of the reactive controls ( $P=0.004$ ). In contrast, nonreactive patients did not improve significantly with prednisone ( $P=0.51$ ). Although specific cytokine levels were not measured in this study, the data suggest that patients with idiopathic dilated cardiomyopathy may have some improvement when given a high dose of prednisone daily. Thus, this early study raises the possibility that suppression of proinflammatory cytokines may play an important role in heart failure. Another potentially important pharmacological



**Figure 5.** Biological and functional effects of etanercept. Panels A-C (left) show the effects of etanercept on tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) bioactivity and on levels of soluble tumor necrosis factor receptor 2 (sTNFR2) and interleukin-6 (IL-6). Panels D-F (right) show the changes in clinical end points for the patients who received either 4 mg/m<sup>2</sup> or 10 mg/m<sup>2</sup> of etanercept

Reproduced from ref 49: Deswal A, Bozkurt B, Seta Y, et al. A phase I trial of tumor necrosis factor receptor (p75) fusion protein (TNFR:Fc) in patients with advanced heart failure. *Circulation*. 1999;99:3224-3226. Copyright © 1999, The American Heart Association.

method for suppressing TNF- $\alpha$  production is through the use of agents that elevate cAMP levels, such as dobutamine. As mentioned above, short-term dobutamine infusion has been shown to suppress TNF- $\alpha$  production.<sup>30</sup> It is therefore tempting to speculate that one of the mechanisms for the sustained benefit of intravenous infusion of dobutamine<sup>41,42</sup> may be through suppression of proinflammatory cytokines such as TNF- $\alpha$ . However, this point of view is not supported by a recent full-length publication, in which it

was shown that treatment with either intravenous dobutamine or milrinone (which also raises cAMP and might therefore be expected to decrease TNF- $\alpha$  levels) had no effect in terms of decreasing circulating TNF- $\alpha$  levels.<sup>18</sup> In contrast to the findings with respect to TNF- $\alpha$ , Deng and colleagues reported that IL-6 levels increased in patients with NYHA class III to IV heart failure.<sup>43</sup> However, the mechanism for this increase in IL-6 levels was not determined. More encouraging results with respect to modulating TNF- $\alpha$  levels through



alterations in intracellular cAMP levels have been reported recently by Wagner and colleagues<sup>44,45</sup> and Sliwa and colleagues.<sup>46</sup> Wagner and colleagues<sup>44,45</sup> showed that adenosine was sufficient to block lipopolysaccharide-induced TNF- $\alpha$  production in cultured neonatal and adult rat myocytes as well as in slices of human myocardium obtained from explanted failing human hearts. The effect of adenosine could be mimicked by PD-125944, a selective adenosine A<sub>2</sub>-receptor agonist (which is known to increase cAMP levels), or forskolin, and antagonized by 3,7-dimethyl-1-propylxanthine (DPMX), an A<sub>2</sub>-selective antagonist. However, adenosine was only able to block TNF- $\alpha$  production if given before lipopolysaccharide challenge. Adenosine has also been shown to suppress intramyocardial TNF- $\alpha$  levels in an ex vivo model of ischemia reperfusion in the rat, as well as improving postischemic myocardial function.<sup>47</sup> More recently, Sliwa and associates<sup>46</sup> studied the effects of pentoxifylline in patients with dilated cardiomyopathy and NYHA class II to III heart failure. All patients were receiving concurrent therapy with digitalis, diuretics, and angiotensin-converting enzyme (ACE) inhibitors for 4 months. The primary end points of the 6-month study were NYHA functional class, left ventricular dimensions, and left ventricular systolic and diastolic function. A total of 14 patients received pentoxifylline and 14 patients received placebo. Four patients in the placebo group died as a result of progressive pump dysfunction during the 6-month study period, whereas no patient in the pentoxifylline group experienced functional deterioration. At the end of 6 months, there was an improvement in functional class in the pentoxifylline group, whereas there was functional deterioration in the placebo group. At the end of 6 months, there was a significant increase in the ejection fraction (from  $22.3 \pm 9.0$  standard deviation [SD] to  $38.7 \pm 15.0$  SD) in the pentoxifylline group, whereas there was no significant change in the placebo group. There was, however, no change in the LV end-diastolic dimension in either group. Relevant to the present discussion is the observation that TNF- $\alpha$  levels fell significantly ( $P < 0.001$ ) from  $6.5 \pm 5.0$  pg/mL to  $2.1 \pm 1.0$  pg/mL in the pentoxifylline group, whereas there was no significant change in the TNF- $\alpha$  levels in the placebo group.

Thus, modulation of TNF- $\alpha$  levels via agents that alter intracellular cAMP levels, and hence block transcriptional activation of TNF- $\alpha$ , may provide one 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.

Mohler and colleagues<sup>23</sup> have employed a different strategy to alter cytokine levels in patients with heart failure. That is, these authors examined the effects of amlodipine on circulating levels of TNF- $\alpha$  and IL-6 in a subset analysis of patients enrolled in the Prospective Randomized Amlodipine Survival Evaluation (PRAISE) trial.<sup>48</sup> They observed that, although treatment with amlodipine had no effect on TNF- $\alpha$  levels, there was a statistically significant decrease in IL-6 levels following 24 weeks of treatment.<sup>23</sup> Finally, in a recent report employing a soluble TNF- $\alpha$  antagonist that neutralizes the biological effects of circulating TNF- $\alpha$ , Deswal et al<sup>49</sup> showed that there was an improvement in the functional status and quality of life for patients with advanced heart failure (*Figure 5*). However, the results of this study must be regarded as provisional because of the small sample size ( $n=12$  patients) involved.

In summary, although the data are limited at the present time, there is increasing evidence that suppression of cytokine levels and/or modulation of cytokine bioactivity is not only possible, but that it may also favorably impact the disease process.

The authors would like to thank (as always) Jana Grana for her secretarial assistance and her forbearance, as well as Dr Andrew I. Schafer for his past and present guidance and support. The research reviewed herein was supported, in part, by research funds from the Department of Veterans Affairs and the NIH. This research was supported by research funds from the Department of Veterans Affairs and the NIH. (P50 HL-O6H and RO1 HL58081-01, RO1 HL61543-01.)

### THREE KEY QUESTIONS

The Expert Answers in the following section offer perceptive insights into the role of the cytokines in heart failure. In the present review, we have focused on recent clinical and experimental evidence that suggests that stress-activated cytokines may play a role in disease progression in heart failure, by virtue of the direct toxic effects that these molecules exert on the heart and the peripheral circulation. Indeed, pathophysiologically relevant concentrations of these molecules appear to be able to mimic many aspects of the so-called heart failure phenotype in experimental animals, including LV dysfunction, LV dilation, activation of fetal gene expression, cardiac myocyte hypertrophy, endothelial dysfunction, and cardiac myocyte apoptosis (*Table II*). Laura Agnoletti and Laura Comini sum up current knowledge on this in the chapter "**Do cytokines and endothe-**

**lial function have an impact on myocardial activity?"** This leads to the conclusion that, analogous to the proposed role for neurohormones, stress-activated cytokines would appear to represent another distinct class of biologically active molecules that can contribute to heart failure progression. In this regard, it will be interesting to determine whether there is synergy or cross-talk between these two biological systems: this point is gone into by Helmut Drexler and Bernard Schieffer, who ask **"What are the implications of the interaction between the neurohumoral system and the cytokines?"** This article has also reviewed clinical evidence that suggests that elevated levels of stress-activated cytokine levels can be modulated in the setting of chronic heart failure. While it is perhaps premature to speculate whether modulating cytokine levels may translate into clinical improvements in morbidity and mortality for patients with heart failure, there is now a growing body of evidence that suggests that modulating cytokine levels may represent a new therapeutic paradigm for treating patients with heart failure; but more on this by Stefan D. Anker, who asks **"Has the time arrived to use anti-cytokine therapy in chronic heart failure?"**

## REFERENCES

- 1. Bozkurt B, Shan K, Seta Y, Oral H, Mann DL.**  
*Tumor necrosis factor- $\alpha$  and tumor necrosis factor receptors in human heart failure.*  
*Heart Fail Rev.* 1996;1:211-219.
- 2. Bozkurt B, Kribbs S, Clubb FJ Jr, et al.**  
*Pathophysiologically relevant concentrations of tumor necrosis factor- $\alpha$  promote progressive left ventricular dysfunction and remodeling in rats.*  
*Circulation.* 1998;97:1382-1391.
- 3. Thaik CM, Calderone A, Takahashi N, Colucci WS.**  
*Interleukin-1 $\beta$  modulates the growth and phenotype of neonatal rat cardiac myocytes.*  
*J Clin Invest.* 1995;96:1093-1099.
- 4. Kubota T, McTiernan CF, Frye CS, et al.**  
*Dilated cardiomyopathy in transgenic mice with cardiac specific overexpression of tumor necrosis factor- $\alpha$ .*  
*Circ Res.* 1997;81:627-635.
- 5. Agnoletti L, Curello S, Bachetti T, et al.**  
*Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: role of tumor necrosis factor- $\alpha$ .*  
*Circulation.* 1999;100:1983-1991.
- 6. Seta Y, Shan K, Bozkurt B, Oral H, Mann DL.**  
*Basic mechanisms in heart failure: the cytokine hypothesis.*  
*J Card Fail.* 1996;2:243-249.
- 7. Kapadia S, Dibbs Z, Kurrelmeyer K, et al.**  
*The role of cytokines in the failing human heart. In: Crawford M, ed. Cardiology Clinics.* Philadelphia, Pa: W.B. Saunders, 1998:645-656.
- 8. Levine B, Kalman J, Mayer L, Fillit HM, Packer M.**  
*Elevated circulating levels of tumor necrosis factor in severe chronic heart failure.*  
*N Engl J Med.* 1990;223:236-241.
- 9. McMurray J, Abdullah I, Dargie HJ, Shapiro D.**  
*Increased concentrations of tumour necrosis factor in "cachectic" patients with severe chronic heart failure.*  
*Br Heart J.* 1991;66:356-358.
- 10. Dutka DP, Elborn JS, Delamere F, Shale DJ, Morris GK.**  
*Tumour necrosis factor alpha in severe congestive cardiac failure.*  
*Br Heart J.* 1993;70:141-143.
- 11. Wiedermann CJ, Beimbold H, Herold M, Knapp E, Braunsteiner H.**  
*Increased levels of serum neopterin and decreased production of neutrophil superoxide anions in chronic heart failure with elevated levels of tumor necrosis factor- $\alpha$ .*  
*J Am Coll Cardiol.* 1993;22:1897-1901.
- 12. Katz SD, Rao R, Berman JW, et al.**  
*Pathophysiological correlates of increased serum tumor necrosis factor in patients with congestive heart failure: relation to nitric oxide-dependent vasodilation in the forearm circulation.*  
*Circulation.* 1994;90:12-16.
- 13. Matsumori A, Yamada T, Suzuki H, Matoba Y, Sasayama S.**  
*Increased circulating cytokines in patients with myocarditis and cardiomyopathy.*  
*Br Heart J.* 1994;72:561-566.
- 14. Ferrari R, Bachetti T, Confortini R, et al.**  
*Tumor necrosis factor soluble receptors in patients with various degrees of congestive failure.*  
*Circulation.* 1995;92:1479-1486.



**15. Pritchett G, Cohen HJ, Rao KMK, Cobb F, Sullivan M, Currie MS.**

*Tumor necrosis factor, natural killer activity and other measures of immune function and inflammation in elderly men with heart failure.*

*Gerontology.* 1995;41:45-56.

**16. Torre-Amione G, Kapadia S, Lee J, Durand JB, Bies RD, Young JB, et al.**

*Tumor necrosis factor- $\alpha$  and tumor necrosis factor receptors in the failing human heart.*

*Circulation.* 1996;93:704-711.

**17. Torre-Amione G, Kapadia S, Benedict CR, Oral H, Young JB, Mann DL.**

*Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the studies of left ventricular dysfunction (SOLVD).*

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

**18. Milani RV, Mehra MR, Endres S, et al.**

*The clinical relevance of circulating tumor necrosis factor- $\alpha$  in acute decompensated chronic heart failure without cachexia.*

*Chest.* 1996;110:992-997.

**19. Munger MA, Johnson B, Amber IJ, Callahan KS, Gilbert EM.**

*Circulating concentrations of proinflammatory cytokines in mild or moderate heart failure secondary to ischemic or idiopathic dilated cardiomyopathy.*

*Am J Cardiol.* 1996;77:723-727.

**20. Testa M, Yeh M, Lee P, et al.**

*Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension.*

*J Am Coll Cardiol.* 1996;28:964-971.

**21. Anker SD, Chua TP, Ponikowski P, et al.**

*Hormonal changes and catabolic/anabolic imbalance in chronic heart failure and their importance for cardiac cachexia.*

*Circulation.* 1997;96:526-534.

**22. MacGowan GA, Mann DL, Kormos RL, Feldman AM, Murali S.**

*Circulating interleukin-6 in severe heart failure.*

*Am J Cardiol.* 1997;79:1128-1131.

**23. Mohler ERI, Sorensen LC, Ghali JK, et al.**

*Role of cytokines in the mechanism of action of amlodipine: The PRAISE heart failure trial.*

*J Am Coll Cardiol.* 1997;30:35-41.

**24. Nishigaki K, Minatoguchi S, Seishima M, et al.**

*Plasma Fas ligand, an inducer of apoptosis, and plasma soluble Fas, an inhibitor of apoptosis, in patients with chronic congestive heart failure.*

*J Am Coll Cardiol.* 1997;29:1214-1220.

**25. Anker SD, Egerer KR, Volk HD, Kox WJ, Poole-Wilson PA, Coats AJS.**

*Elevated soluble CD14 receptors and altered cytokines in chronic heart failure.*

*Am J Cardiol.* 1997;79:1426-1429.

**26. Tsutamoto T, Hisanaga T, Wada A, et al.**

*Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure.*

*J Am Coll Cardiol.* 1998;31:391-398.

**27. Dibbs Z, Thornby J, White BG, Mann DL.**

*Natural variability of circulating levels of cytokines and cytokine receptors in patients with heart failure: implications for clinical trials.*

*J Am Coll Cardiol.* 1999;33:1935-1942.

**28. Hirano T. Interleukin-6.** In: Thompson A, ed.

*The cytokine Handbook.* Boston, Mass: Academic Press; 1991:169-190.

**29. Torre-Amione G, Kapadia S, Lee J, Bies RD, Lebovitz R, Mann DL.**

*Expression and functional significance of tumor necrosis factor receptors in human myocardium.*

*Circulation.* 1995;92:1487-1493.

**30. Sindhvani R, Yuen J, Hirsch H, et al.**

*Reversal of low flow state attenuates immune activation in severe decompensated congestive heart failure.*

*Circulation.* 1993;88(suppl):1255. Abstract.

**31. Niebauer J, Volk HD, Kemp M, et al.**

*Endotoxin and immune activation in chronic heart failure: a prospective cohort study.*

*Lancet.* 1999;353:1838-1842.

**32. Oddis CV, Simmons RL, Hattler BG, Finkel MS.**

*cAMP enhances inducible nitric oxide synthase mRNA stability in cardiac myocytes.*

*Am J Physiol.* 1995;269:H2044-H2050.

**33. Kapadia S, Oral H, Lee J, Nakano M, Taffet GE, Mann DL.**

*Hemodynamic regulation of tumor necrosis factor- $\alpha$  gene and protein expression in adult feline myocardium.*

*Circ Res.* 1997;81:187-195.

**34. Zabel P, Schade FU, Schlaak M.**

*Inhibition of endogenous TNF formation by pentoxifylline.*

**Immunobiology.** 1993;187:447-463.

**35. Zabel P, Greinert U, Entzian P, Schlaak M.**

*Effects of pentoxifylline on circulating cytokines (TNF and IL-6) in severe pulmonary tuberculosis.* In: Fiers W, Buurman WA, eds.

**Tumor Necrosis Factor: Molecular and Cellular Biology and Clinical Relevance.** Basel, Switzerland: S. Karger; 1993:178-181.

**36. Remick DG, Strieter RM, Lynch IJP, et al.**

*In vivo dynamics of murine tumor necrosis factor- $\alpha$  gene expression. Kinetics of dexamethasone-induced suppression.*

**Lab Invest.** 1989;60:766-771.

**37. Mohler KM, Sleath PR, Fitzner JN, et al.**

*Protection against a lethal dose of endotoxin by an inhibitor of tumor necrosis factor processing.*

**Nature.** 1994;370:218-220.

**38. Sampaio EP, Sarno EN, Galilly R, Cohn ZA, Kaplan G.**

*Thalidomide selectively inhibits tumor necrosis factor  $\alpha$  production by stimulated human monocytes.*

**J Exp Med.** 1991;173:699-703.

**39. Moreira AL, Sampaio EP, Zmuidzinas A, Frindt P, Smith KA, Kaplan G.**

*Thalidomide exerts its inhibitory action on tumor necrosis factor- $\alpha$  by enhancing messenger RNA degradation.*

**J Exp Med.** 1993;177:1675-1680.

**40. Parrillo JE, Cunnion RE, Epstein SE, et al.**

*A prospective randomized controlled trial of prednisone for dilated cardiomyopathy.*

**N Engl J Med.** 1989;321:1061-1068.

**41. Leier CV, Huss P, Lewis RP, Unverferth DV.**

*Drug-induced conditioning in congestive heart failure.*

**Circulation.** 1982;65:1382-1387.

**42. Unverferth DV, Magorien RD, Lewis RP, Leier CV.**

*Long-term benefit of dobutamine in patients with congestive cardiomyopathy.*

**Am Heart J.** 1980;100:622-630.

**43. Deng MC, Erren M, Lutgen A, Zimmermann P, Brisse B, Schmitz W, et al.**

*Interleukin-6 correlates with hemodynamic impairment during dobutamine administration in chronic heart failure.*

**Int J Cardiol.** 1996;57:129-134.

**44. Wagner DR, Combes A, McTiernan CF, Sanders VJ,**

**Lemster B, Feldman AM.**

*Adenosine inhibits lipopolysaccharide-induced cardiac expression of tumor necrosis factor- $\alpha$ .*

**Circ Res.** 1998;82:47-56.

**45. Wagner DR, McTiernan CF, Sanders VJ, Feldman AM.**

*Adenosine inhibits lipopolysaccharide-induced secretion of tumor necrosis factor- $\alpha$  in the failing human heart.*

**Circulation.** 1998;97:521-524.

**46. Sliwa K, Skudicky D, Candy G, Wisenbaugh T, Sareli P.**

*Randomized investigation of effects of pentoxifylline on left ventricular performance in idiopathic dilated cardiomyopathy.*

**Lancet.** 1998;351:1091-1093.

**47. Meldrum DR, Cain BS, Cleveland JC Jr, et al.**

*Adenosine decreases post-ischemic cardiac TNF $\alpha$  production: anti-inflammatory implications for preconditioning and transplantation.*

**Immunology.** 1997;92:472-477.

**48. Packer M, O'Connor CM, Ghali JK, et al.**

*Effect of amlodipine on morbidity and mortality in severe chronic heart failure. Prospective Randomized Amlodipine Survival Evaluation Study Group.*

**N Engl J Med.** 1996;335:1107-1114.

**49. Deswal A, Bozkurt B, Seta Y, et al.**

*A phase I trial of tumor necrosis factor receptor (p75) fusion protein (TNFR:Fc) in patients with advanced heart failure.*

**Circulation.** 1999;99:3224-3226.

**50. Medina J, Baud L, Escribano CG, Gila JA, Puyol DR, Puyol MR.**

*Tumor necrosis factor-induced contraction of cultured rat mesangial cells—interaction with angiotensin-II.*

**J Lab Clin Med.** 1993;122:164-172.



# Do cytokines and endothelial function have an impact on myocardial activity?

Laura Agnoletti, MSc, Laura Comini, PhD

Fondazione S. Maugeri - Clinica del Lavoro e della Riabilitazione - IRCCS - Istituto Scientifico di Gussago - Cardiovascular Research Center - Gussago - Brescia - and University of Ferrara - ITALY

**Background:** cytokines mediate cell-to-cell interactions via specific surface receptors regulating activities such as growth and cell death. The cardiac endothelium separates circulating blood from the myocardium; dysfunction of vascular endothelium upregulates enzymatic processes ascribed to modulation of nitric oxide (NO). **Aims:** to assess the role of cytokine-mediated immunologic responses in heart failure (HF). **Results:** patients with HF have high levels of cytokines that correlate with HF severity. Cytokines can affect endothelial function, reducing the vasodilator response, this action being exerted through NO, oxidative stress, and apoptosis. **Conclusions:** cytokines may contribute to the progression of HF through endothelial dysfunction, left ventricular dysfunction, and remodeling. New treatments capable of modulating cytokines should be investigated for the management of HF.

**Keywords:** cytokine, endothelial function, heart failure, nitric oxide synthase, oxidative stress, apoptosis

**Address for correspondence:**

Dr Laura Agnoletti, Cardiovascular Pathophysiology Research Center, S. Maugeri Foundation, Via Pinidolo 23, 25064 Gussago, Brescia, Italy (e-mail: Ferrari@master.cci.unibs.it)

This article looks at the hypothesis that the immunologic responses mediated by cytokines play an important pathogenetic role in the development of heart failure (HF). This “cytokine hypothesis” does not imply that cytokines cause HF per se, but rather that they contribute to the progression of the disease through endothelial dysfunction, left ventricular (LV) dysfunction, and remodeling.

Growing evidence of the complexity of the endothelium has led a large number of cardiovascular diseases to be reinterpreted with regard to their pathophysiology. In particular, endothelial dysfunction is now considered as a very important initial etiopathogenetic event in HF, both at the cardiac and vascular levels (Figure 1, next page).

## CYTOKINES

Cytokines are small soluble protein molecules with molecular weights between 6000 and 60 000. They mediate cell-to-cell interactions via specific cell surface receptors, and regulate the activation, differentiation, growth, death, or acquisition of effector functions of immune cells. They are not produced by specialized glands, but are discontinuously produced by individual cells and different tissues in response to specific stimuli. Cytokines do not have general systemic effects, but

exert their effects mainly in either a paracrine (toward adjacent cells) or autocrine (toward the producing cell itself) way. It was originally thought that each cytokine exerted a specific effect on its specific target cell. However, it is today well established that most cytokines exhibit a wide range of biological effects on various tissues and cells. The manifestations of the actions of the cytokines are attributable to the functional pleiotropism due to the similarities among their receptors. The classification of cytokines is based on the structural relationships among the molecules. Some of them retain their historical name,

### SELECTED ABBREVIATIONS AND ACRONYMS

eNOS	constitutive (endothelial) nitric oxide synthase
HF	heart failure
HUVECs	human umbilical vein endothelial cells
IFN- $\gamma$	interferon gamma
IL	interleukin
iNOS	inducible nitric oxide synthase
LV	left ventricle/ left ventricular
NO	nitric oxide
TNF- $\alpha$	tumor necrosis factor $\alpha$

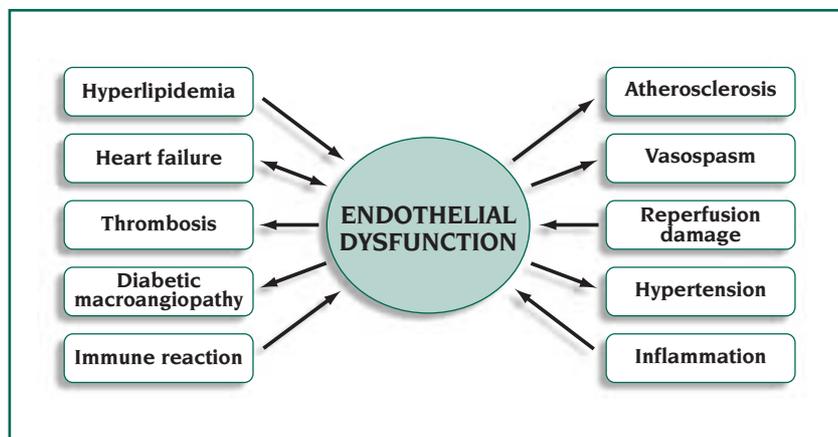


Figure 1. Causes and consequences of endothelial dysfunction.

eg, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), while others have been termed interleukins (IL) and have been assigned numbers in sequence (IL-1 to IL-18). To date, more than 100 genetically unrelated cytokines have been identified (Table I).

### ENDOTHELIAL FUNCTION

At the cardiac level, the endocardial endothelium plays an important role, as it covers the complex inner surface of the cardiac cavities, separating the circulating blood from the myocardium itself. It modulates the mechanical performance of the adjacent myocardium through its electrophysiological and transendothelial transport properties and through the release of a number of mediators, such as nitric oxide (NO), prostacyclin, and endothelin. These mediators may also play a role as growth factors, promoting or inhibiting the proliferation of endocardial interstitial cells and myocardial cells. In addition, the endocardium plays a role in coagulation and thrombotic processes, and expresses adhesion molecules and other antigens during inflammatory processes. Impairment of any of these functions may result in endocardium dysfunction. At the vascular level, activation, hence dysfunction, of the vascular

endothelium results in upregulation of adhesion molecules and enzymatic processes, which are mainly ascribed to the modulation of the NO pathway (ie, activation of inducible NO synthase [iNOS]).

### CYTOKINES AND ENDOTHELIAL FUNCTION IN HEART FAILURE

Even though several symptoms of HF can be explained or mimicked by cytokines, only few among these (TNF- $\alpha$ , IL-1, and IL-6) have generated interest among cardiologists with respect to their potential role in the pathogenesis, clinical course, and treatment of congestive HF.

HF is considered as a systemic syndrome rather than a disease of the heart alone. In fact, the clinical features of HF are the resultant of structural and functional changes occurring both at the cellular and molecular levels in different systems such as heart, skeletal muscle, kidney, and endothelial cells.

Proinflammatory cytokines modulate cardiovascular function through a variety of mechanisms, resulting in progressive LV dysfunction and remodeling, pulmonary edema, fetal gene expression, and cardiomyopathy. Thus, the production of cy-

tokines—similar to neurohormone production—represents one of the possible biochemical mechanisms responsible for the development of symptoms in patients with HF.

A number of clinical studies have demonstrated that patients with HF express excessive levels of cytokines in plasma.<sup>1</sup> The systemic increase in cytokines produces a series of pathologic reactions. Thus, continuous infusion of TNF- $\alpha$  in rat was shown to induce a time-dependent depression in LV function and structure.<sup>2</sup> Injection of recombinant TNF- $\alpha$  increased mortality in the murine model of myocarditis.<sup>3</sup> It has been reported that serum levels of TNF- $\alpha$  may correlate with the severity of HF, but the net biologic effect of TNF- $\alpha$  seems to be determined primarily by the body compartment in which it is produced.<sup>4-6</sup> The source of cytokines in patients with HF is controversial; clinical studies suggest that the heart may be the source of intracardiac TNF- $\alpha$ , as evidenced in excised hearts, right atrial specimens from patients during cardiac surgery, and, recently, in failing human cardiac myocytes.

Moreover, during HF, cytokines affect endothelial function, contributing to a marked reduction in the vascular dilator response. This action is exerted through several pathways, which involve NO, oxidative stress, and apoptosis.

### NO-dependent pathway

There is clear evidence that, both in experimental animals with HF of differing etiology<sup>7</sup> and in patients with HF,<sup>8,9</sup> the vasodilator response to acetylcholine is reduced, compared with control groups. Several reasons may explain this reduced NO production, the main one being reduced activity of constitutive (endothelial) nitric oxide synthase



(eNOS), which produces NO from arginine. This hypothesis is supported by evidence of reduced synthesis and expression of this enzyme in experimental models of HF.<sup>7</sup> Such NO synthase downregulation seems to be the resultant of chronic cytokine activation, as suggested by experimental data showing that TNF- $\alpha$ , IL-6, interferon gamma (IFN- $\gamma$ ), and other cytokines<sup>10</sup> can inhibit the transcription of this enzyme in vitro.

Furthermore, several studies from different groups of researchers have shown that, in the advanced stages of HF, an increase in the circulating levels of these cytokines occurs. Fi-

nally, cytokines have been shown to be the most powerful indicator of short-term negative prognosis in patients with HF.<sup>11,12</sup>

In vivo expression of the iNOS messenger RNA and protein has been evidenced in most cardiovascular tissues, including vascular smooth muscle, endothelial cells, and cardiac myocytes.<sup>13-16</sup> Overproduction of NO could, at least in theory, account for many of the clinical features described in HF, since NO is a potent vasodilator and is involved in blood flow regulation; it can also depress myocardial function and impair cellular respi-

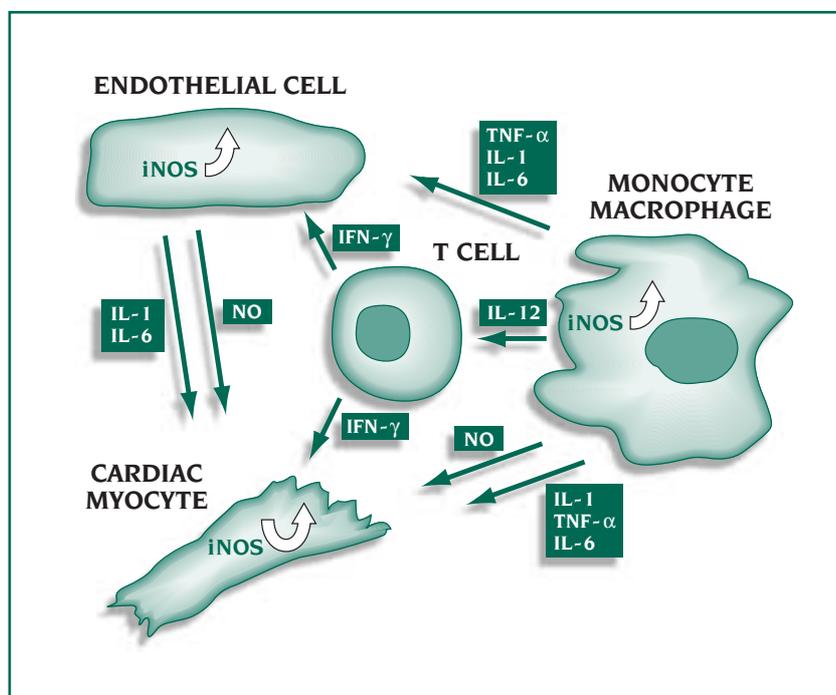
ration. However, in many in vivo settings—especially in the heart—a major proportion of iNOS expression and activity is present in infiltrating inflammatory cells (*Figure 2, next page*). Therefore, in the case of cardiac iNOS expression in vivo, it is quite difficult to distinguish the actual cellular contribution of iNOS and determine the corresponding functional consequences.

### Oxidative stress

IL-1, IL-6, and particularly TNF- $\alpha$  may induce endothelial and LV dysfunction and remodeling either directly or via oxidative stress, ie,

TNF- $\alpha$	<i>Tumor necrosis factor <math>\alpha</math></i>	Contains highly conserved carboxy terminal domains; can induce receptor trimerization influencing signaling pathways
IL-1	<i>Interleukin-1</i>	Synthesized as glycosylated proforms lacking signal peptides
IL-6 family	<i>Interleukin-6</i>	Uses receptors homologous to or containing the gp 130 subunit as the common signaling component
IL-4 IL-13	<i>Interleukins 4 and 13</i>	Bind to shared heteromultimeric receptor complexes
IGF	<i>Insulin-like growth factor</i>	Shares sequence homology with the insulin family of proteins
HGF MSP	<i>Hepatocyte growth factor</i> <i>Macrophage-stimulating protein</i>	Contain a 4-kringle domain and a pseudoserine protease domain that lacks enzymatic activity
FGF	<i>Fibroblast growth factor</i>	Heparin-binding polypeptide
NGF BDNF	<i>Nerve growth factor</i> <i>Brain-derived neurotrophic factor</i>	Induce signal transduction through ligand-induced dimerization and activation of trk receptors
Tpo Epo	<i>Thrombopoietin</i> <i>Erythropoietin</i>	Share sequence homology
PDGF VEGF PlGF	<i>Platelet-derived growth factor</i> <i>Vascular endothelial growth factor</i> <i>Placenta growth factor</i>	Dimeric angiogenic factors containing an 8-cysteine motif
EGF	<i>Epidermal growth factor</i>	Contains at least one extracellular EGF structural unit (conserved 6-cysteine motif that forms 3 disulfide bonds)
SCF Flt-3L, M-CSF	<i>Stem cell factor</i> ; <i>Fms-like tyrosine kinase 3 ligand</i> <i>Macrophage colony-stimulating factor</i>	Contain a 4-helix bundle structure in the extracellular domain and 4 conserved cysteines; receptors are tyrosine kinases
MK PTN	<i>Midkine</i> <i>Pleiotrophin</i>	Products of retinoic acid-responsive genes; developmentally regulated molecules
TGF- $\beta$ superfamily	<i>Transforming growth factor-<math>\beta</math></i>	Contain a highly conserved 7-cysteine domain that forms a characteristic cysteine knot

**Table 1.** Cytokine family key.



**Figure 2.** Relation between nitric oxide production and cytokine network in myocardium.

Abbreviations: IL-1, -6, -12, interleukin-1, -6, -12; iNOS, inducible nitric oxide synthase; IFN- $\gamma$ , interferon gamma; NO, nitric oxide; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ .

through the toxic effect of reactive oxygen species. Oxidative stress occurs in patients with HF as a consequence of increased production of reactive oxygen free radicals and/or alteration of cellular mechanisms of antioxidant protection.<sup>17</sup> Oxidative stress activates a family of transcription factors involved in cardiac and vascular remodeling. Oxygen free radicals are also involved in apoptosis, which is characterized by a continuous loss of myocardial and endothelial cells. This phenomenon may result in a progressive decrease in myocardial and endothelial function over time in patients with HF, and is a hallmark of the syndrome.

The myocardium is exposed to oxidative stress both during myocardial ischemia, where the antioxidant reserve of the heart is consumed and oxygen free radical production

increases, and particularly during reperfusion, where the heart is re-exposed to molecular oxygen.<sup>18</sup> It has also been suggested that a mismatch between oxygen free radical production and antioxidant defense mechanisms may play a role in the transition from hypertrophy to the decompensated state of the heart. Interestingly, compensatory hypertrophy is accompanied by an increased myocardial antioxidant reserve, a redox state, and a greater resistance to oxidative stress.<sup>19</sup> Conversely, in the decompensated state, there is a relative deficit in the myocardium's antioxidant capacity, accompanied by a decrease in its redox state.<sup>20</sup>

### Apoptosis

In contrast to necrosis, apoptosis is a genetically regulated death process characterized by cell shrinkage,

DNA fragmentation, cytoplasmic blebbing, and cellular disassembling into small apoptotic bodies that are then digested by neighboring cells and macrophages.<sup>21</sup> Apoptosis has been demonstrated in pathologic conditions affecting the endothelium and the adult human heart. In vitro studies have shown that the incubation of human umbilical vein endothelial cells (HUVECs) with serum from patients with severe HF induces a downregulation of constitutive eNOS protein expression and an increase in apoptosis, and that these effects are partially counteracted by the addition of an anti-TNF- $\alpha$  antibody.<sup>22</sup> Apoptosis has also been demonstrated in the heart of patients with severe end-stage HF.<sup>23,24</sup> However, in spite of the evidence of apoptosis in the failing human heart, its clinical significance remains controversial.

Recent findings suggest that apoptosis, which is associated with HF and thus has a negative prognosis, may be counteracted by the activity of "good" cytokines, such as cardiotrophin 1. The latter acts on different cellular pathways than the other cytokines (gp 130 receptor), resulting in hypertrophy, thereby favoring cell survival instead of apoptosis.

### CONCLUSIONS

The interest in the role of stress-induced cytokines in myocyte function in HF is increasing. Researchers are currently investigating new treatments designed to modulate the actions of the cytokines, by either neutralizing the proteins secreted or inhibiting their synthesis.<sup>25</sup> Such treatments, should they prove effective, hold the prospect of becoming the future gold standard in the management of HF.



## REFERENCES

- 1. Levine B, Kalman J, Mayer L, Fillit HM, Packer M.**  
*Elevated circulating levels of tumor necrosis factor in severe chronic heart failure.*  
*N Engl J Med.* 1990;323:236-241.
- 2. Bozkurt B, Kribbs SB, Clubb FJ, et al.**  
*Pathophysiologically relevant concentrations of tumor necrosis factor  $\alpha$  promote progressive left ventricular dysfunction and remodeling in rats.*  
*Circulation.* 1998;97:1382-1391.
- 3. Yamada T, Matsumori A, Sasayama S.**  
*Therapeutic effect of anti-tumor necrosis factor  $\alpha$  antibody on the murine model of viral myocarditis induced by encephalomyocarditis virus.*  
*Circulation.* 1994;89:846-851.
- 4. Kubota T, McTiernan CF, Frye CS, et al.**  
*Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor  $\alpha$ .*  
*Circ Res.* 1997;81:627-635.
- 5. Bryant D, Becker L, Richardson J, et al.**  
*Cardiac function in transgenic mice with myocardial expression of tumor necrosis factor  $\alpha$ .*  
*Circulation.* 1998;97:1375-1381.
- 6. Kapadia SR, Oral H, Lee J, Nakano M, Taffet GE, Mann DL.**  
*Hemodynamic regulation of tumor necrosis factor  $\alpha$  gene and protein expression in adult feline myocardium.*  
*Circ Res.* 1997;81:187-195.
- 7. Smith CJ, Sun D, Hoegler C, et al.**  
*Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure.*  
*Circ Res.* 1996;78:58-64.
- 8. Kubo SH, Rector TS, Bank AL, Williams RE, Heifetz SM.**  
*Endothelium-dependent vasodilation is attenuated in patients with heart failure.*  
*Circulation.* 1991;84:1589-1596.
- 9. Drexler H, Hayoz D, Munzel T, et al.**  
*Endothelial function in chronic congestive heart failure.*  
*Am J Cardiol.* 1992;69:1596-1601.
- 10. Wiedermann CJ, Beimbold H, Herold M, Knapp E, Braunsteiner H.**  
*Increased levels of serum neopterin and decreased production of neutrophil superoxide anions in chronic heart failure with elevated levels of tumor necrosis factor- $\alpha$ .*  
*J Am Coll Cardiol.* 1993;22:1897-1901.
- 11. Ferrari R, Bachetti T, Confortini R, et al.**  
*Tumor necrosis factor-soluble receptors in patients with various degrees of congestive heart failure.*  
*Circulation.* 1995;92:1479-1486.
- 12. Vanderheyden M, Kersschot E, Paulus WJ.**  
*Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm.*  
*Eur Heart J.* 1998;19:747-752.
- 13. Balligand JL, Ungureanu-Longrois D, Simmons WW, et al.**  
*Cytokine-inducible nitric oxide synthase I (iNOS) expression in cardiac myocytes.*  
*J Biol Chem.* 1994;269:27580-27588.
- 14. Balligand JL, Ungureanu-Longrois D, Simmons WW, et al.**  
*Induction of NO synthase in rat cardiac microvascular endothelial cells by IL-1 $\beta$  and IFN- $\gamma$ .*  
*Am J Physiol.* 1995;268:H1293-H1303.
- 15. Haywood GA, Tsao PS, von der Leyen HE, et al.**  
*Expression of inducible nitric oxide synthase in human heart failure.*  
*Circulation.* 1996;93:1087-1094.
- 16. Habib FM, Springall DR, Daies GJ, et al.**  
*Tumor necrosis factor and inducible nitric oxide synthase in dilated cardiomyopathy.*  
*Lancet.* 1996;347:1151-1155.
- 17. Ferrari R, Ceconi C, Curello S, et al.**  
*Intracellular effects of myocardial ischaemia and reperfusion: role of calcium and oxygen.*  
*Eur Heart J.* 1986;7:3-12.
- 18. Prasad K, Gupta JB, Kalra J, Lee P, Mantha SV, Bharadwaj B.**  
*Oxidative stress as mechanism of cardiac failure in chronic volume overload in canine model.*  
*J Mol Cell Cardiol.* 1996;28:375-385.
- 19. Kirshenbaum LA, Singal PK.**  
*Antioxidant changes in heart hypertrophy: significance during hypoxia-reoxygenation injury.*  
*Can J Physiol Pharmacol.* 1992;70:1330-1335.
- 20. Galinanes M, Ferrari R, Qui Y, Cargnoni A, Ezrin A, Hearse DJ.**  
*PEG-SOD and myocardial antioxidant status during ischaemia and reperfusion: dose-response studies in isolated blood perfused rabbit heart.*  
*J Mol Cell Cardiol.* 1992;24:1021-1030.
- 21. Hetts SW.**  
*To die or not to die. An overview of apoptosis and its role in disease.*  
*JAMA.* 1998;279:300-307.
- 22. Agnoletti L, Curello S, Bachetti T, et al.**  
*Serum from patients with severe heart failure downregulates eNOS and is proapoptotic. Role of tumor necrosis factor  $\alpha$ .*  
*Circulation.* 1999;100:1983-1991.
- 22. Saraste A, Voipio-Pulkki LM, Parvinen M, et al.**  
*Apoptosis in the heart.*  
*N Engl J Med.* 1997;336:1025-1026. Letter.
- 24. Olivetti G, Abbi R, Quaini F, et al.**  
*Apoptosis in the failing human heart.*  
*N Engl J Med.* 1997;336:1131-1141.
- 25. Sasayama S.**  
*Immune modulation of cardiac function: a new frontier. Role of the immune system in cardiac dysfunction.*  
*J Cardiac Fail.* 1995;1:331-335.



# What are the implications of the interaction between the neurohumoral system and the cytokines?

Helmut Drexler, MD; Bernhard Schieffer, MD

Department of Cardiology and Angiology - Medical University School of Hannover - GERMANY

*The neurohumoral system is critical to the pathophysiology of atherosclerosis and congestive heart failure; cytokines are elevated in unstable angina and heart failure. Cytokine-neurohumoral system interaction can be considered a pathophysiologic hallmark of certain cardiovascular diseases. Although interaction between, eg, the renin-angiotensin system and proinflammatory cytokines has yet to be demonstrated at cellular or molecular level, there is accumulating circumstantial evidence that the protective effect of chronic angiotensin-converting enzyme inhibition against cardiovascular events is mediated, directly or indirectly, by the cytokines involved in remodeling of the atherosclerotic plaque and myocardium. Not least, interaction is providing a promising focus for new treatment concepts in atherosclerosis and heart failure.*

**Keywords:** angiotensin II; interleukin-6; matrix metalloproteinase; plasminogen activator inhibitor-1; C-reactive protein; atherosclerosis, acute coronary syndrome; myocardial infarction; renin-angiotensin system; angiotensin-converting enzyme

**Address for correspondence:**

Prof Helmut Drexler; Abteilung Kardiologie und Angiologie, Medizinische Hochschule Hannover, Carl Neuberg Straße 1, 30625 Hannover, Germany  
(e-mail: Drexler.Helmut@MH-Hannover.de)

**T**he neurohumoral system plays a critical role in the pathophysiology of atherosclerosis and congestive heart failure.

Cytokines are elevated in congestive heart failure and unstable angina. These two observations form the basis for the following review, which examines the implications of the interaction between the neurohumoral system and the cytokines and why this interaction can be considered as a pathophysiological

hallmark of certain cardiovascular diseases such as atherosclerosis and congestive heart failure.

Acute or chronic clinical cardiovascular events remain the main cause of morbidity and mortality in industrialized societies. Evidence from both classic pathological observations and state-of-the-art imaging shows that fatal events are mainly due to pathological vascular or myocardial remodeling processes.

## SELECTED ABBREVIATIONS AND ACRONYMS

ACE	angiotensin-converting enzyme
Ang II	angiotensin II
apo E	apolipoprotein E
AT <sub>1</sub>	angiotensin receptor-1
CRP	C-reactive protein
HOPE	Heart Outcomes Prevention Evaluation
IL-1 $\beta$	interleukin-1 $\beta$
IL-6	interleukin-6
JAK	Janus tyrosine kinase
MAPK	mitogen-activated protein kinase
MCP-1	macrophage chemoattractant protein-1
MMP	matrix metalloproteinase
PAI-11	plasminogen activator inhibitor-1
RAS	renin-angiotensin system
SAVE	Survival And Ventricular Enlargement
SOLVD	Studies Of Left Ventricular Dysfunction
STAT	signal transducer and activator of transcription
TIMP	tissue inhibitor of metalloproteinases



These remodeling processes have been observed both in the atherosclerotic plaque, predisposing it to rupture, as well as in the noninfarcted and infarcted left ventricular myocardium, promoting the progression of heart failure. Neurohumoral systems, such as the endothelin, renin-angiotensin (RAS), and sympathoadrenergic systems have been conclusively shown to be involved in the progression of congestive heart failure, and, at least in part, in the development or progression of atherosclerosis.

Past experimental and clinical studies have almost exclusively looked at the effects of RAS blockers, and recent experimental and clinical evidence points to inflammatory processes as promoters/predictors of cardiovascular events. This review will focus instead on the interaction between the neurohumoral systems (in particular the RAS) and the cytokines, and its potential impact on the remodeling processes involved in the development of the atherosclerotic plaque in the coronary arteries and congestive heart failure.

### NEUROHUMORAL SYSTEM / CYTOKINE INTERACTION AND CORONARY ARTERY DISEASE

Atherosclerosis is an inflammatory disease.<sup>1,2</sup> Atherosclerotic plaques are chronic inflammatory lesions consisting of dysfunctional endothelium, smooth muscle cells, lipid-laden macrophages, and T lymphocytes.<sup>3</sup> The lipid-laden activated macrophages and the T lymphocytes stimulate the neighboring cells, thereby eroding the collagen and elastin framework that forms the plaque's cap.<sup>4,5</sup> Myocardial infarction is one of the fatal end points of progressive atherosclerosis and is thought to result from these

pathological remodeling processes.<sup>2</sup> The impact of the activated RAS on the development of acute myocardial infarction was first demonstrated in epidemiological studies carried out by Aldermann and colleagues.<sup>6</sup> These authors showed that subjects with an elevated renin-sodium profile (a surrogate marker of RAS activation) had a fivefold higher risk of myocardial infarction compared with those with a low renin-sodium profile.<sup>6</sup> In addition, genetic data indicate that the deletion polymorphism *D/D* genotype of the angiotensin-converting enzyme (ACE) gene is associated with higher tissue and plasma levels of ACE, whereas the insertion polymorphism *I/I* genotype is associated with lower ACE levels, and the *I/D* genotype with intermediate levels.<sup>7</sup> Moreover, Cambien and colleagues reported the prevalence of ACE *DD* genotype in a population with no other significant cardiovascular risk factors to be greater in subjects with prior myocardial infarction than in those with no previous infarction.<sup>8</sup> Although the results of these studies are difficult to reconcile, some observations indicate that genetically defined polymorphisms of components of the RAS may be associated with an increased risk of myocardial infarction.<sup>9</sup>

These epidemiological observations stimulated a number of in vitro studies to determine through which mechanisms angiotensin II (Ang II) contributed to the development of acute coronary syndromes. Some of these studies pointed to the potential interaction between Ang II and inflammatory cells.<sup>2,10</sup> Ang II was shown to enhance the migration of blood-derived monocytes and macrophages into atherosclerotic lesions in the coronary vessels,<sup>11</sup> a mechanism thought to play an essential role both in the progression of atherosclerosis and in the devel-

opment of acute coronary syndromes.<sup>2</sup> Ang II was further shown to stimulate the generation of reactive oxygen species in vascular cells and macrophages,<sup>12,13</sup> which are known activators of cytoplasmic signaling cascades, such as nuclear factor kappa B (NF- $\kappa$ B), mitogen-activated protein kinases (MAPK), or the Janus tyrosine kinase / signal transducer and activator of transcription (JAK/STAT) cascade.<sup>14</sup> Together, these mechanisms may enhance oxidative stress within the vascular wall and lead to the activation of redox-sensitive genes, such as those of proinflammatory cytokines,<sup>15,16</sup> as in the case of interleukin-6 (IL-6) transcription, which was shown to be regulated by a redox-sensitive mechanism.<sup>17</sup> These observations suggest that Ang II (via redox-sensitive mechanisms) may activate IL-6 synthesis and release.

In contrast, experimental studies carried out in a rabbit model of atherosclerosis and apolipoprotein E (apo E)-deficient mice have shown that blocking Ang II-induced effects by chronic ACE inhibition abolishes macrophage recruitment into the vessel wall.<sup>11,17</sup> Moreover, blockade of the angiotensin receptor AT<sub>1</sub> by losartan even prevented the accumulation of oxidative reactants in the atherosclerotic vessel wall and reduced the quantity of atherosclerotic lesions in an apo E-deficient animal model.<sup>18</sup> Thus, the interaction between reactive oxygen species, inflammatory cells, and the RAS appears to play an important role not only in the development of acute coronary syndromes, but also in the progression of atherosclerosis. With regard to the progression of experimental atherosclerosis, evidence from other animal models—including rodents and primates—showed that ACE inhibition may reduce the extent of

vascular lesions.<sup>19-21</sup> Additional mechanisms by which the RAS, via Ang II, may enhance the development of atherosclerosis include the activation of thrombosis pathways via plasminogen activator inhibitor-1 (PAI-1) or the stimulation of proinflammatory cytokines.<sup>22</sup> PAI-1 serum levels were shown to be elevated and associated with a higher risk of myocardial infarction in formerly healthy volunteers of the Physician Health Study.<sup>23</sup>

If Ang II does trigger some or all of these mechanisms, one would expect that blockade of the RAS by chronic ACE inhibition would reduce the risk of myocardial infarction. Indeed, retrospective analyses in patients with left ventricular dysfunction, such as in the Studies Of Left Ventricular Dysfunction (SOLVD) and the Survival And Ventricular Enlargement trial (SAVE) have consistently demonstrated a reduction in myocardial infarction with long-term ACE inhibition.<sup>24,25</sup> Nevertheless, it was not clear until recently whether ACE inhibitors could prevent ischemic cardiovascular events in patients with normal ventricular function and no history of cardiovascular disease. The Heart Outcomes Prevention Evaluation (HOPE) showed a reduction in all cardiovascular events when patients with normal left ventricular function and a history of coronary artery disease were treated with an ACE inhibitor (10 mg ramipril/day).<sup>26</sup> These findings suggest that the RAS is implicated in the progression of atherosclerosis, leading to development of acute coronary syndromes. Given the central role of inflammatory processes in the pathophysiology of the atherosclerotic plaque,<sup>2</sup> these findings also suggest that chronic ACE inhibition may interfere with these inflammatory processes, thereby stabilizing the atherosclerotic plaque. However, the exact

nature of the mechanism of the interaction between the RAS activated in the atherosclerotic plaque and the cytokines as well as that of the cytokines involved remain unknown.

Recent evidence has accumulated that tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) are expressed at the shoulder region of atherosclerotic plaques.<sup>27,28</sup> These two cytokines are known stimulators of extracellular matrix-degrading metalloproteinases (MMP), eg, MMP-1, MMP-2, MMP-3, and MMP-9 (for summary see ref 29). However, their potential interaction with neurohumoral systems such as the RAS and their overall impact on the progression of atherosclerosis or the development of acute coronary syndromes are still unclear. Unpublished experiments from our laboratory demonstrated that Ang II does NOT stimulate TNF- $\alpha$  or IL-1 $\beta$  expression in human coronary vascular smooth muscle cells or macrophages.

In contrast, IL-6 serum levels have been shown to be elevated in patients with unstable angina<sup>30,31</sup> and have been implicated in the onset of acute coronary syndromes.<sup>31</sup> IL-6 is involved in a variety of physiologic functions, including the stimulation of acute-phase protein synthesis ( $\alpha_2$ -macroglobulin and C-reactive protein [CRP], leading to the activation of the complement cascade), the induction of prothrombotic factors, eg, PAI-1, and the stimulation of matrix degrading enzymes, such as MMP-1 and MMP-9.<sup>16,32</sup>

Interestingly, a recent report by Rekhter and coworkers indicated that the delicate balance of plaque stability is controlled, on the one hand, by the intrinsic properties of the tissue, and, on the other hand, by the external forces to which the plaque is subjected.<sup>33</sup> On the basis

of circumstantial evidence, it has long been suspected that an increased content of lipid and a decreased content of collagen decrease the mechanical strength of the plaque.<sup>34,35</sup> Thus, a potential plaque-rupturing "cocktail" of factors may include a powerful vasoconstrictor, eg, Ang II, responsible for an increase in local circumferential stress, and cytokines, such as IL-6, stimulating extracellular matrix degradation. Interestingly, the study by Rekhter and colleagues demonstrated that collagen loss was focal, a feature that may have important consequences in the formation of a plaque's weak spots,<sup>33</sup> known to be vulnerable to rupture.<sup>36</sup> Collagen loss was also found to occur preferentially in areas rich in macrophage-derived foam cells (CD68 positive cells),<sup>37</sup> in agreement with previous observations that human atheroma tends to rupture in areas with inflammatory infiltrates.<sup>37-39</sup>

In this regard, Diet and coworkers were the first to show that the Ang II-forming protease ACE is expressed in human atherosclerotic plaques.<sup>40</sup> The authors demonstrated that in early- and intermediate-stage atherosclerotic lesions, ACE was predominantly expressed in lipid-laden macrophages (similarly to proinflammatory cytokines), whereas in advanced lesions, it was localized throughout the plaque microvasculature.<sup>40</sup> Potter and coworkers further demonstrated, in a primate model of atherosclerosis, that lipid-laden macrophages contained Ang II.<sup>41</sup> In humans, at least two major enzymes—ACE and chymase—are involved in the conversion of Ang I into Ang II and may contribute to Ang II formation in coronary arteries. Further investigations in normal and atheromatous coronary artery segments of patients dying of malignant diseases demonstrated that only ACE (not chymase)



was colocalized with Ang II in the intima of stable atherosclerotic lesions with diffuse intimal thickening.<sup>42</sup> In contrast, double immunostaining of Ang II with chymase did not show any colocalization.<sup>42</sup> These findings suggest that ACE is the primary source of Ang II in human atherosclerotic coronary arteries. In addition, recent analyses of coronary arteries obtained during transplantation reveal that chymase-containing mast cells are consistently present in the adventitia, but do not stain for Ang II.<sup>43</sup> Findings from our laboratory do not rule out that chymase secreted by activated mast cells provides an alternative pathway for Ang II formation, but the cellular colocalization and abundance of Ang II in macrophage-rich areas suggests that mast cell-derived chymase is not the major contributor to Ang II formation in human atherosclerotic coronary arteries.<sup>43</sup>

Recent evidence suggests that proinflammatory cytokines are increased in patients with acute coronary syndromes,<sup>31</sup> while Ang II and ACE are expressed predominantly in areas of clustered macrophages (which release proinflammatory cytokines) in atherosclerotic coronary segments<sup>40-42</sup> Therefore, we hypothesized that the two peptides may interact and together enhance the development of acute coronary syndromes. Recent results demonstrate that Ang II induces the synthesis and release of IL-6 in smooth muscle cells and human macrophages.<sup>43</sup> In vivo experiments further showed that Ang II, the AT<sub>1</sub>-receptor, and ACE are expressed at strategically relevant sites of human coronary atherosclerotic plaques, that is, at the shoulder of macrophage-rich atherosclerotic plaques. Furthermore, Ang II was detected in close proximity to the potential plaque rupture site in coronary artery sections from pa-

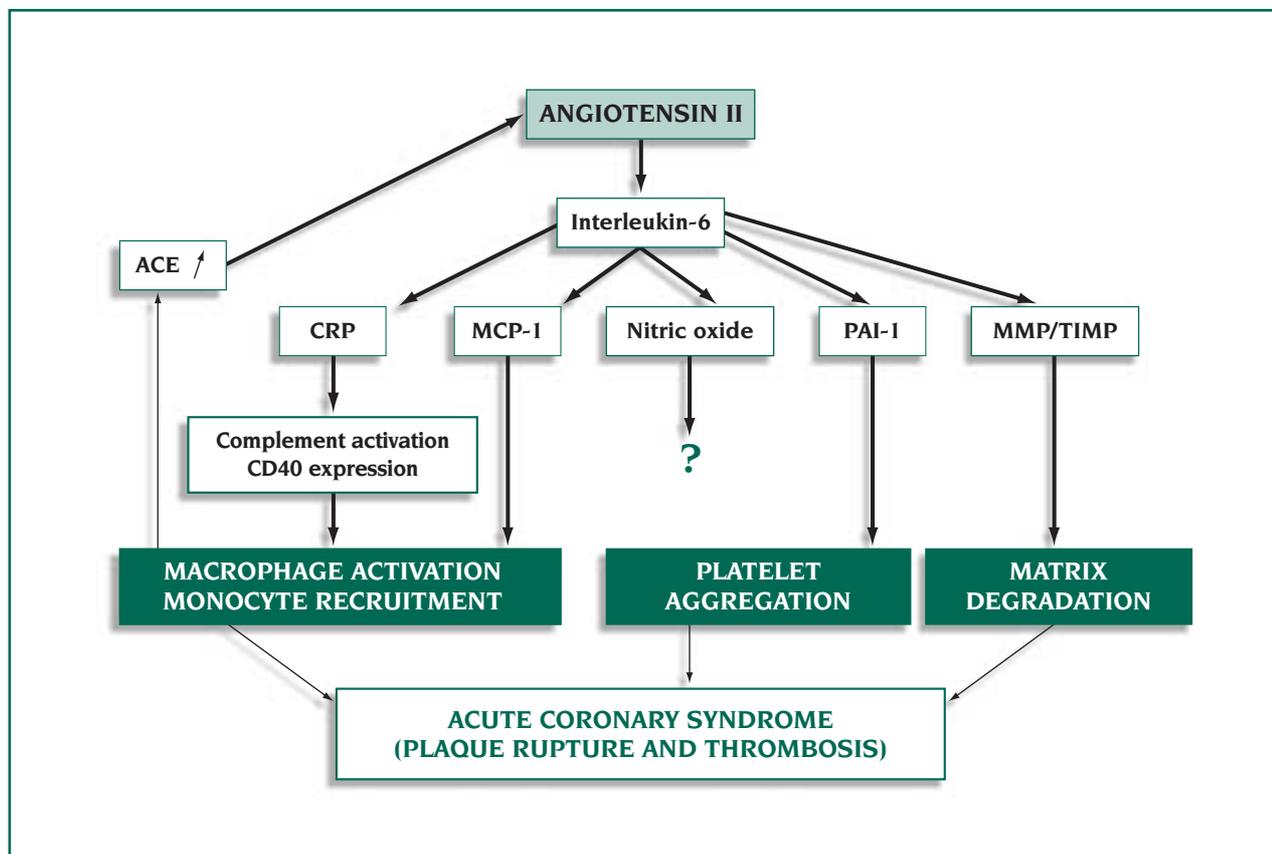
tients having died of acute myocardial infarctions. Colocalization of components of the RAS with IL-6 was observed in stable coronary plaques and atherectomy tissues.<sup>43</sup> These findings suggest that the RAS may contribute to inflammatory processes within the atherosclerotic vascular wall and thereby to the development of acute coronary syndromes.<sup>43</sup> However, the colocalization of Ang II and IL-6 raises the issue of its effect on plaque stability. As a mediator of inflammation, IL-6 stimulates a variety of intracellular signaling mechanisms, including the traditional cytokine signaling cascade of the JAK kinases and STAT transcription factors.<sup>16</sup> The physiologic functions of IL-6 (macrophage differentiation, B-cell maturation, acute phase protein production, and smooth muscle cell proliferation) are mediated via this signaling cascade. Moreover, as indicated above, cytokine-stimulated smooth muscle cells synthesize and release enzymes responsible for extracellular matrix degradation, which may destabilize the plaque's fibrous cap.<sup>29</sup> This is important to take into consideration with respect to the role of proinflammatory cytokines such as IL-6 in the development of acute coronary syndromes. Finally, IL-6 regulates the expression of adhesion molecules and other cytokines, eg, IL-1 $\beta$  and TNF- $\alpha$ , which together may enhance an inflammatory reaction at the atherosclerotic plaque.

Since both Ang II and IL-6 activate identical cellular signaling events, we investigated whether Ang II induced the release of PAI-1 and CRP via the activation of the JAK/STAT cascade. Preliminary results indicated that PAI-1 and CRP were released when smooth muscle cells were stimulated with Ang II.<sup>44</sup> Therefore, we here suggest a model in which the combined action of Ang II and

IL-6 may amplify the development of acute coronary syndromes via the induction of PAI-1, CRP, and/or other potential atherogenic factors, such as macrophage chemoattractant protein-1 [MCP-1] and MMPs (Figure 1, next page).

Whether or not the latter involves the generation of oxygen free radicals needs further investigation. Griending and coworkers, however, demonstrated that Ang II is capable of stimulating superoxide anions via the NADH/NADPH oxidase system, a free radical-forming enzyme formerly known to occur exclusively in leukocytes<sup>12,13</sup> Superoxide anions are known activators of signaling systems such as the JAK/STAT or the NF- $\kappa$ B system.<sup>45,46</sup> Thus, it is reasonable to assume that IL-6 induction by Ang II is redox-sensitive and is stimulated by one of these signaling cascades, as appears to be confirmed by recent observations that blockade of the superoxide anion generation by Ang II abolishes Ang II-induced IL-6 release in vitro.<sup>47</sup> In summary, the generation of superoxide anions via the AT<sub>1</sub> receptor appears to be a crucial signaling step in relation to the proinflammatory potency of Ang II.

In conclusion, components of the activated RAS are present at the shoulder region of coronary atherosclerotic plaques, which are areas with increased risk of plaque rupture. In vitro observations suggest that Ang II stimulates synthesis and release of IL-6, increased serum concentrations of which have been consistently observed in unstable angina. This interaction of Ang II and IL-6 may play a role in vessel wall inflammation and possibly contribute to the development of acute coronary syndromes. Whether or not the impact of Ang II is predominantly subjected to its interaction with cytokines or to other ef-



**Figure 1.** Summary of potential intermediates activated by angiotensin II via interleukin 6. Interleukin-6 induces macrophage chemoattractant protein-1 (MCP-1) and C-reactive protein (CRP) synthesis and release, and both factors together may lead to macrophage accumulation in atherosclerotic plaques. Plasminogen activator inhibitor-1 (PAI-1), if released from the atherosclerotic lesion, leads to the immediate aggregation of platelets. In addition, CRP and PAI-1 are known activators of matrix-degrading enzymes, such as the matrix metalloproteinases (MMPs) and their negative regulators, called (tissue inhibitors of metalloproteinases (TIMPs). Together, these factors initiate the process of plaque destabilization that leads to the development of an acute coronary syndrome. Interestingly, the more macrophages accumulate in an atherosclerotic lesion, the more angiotensin II is expressed (via macrophage-specific angiotensin-converting enzyme [ACE], first demonstrated by Diet et al<sup>40</sup>) and the more impact this plaque-destabilizing pathway has.

facts, such as biomechanical stress, needs further investigation. Notwithstanding, the overall pathophysiologic importance of blocking locally secreted Ang II in atherosclerotic coronary arteries was convincingly shown by the results of the HOPE trial.

### NEUROHUMORAL SYSTEM / CYTOKINE INTERACTION AND CONGESTIVE HEART FAILURE

Myocardial remodeling is a pivotal process in the development of congestive heart failure.<sup>48</sup> Myocardial dysfunction results in an increase

in mechanical stress and Ang II production, triggering a range of molecular and cellular alterations such as hypertrophy and cellular apoptosis of myocytes, changes in the molecular phenotype of the myocardium with reinduction of fetal gene programs, and alterations in the quantity and composition of the extracellular matrix. These alterations together elicit the changes in myocardial structure and function (or phenotype) that define myocardial remodeling.<sup>48</sup> SAVE and SOLVD have demonstrated that, similarly to what is observed in coronary artery atherosclerotic plaques, ACE inhibition is able to counteract this process, thereby slowing the pro-

gression of myocardial failure and reducing mortality in patients with impaired left ventricular function. Other important mechanisms involved in the myocardial remodeling process have been recently identified, such as oxidative stress and inflammatory cytokines (IL-6 and TNF- $\alpha$ ). The latter may be used as predictors of survival of patients with impaired left ventricular function.<sup>49</sup> However, the existence of a direct interaction between the RAS and proinflammatory cytokines at the cellular or molecular level in the myocardium in congestive heart failure has not yet been demonstrated. Nevertheless, indirect evidence from small clinical trials shows that



ACE inhibition with high-dose enalapril (40 mg/d) is able to reduce circulating levels of IL-6/IL-6 receptor over a 34-week treatment period.<sup>50</sup> This decrease in IL-6 bioactivity was significantly associated with decreased interventricular septum thickness, as assessed by echocardiography.

Furthermore, recent studies indicate that TNF- $\alpha$  plays an important role in the pathogenesis of congestive heart failure and that drugs used in the treatment of heart failure can modulate TNF- $\alpha$  production. Liu and coworkers,<sup>51</sup> in a small population of 31 patients with severe chronic heart failure, evaluated cardiac function before and after 72 h of treatment. The patients were randomized into three groups according to their management: Group A (n=14) received milrinone and an ACE inhibitor; Group B (n=6) received milrinone, but no ACE inhibitor; and Group C (n=11) received an ACE inhibitor and dobutamine, but no milrinone. The results showed that circulating TNF- $\alpha$  concentrations significantly decreased after therapy only in those patients whose heart function was improved by one class or more after therapy. No statistically significant change in TNF- $\alpha$  concentration was found in Group B or C patients, although a trend toward decline was present. These observations suggest that the decrease in plasma TNF- $\alpha$  levels is accompanied by an improvement in heart function. The authors speculate that the observed phenomenon is due to the ability of agents such as ACE inhibitors to

inhibit TNF- $\alpha$  production. Moreover, it is tempting to speculate that an increase in perfusion as a result of afterload reduction would reduce muscle and organ hypoperfusion, a hallmark of severe congestive heart failure. In this regard, recent observations indicate that cytokine levels are more elevated in venous blood samples of patients with congestive heart failure than in arterial blood samples, and that the peripheral skeletal muscle may thus significantly contribute to the circulating cytokine levels in congestive heart failure. In contrast, recent work from our laboratory demonstrated that chronic ACE inhibition dose-dependently prevented the pathological remodeling process in skeletal muscle in experimental congestive heart failure.<sup>52</sup>

## CONCLUSION

Although the interaction between neurohumoral systems like the RAS and proinflammatory cytokines at the cellular or molecular level has not yet been conclusively demonstrated, reduction of proinflammatory cytokine levels in response to ACE inhibition suggests that a direct or indirect interaction between the neurohumoral system and the cytokines does take place in congestive heart failure and atherosclerosis, thus providing a potentially promising target for new treatment concepts in atherosclerosis and congestive heart failure.

Studies summarized in this manuscript were supported by DFG-grants Dre 486/8-4 and Schie 386/3-1 as well as by grant 244/C9 from the DFG-Sonderforschungsbereich.

## REFERENCES

### 1. Ross R.

*Pathogenesis of atherosclerosis: a perspective for the 1990s.*

*Nature.* 1993;362:801-809.

### 2. Ross R.

*Atherosclerosis—an inflammatory disease.*

*N Engl J Med.* 1999;340:115-126.

### 3. Van der Waal AC, Becker AE, Loos CM, Das PK.

*Site of intimal rupture or erosion of thrombotic coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology.*

*Circulation.* 1994;89:34-44.

### 4. Libby P.

*Molecular bases of the acute coronary syndrome.*

*Circulation.* 1995;21:2844-2850.

### 5. Davies MJ, Thomas AC.

*Plaque fissuring: the cause of acute myocardial infarction, sudden ischemic death, and crescendo angina.*

*Br Heart J.* 1985;53:363-373.

### 6. Aldermann MH, Madhavan S, Ooi WL, Cohen H, Sealy JE, Laragh JH.

*Association of the renin-sodium profile with the risk of myocardial infarction in patients with hypertension.*

*N Engl J Med.* 1991;324:1098-1104.

### 7. Cambien F, Poirier O, Lecerf L, et al.

*Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction.*

*Nature.* 1992;359:641-644.

### 8. Cambien F, Costerousse O, Tirret L, et al.

*Plasma levels and gene polymorphism of angiotensin-converting enzyme in relation to myocardial infarction.*

*Circulation.* 1994;90:669-676.

**9. Danser AH, Schalekamp MA, Bax WA, et al.**

Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism.

*Circulation.* 1995;92:1387-1388.

**10. Alexander RW.**

Inflammation and coronary artery disease.

*N Engl J Med.* 1994;331:468-469.

**11. Hernandez-Presa M, Bustos C, Ortega M, et al.**

Angiotensin-converting enzyme inhibition prevents arterial nuclear factor- $\kappa$ B activation, monocyte chemoattractant protein-1 expression and macrophage infiltration in a rabbit model of early accelerated atherosclerosis.

*Circulation.* 1997;95:1532-1541.

**12. Griendling KK, Alexander RW.**

Oxidative stress and cardiovascular disease.

*Circulation.* 1997;96:3264-3265.

**13. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW.**

Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells.

*Circ Res.* 1994;74:1141-1148.

**14. Chakraborti S, Chakraborti T.**

Oxidant-mediated activation of mitogen-activated protein kinases and nuclear transcription factors in the cardiovascular system: a brief overview.

*Cell Signal.* 1998;10:675-683.

**15. Northermann W, Braciak TA, Hattori M, Lee F, Fey GH.**

Structure of the rat IL-6 gene and its expression in macrophage-derived cells.

*J Biol Chem.* 1989;264:16072-16082.

**16. Kishimoto T, Akira S, Narazaki M, Taga T.**

IL-6 family of cytokines and gp130.

*Blood.* 1995;86:1243-1254.

**17. Fuhrman B, Oiknine J, Aviram M.**

Iron induces lipid peroxidation in cultured macrophages, increases their ability to oxidatively modify LDL and affect their secretory properties.

*Atherosclerosis.* 1994;111:65-78.

**18. Keidar S, Attias J, Smith J, Breslow JL, Hayek T.**

The angiotensin II receptor antagonist, losartan, inhibits LDL lipid peroxidation and atherosclerosis in apolipoprotein E deficient mice.

*Biochem Biophys Res Commun.*

1997;236:622-625.

**19. Chobanian AV, Haudenschild CC, Nickerson C, Drago R.**

Antiatherogenic effect of captopril in Watanabe heritable hyperlipidemic rabbits.

*Hypertension.* 1990;15: 327-331.

**20. Hayek T, Keidar S, Mei Y, Oikine J, Breslow J.**

Effect of angiotensin converting enzyme inhibitors on LDL lipid peroxidation and atherosclerosis progression in apoE deficient mice.

*Circulation.* 1995;92 (suppl I):I625.

**21. Aberg G, Ferrer P.**

Effects of captopril on atherosclerosis in cynomolgus monkeys.

*J Cardiovasc Pharmacol.*

1990;15(suppl I):S65-S72.

**22. Ridker PM, Gaboury CJL, Conlin PR, Seely EW, Williams GH, Vaughan DE.**

Stimulation of plasminogen activator inhibitor in vivo by infusion of angiotensin II: evidence of a potential interaction between the renin-angiotensin system and fibrinolytic function.

*Circulation.* 1993;87:1969-1973.

**23. Ridker PM, Vaughan DE.**

Hemostatic factors and the risk of myocardial infarction.

*N Engl J Med.* 1995;333:389-390.

**24. Pfeffer M, Braunwald E, Moye L, et al.**

Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: results of the Survival And Ventricular Enlargement trial.

*N Engl J Med.* 1992;327:669-677.

**25. The SOLVD Investigators.**

Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fraction.

*N Engl J Med.* 1992;327:568-574.

**26. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G.**

Effects of an angiotensin-converting enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators.

*N Engl J Med.* 2000;342:145-153.

**27. Mach F, Schonbeck U, Sukhova GK, et al.**

Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis.

*Proc Natl Acad Sci USA.*

1997;94:1931-1936.

**28. Fiotti N, Giansante C, Ponte E, et al.**

Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease.

*Atherosclerosis.* 1999;145:51-60.

**29. Rabbani R, Topol EJ.**

Strategies to achieve coronary artery plaque stabilization.

*Cardiovasc Res.* 1999;41:401-417.

**30. Biassuci L, Vitelli A, Liuzzo G, et al.**

Elevated levels of interleukin-6 in unstable angina.

*Circulation.* 1996;94:874-877.

**31. Marx N, Neumann FJ, Ott I, et al.**

Induction of cytokine expression in leukocytes in acute myocardial infarction.

*J Am Coll Cardiol.* 1997;30:165-170.

**32. Solis-Herruzo JA, Rippe A, Schrum LW, et al.**

Interleukin 6 increases rat metalloproteinase-13 gene expression through stimulation of activator protein 1 transcription factor in cultured fibroblasts.

*J Biol Chem.* 1999;274:30919-30926.

**33. Rekhter MD, Hicks GW, Brammer DW, et al.**

*Hypercholesterolemia causes mechanical weakening of rabbit atheroma: local collagen loss as a prerequisite of plaque rupture*

*Circ Res.* 2000;86:101-108.

**34. Richardson PD, Davies MJ, Born GV.**

*Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques.*

*Lancet.* 1989;2:941-944.

**35. Davies MJ, Woolf N, Rowles P, Richardson PD.**

*Lipid and cellular constituents of unstable human aortic plaques.*

*Basic Res Cardiol.* 1994;89:33-39.

**36. Galis ZS.**

*Metalloproteinases in remodeling of vascular extracellular matrix.*

*Fibrin Proteol.* 1999;13:54-63.

**37. Galis ZS, Sukhova GK, Lark MW, Libby P.**

*Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques.*

*J Clin Invest.* 1994;94:2493-2503.

**38. van der Wal AC, Becker AE, van der Loos CM, Das PK.**

*Site of intimal rupture or erosion of thrombotic coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology.*

*Circulation.* 1994;89:36-44.

**39. Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, et al.**

*Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture.*

*Circulation.* 1995;92:1565-1569.

**40. Diet F, Pratt R, Berry GJ, Momose N, Gibbons G, Dzau VJ.**

*Increased accumulation of tissue ACE in human atherosclerotic coronary artery disease.*

*Circulation.* 1996;94:2576-2767.

**41. Potter DD, Sobey CG, Tompkins PK, Rossen JD, Heistad DD.**

*Evidence that macrophages in atherosclerotic lesions contain angiotensin II.*

*Circulation.* 1998;98:800-807.

**42. Ohishi M, Ueda M, Rakugi H, et al.**

*Relative localization of angiotensin-converting enzyme, chymase and angiotensin II in human coronary atherosclerotic lesion.*

*J Hypertens.* 1999;17:547-553.

**43. Schieffer B, Schieffer E, Hilfiker-Kleiner D, et al.**

*Expression of angiotensin II in human coronary atherosclerotic plaques—potential implications for inflammation and plaque instability.*

*Circulation.* 2000;101:1372-1378.

**44. Han Y, Runge M, Brasier A.**

*Angiotensin II induces interleukin-6 transcription in vascular smooth muscle cells through pleiotropic activation of nuclear factor- $\kappa$ B transcription factor.*

*Circ Res.* 1999;84:695-703.

**45. Schieffer B, Kowert A, Brauer N, et al.**

*Irbesartan inhibits angiotensin II-induced pro-inflammatory and prothrombotic factors in human coronary arteries: role of the JAK/STAT cascade.*

*Eur Heart J.* 1998;(suppl II):P322.

**46. Pagano PJ, Clark JK, Cifuentes-Pagano ME, Clark SM, Callis GM, Quinn MT.**

*Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II.*

*Proc Natl Acad Sci USA.*

1997;94:14483-14488.

**47. Schieffer B, Wagner M, Hilfiker-Kleiner D, Hilfiker A, Drexler H.**

*NADPH-oxidase-dependent activation of JAK/STAT cascade by angiotensin II: role of p47phox.*

*Circ Res.* 2000;87. In press.

**48. Colucci WS.**

*Molecular and cellular mechanisms of myocardial failure.*

*Am J Cardiol.* 1997;80:15L-25L.

**49. Tsutamoto T, Hisanaga T, Wada A, et al.**

*Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure.*

*J Am Coll Cardiol.* 1998;34:391-398.

**50. Gullestad L, Aukrust P, Ueland T, et al.**

*Effect of high- versus low-dose angiotensin converting enzyme inhibition on cytokine levels in chronic heart failure.*

*J Am Coll Cardiol.* 1999;34:2061-2067.

**51. Liu L, Zhao SP.**

*The changes of circulating tumor necrosis factor levels in patients with congestive heart failure influenced by therapy.*

*Int J Cardiol.* 1999;69:77-82.

**52. Schieffer B, Wollert KC, Berchtold M, et al.**

*Development and prevention of skeletal muscle structural alterations in experimental chronic heart failure.*

*Am J Physiol.* 1995;269:H1507-H1513.



# Has the time arrived to use anticytokine therapy in chronic heart failure?

Stefan D. Anker, MD, PhD

Cardiac Medicine - National Heart & Lung Institute - Imperial College School of Medicine - London - UK  
and Franz Volhard Klinik (Charité, Campus Berlin-Buch) at Max Delbrück Center for Molecular Medicine - Berlin - GERMANY

*Inflammatory cytokines contribute to the progression of chronic heart failure (CHF), and they are related to patients' prognosis. Advanced CHF can be considered a state of chronic (low-grade) inflammation, and there are many reasons to think that anticytokine (and particularly anti-tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ]) therapy could be successful in these patients. If anti-TNF- $\alpha$  therapies could be shown to be successful in CHF patients, the etiological link between TNF- $\alpha$  and CHF deterioration could finally be considered proven. Many questions remain to be answered before we can make a final decision about the use of anticytokine treatment in patients with CHF. In my opinion, it is very likely that one day in the not so distant future we will routinely use anticytokine-directed therapies in CHF patients. Whatever the outcome, we are experiencing the beginning of a new era of therapeutic research in heart failure.*

**Keywords:** chronic heart failure; cytokine; tumor necrosis factor; interleukin; anticytokine therapy

**Address for correspondence:**

Dr Stefan D. Anker, Cardiac Medicine,  
NHLI London, Dovehouse Street, London  
SW3 6LY, UK  
(e-mail: s.anker@ic.ac.uk)

**P**rogress in understanding the pathophysiological mechanisms of disease development and progression is necessary to steer major changes in therapy. Cytokines contribute to the development of cardiac dysfunction and to the processes of deterioration in patients with chronic heart failure (CHF). This has been summarized elsewhere in this issue of *Dialogues in Cardiovascular Medicine* and in other recent reviews.<sup>1-4</sup> Pathophysiological correlates and mechanistic plausibility suggest that cytokines can contribute causally to the syndrome of CHF. With the availability of pharmacological compounds that counteract cytokines, the time has clearly come to also consider and test anticytokine treatment strategies in CHF. Anti-tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) therapy has shown

some success in other chronic illnesses associated with inflammatory cytokine activation such as rheumatoid arthritis<sup>5</sup> and Crohn's disease.<sup>6,7</sup> However, anti-TNF- $\alpha$  strategies have failed to reduce mortality in another clinical setting, sepsis,<sup>8-10</sup> which is also clearly linked to a variety of cytokine abnormalities. Therefore, many questions remain to be answered before we can make a final decision about the above question. In my opinion, it is very likely that one day in the not so distant future we will routinely use anticytokine-directed therapies in CHF patients. We are experiencing the early stages of a completely new therapeutic approach to chronic heart failure.

Some of the cytokines have been labeled "proinflammatory" because of their role in the activation and regulation of the body's inflamma-

## SELECTED ABBREVIATIONS AND ACRONYMS

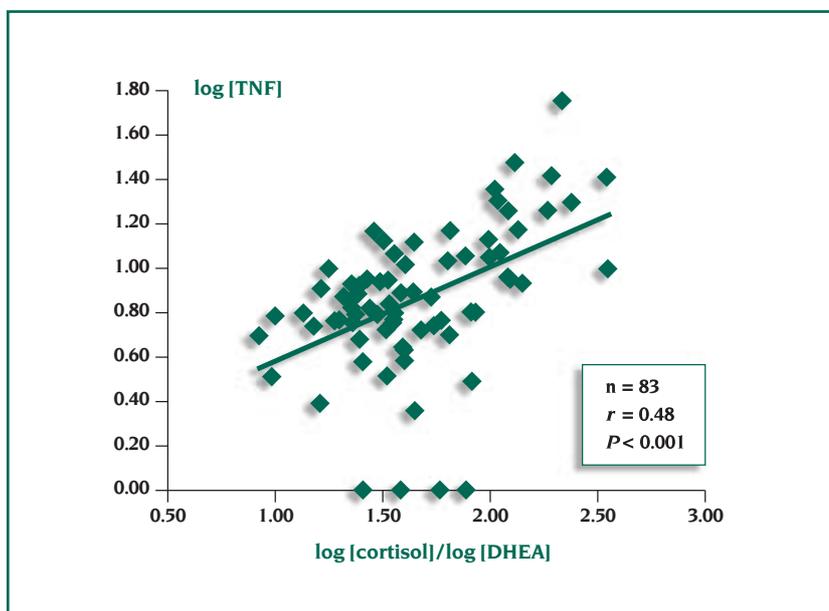
<b>CHF</b>	chronic heart failure
<b>CONSENSUS</b>	COoperative North Scandinavian ENalapril SURvival Study
<b>CRP</b>	C-reactive protein
<b>DHEA</b>	dehydroepiandrosterone
<b>IL-6</b>	interleukin-6
<b>NO</b>	nitric oxide
<b>P38MAPK</b>	P38 mitogen-activated protein kinase
<b>TGF-<math>\beta</math></b>	transforming growth factor $\beta$
<b>TNF-<math>\alpha</math></b>	tumor necrosis factor $\alpha$



tory response to injury. TNF- $\alpha$ , the interleukin (IL)-1 family, IL-6, certain chemokines, transforming growth factor  $\beta$  (TGF- $\beta$ ), and other molecules can be included in this group. In the context of CHF, TNF- $\alpha$  is the most intensely studied cytokine. Anti-TNF- $\alpha$  treatment strategies are the first anticytokine strategies that are now being evaluated in CHF, and hence it appears appropriate to discuss the above question and focus on TNF- $\alpha$ , and anti-TNF- $\alpha$  treatment in particular.

### TNF- $\alpha$ LEVELS: WHOM TO TREAT

Levine et al were the first to demonstrate elevated levels of TNF- $\alpha$  in patients with severe CHF, specifically in patients with cardiac cachexia.<sup>11</sup> This has been confirmed repeatedly,<sup>12,13</sup> and there appears to be a direct relationship between the degree of weight loss and TNF- $\alpha$  plasma concentration.<sup>14</sup> Plasma levels of TNF- $\alpha$  also correlate with the clinical severity of CHF (ie, New York Heart Association [NYHA]), and they can be found to be elevated in a proportion of patients with mild CHF.<sup>15</sup> Apart from cachectic patients, TNF- $\alpha$  levels are highest in NYHA class IV CHF patients, in patients with peripheral edema,<sup>16,17</sup> and in the particular subgroup of patients in cardiogenic shock.<sup>18</sup> TNF- $\alpha$  could contribute to activation of nitric oxide (NO) synthase,<sup>19</sup> generation of oxygen free radicals,<sup>20</sup> and apoptosis.<sup>21,22</sup> By these actions and by its effects on cellular calcium handling and mitochondrial function,<sup>2</sup> TNF- $\alpha$  contributes to cardiomyocyte<sup>23,24</sup> and endothelial dysfunction,<sup>25</sup> with subsequent cardiac and global skeletal muscle dysfunction and fatigability,<sup>26,27</sup> reduced peripheral blood supply,<sup>28</sup> poor oxygen consumption, and tissue wasting,<sup>29,30</sup> all of which are hallmarks of patients with advanced heart failure



**Figure 1.** Relationship between tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and the cortisol/dehydroepiandrosterone (DHEA) ratio in 63 chronic heart failure patients and 20 control subjects (group as a whole).

Adapted from ref 35: Anker SD, Clark AL, Kemp M, et al. Tumor necrosis factor and steroid metabolism in chronic heart failure: possible relation to muscle wasting. *J Am Coll Cardiol.* 1997;30:997-1001. Copyright © 1997, The American College of Cardiology.

and a very poor prognosis.<sup>31,32</sup> The diversity of the pathophysiology of CHF attributable to elevated TNF- $\alpha$  levels also extends to metabolic pathways. Insulin resistance is well documented to occur in CHF patients,<sup>33</sup> and TNF- $\alpha$  levels correlate positively with the degree of insulin resistance in these patients.<sup>34</sup> Disorders of steroid metabolism are also seen in patients with CHF. The cortisol/dehydroepiandrosterone (DHEA) ratio (a measure of catabolic/anabolic imbalance) is raised in CHF patients and correlates positively with TNF- $\alpha$  levels (*Figure 1*).<sup>35</sup> When considering anti-TNF- $\alpha$  treatment, intuitively it appears, therefore, that the subgroups of patients with the highest TNF- $\alpha$  levels are the strongest candidates for this type of treatment.

In absolute terms, the TNF- $\alpha$  levels measured in CHF patients are not as high as can be found in patients with sepsis. This is not surprising as the vast majority of CHF patients

do not show classic clinical signs of inflammation. Even in patients with cardiogenic shock and pyrexia (mean temperature 38.4°C), levels of TNF- $\alpha$ , IL-6, neopterin, and C-reactive protein (CRP) are, on average, significantly lower than in septic patients.<sup>18</sup> However, the chronic and less severe degree of cytokine activation in CHF patients may increase the chances of success of anticytokine treatment strategies in CHF, compared with sepsis. The pathophysiological processes in heart failure may be less rapid and less "chaotic," making any anti-inflammatory intervention potentially more predictable and less likely to cause harm.

But are TNF- $\alpha$  levels reliable measures of inflammatory immune status? Little is known about the "volume of distribution" of TNF- $\alpha$  and other cytokines in CHF patients. What we usually assess is the concentration of cytokines in the patients' serum or plasma. TNF- $\alpha$

and TNF- $\alpha$  receptors have also been assessed in cardiac muscle tissue.<sup>36</sup> A direct relationship between these measures and circulating TNF- $\alpha$  has not yet been established. Tissue TNF- $\alpha$  levels in skeletal muscle and fat tissue levels are currently the focus of research efforts in several laboratories—one would expect them to be raised in CHF patients, particularly in those with some degree of muscle wasting. Nothing is known about TNF- $\alpha$  or other cytokines in edema or ascitic fluid of CHF patients. Nevertheless, the patients' blood will remain the key source to assess inflammatory status in CHF. The future will show whether taking into account alterations of kidney function and blood or plasma volume has any advantage. This has not yet been studied.

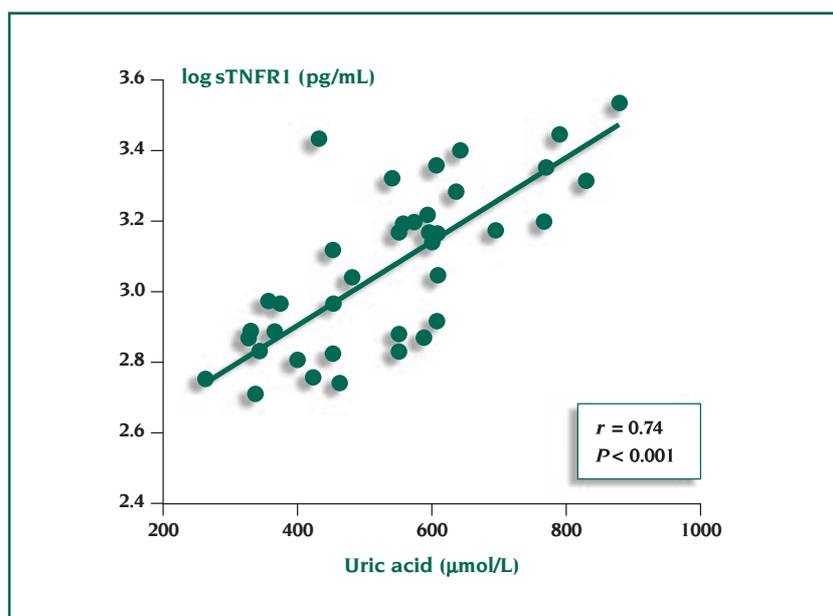
A further problem for the assessment of TNF- $\alpha$  (and even more so for IL-6) lies in the relatively high variability of plasma levels over days to months, which can be improved by analyzing the soluble fraction of TNF- $\alpha$  receptors 1 and 2.<sup>37</sup> Soluble TNF- $\alpha$  receptors appear to be a measure of the history of inflammatory immune activation. In many patients without cachexia and edema, levels of TNF- $\alpha$  receptors are already raised compared with healthy subjects,<sup>30,36,38</sup> and they closely (and better than TNF- $\alpha$  itself) relate to short-term<sup>39</sup> and long-term prognosis in CHF patients.<sup>40</sup> In the setting of pathophysiological research and focused clinical trials (in specialized centers), measurement of soluble TNF- $\alpha$  receptors may be the ideal means to define patients who are likely to benefit from anti-TNF- $\alpha$  treatment. Only in very few institutions, however, will such assessments be available on a routine basis, as they are relatively expensive and time-consuming. In a standard outpatient hospital and clinical trial

setting, therefore, one is forced to use conventional patient characteristics or biochemical surrogate measures. The latter could be CRP,<sup>41</sup> serum uric acid (Figure 2),<sup>42</sup> or the erythrocyte sedimentation rate (Figure 3).<sup>43</sup>

### COULD IT WORK? INDIRECT EVIDENCE

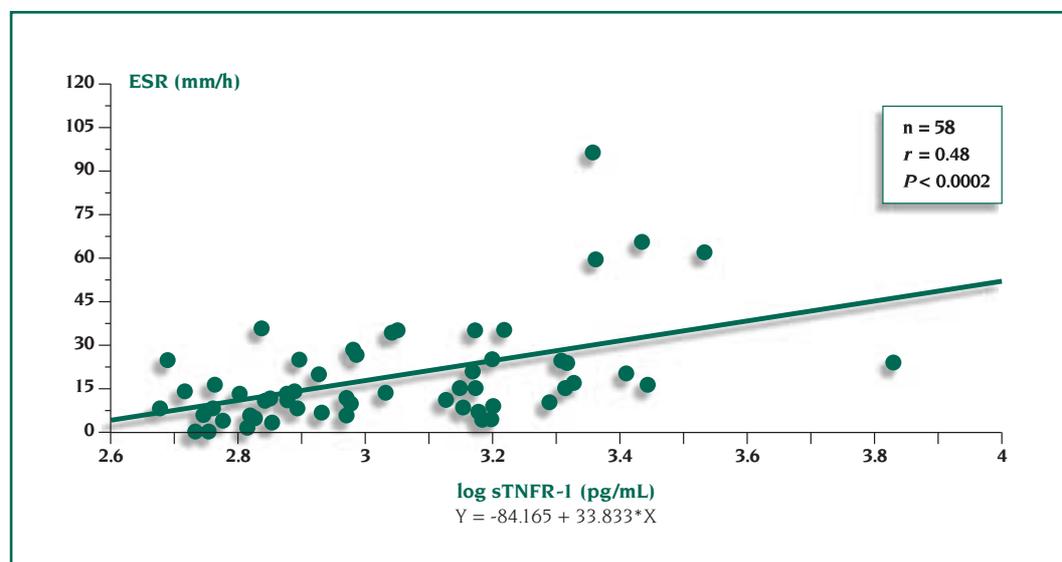
Several well-known treatments for CHF patients that lead to improved survival and/or better symptomatology have been shown to have immune-modulating properties. The angiotensin-converting enzyme (ACE) inhibitor enalapril has been linked to lowering of CRP.<sup>44</sup> The latter study (on historic blood samples of patients from the COoperative North Scandinavian ENalapril SURvival Study [CONSENSUS]) could not document a reduction of TNF- $\alpha$  levels. However, higher doses of enalapril have been shown to reduce IL-6 levels.<sup>45</sup> Vesnarinone can lower cellular TNF- $\alpha$  production in vitro,<sup>46</sup>

as is also the case for amrinone<sup>47</sup> and amiodarone.<sup>48</sup> In the clinical setting, vesnarinone does not influence TNF- $\alpha$  levels (D.L. Mann, personal communication). For amiodarone, in a small study, it was shown that in the subgroup of patients with ischemic cardiomyopathy in NYHA class III, TNF- $\alpha$  levels increased during the treatment period compared with placebo, but this was not linked to an impaired outcome in these patients.<sup>49</sup> Changes in left ventricular wall stress in CHF may result in upregulation of cytokine production. This process can be reversed by implantation of a left ventricular assist device, which results in a decrease in cardiac tissue TNF- $\alpha$ ,<sup>50</sup> IL-6, and IL-8 plasma levels.<sup>51,52</sup> TNF production in CHF may in part be due to the chronic oxidative stress that is seen in this condition,<sup>53,54</sup> possibly due to hypoperfusion of metabolically active tissues. The  $\beta$ -blocker carvedilol has recently been shown to be able to reduce



**Figure 2.** Relationship between serum uric acid and soluble tumor necrosis factor  $\alpha$  receptor-1 (sTNFR1) in 39 patients with chronic heart failure.

Adapted from ref 42: Leyva F, Anker SD, Godsland I, et al. Uric acid in chronic heart failure: a marker of chronic inflammation. *Eur Heart J.* 1998;19:1814-1822. Copyright © 1998, The European Society of Cardiology.



**Figure 3.** Correlation between the erythrocyte sedimentation rate (ESR) and soluble tumor necrosis factor  $\alpha$  receptor-1 plasma levels in 58 patients with chronic heart failure.

Data based on ref 43.

cellular free radical generation.<sup>55</sup> Additionally, oxygen free radicals are known to activate P38 mitogen-activated protein kinase (P38MAPK) and nuclear factor- $\kappa$ B,<sup>56,57</sup> which are key constituents in the macrophage intracellular pathway leading to TNF- $\alpha$  liberation. Carvedilol has also been shown to be able to inhibit P38MAPK.<sup>58</sup> In the short term, diuretic treatment of decompensated patients does not lower cytokine levels within 2 to 3 weeks,<sup>16,59</sup> but there is some limited evidence that, after a longer period (>3 months) of clinical stability, TNF- $\alpha$  levels can decrease.<sup>16</sup>

For none of the above treatment strategies has it as yet been claimed that influencing cytokines would be the main cause of therapeutic benefit in CHF; all have a variety of other effects as well. For pentoxifylline (a phosphodiesterase inhibitor), however, it has recently been suggested that lowering TNF- $\alpha$  could lead to improvement in left ventricular function and clinical status. Sliwa et al<sup>60</sup> reported that pentoxifylline given for 6 months improves left ventricular function and symptoms, based on the finding of significant differences in left

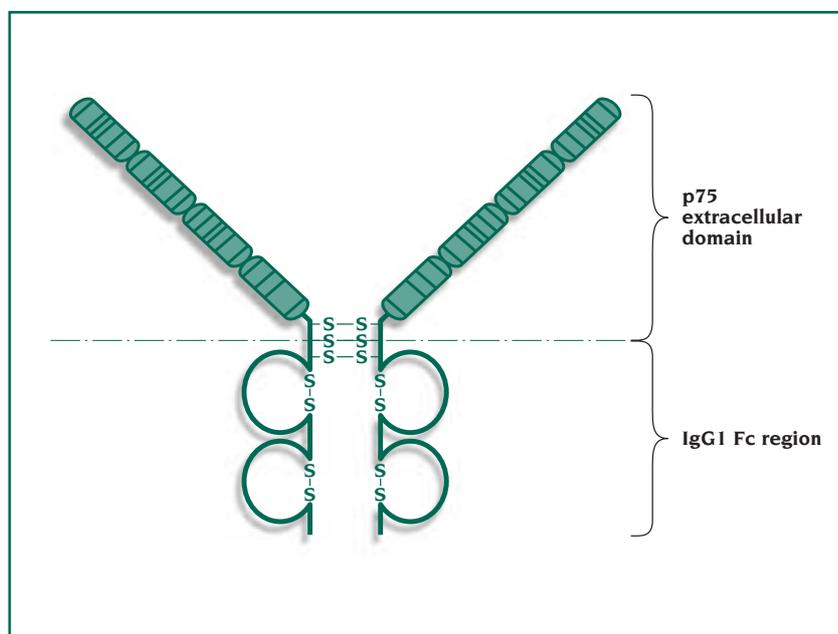
ventricular ejection fraction and clinical status (survival/NYHA class) between groups following treatment. The evaluation of the relevance of TNF- $\alpha$  alterations is, however, difficult. Although the reduction in TNF- $\alpha$  levels was only statistically significant within the treatment group, the approximate differences between the groups in terms of mean TNF- $\alpha$  levels before and after treatment in both study arms were very similar (-4.4 pg/mL in the pentoxifylline group vs -4.3 pg/mL in the placebo group), as calculated from data given in the publication.<sup>60</sup> A preliminary report on 49 CHF patients (52 $\pm$ 11 years) with 12 months' placebo-controlled pentoxifylline treatment (400 mg tds), also suggested improvement in clinical outcome, cardiac function, and reduction in TNF- $\alpha$  and IL-6 plasma levels.<sup>61</sup> In rheumatoid arthritis, a recent study failed to demonstrate any benefit with pentoxifylline (1200 mg od for at least 1 month).<sup>62</sup> Finally, with the exception of amiodarone in patients with an ischemic etiology,<sup>49</sup> to date, none of the proven beneficial treatments of CHF is known to impair immune parameters or raise levels of inflammatory

cytokines. In addition, no treatment modality that has been shown to be able to lower inflammatory cytokine levels in vivo in CHF patients has caused harm in these CHF patients. Although this is no evidence for the benefit of anticytokine strategies, these findings fuel optimism with respect to specific anticytokine treatment in CHF patients.

### DOES IT WORK? DIRECT EVIDENCE

Drugs with anticytokine effects can be divided into: (i) those that reduce the stimulus for cytokine production; (ii) those that disable the cellular mechanisms responsible for the generation of cytokines; and (iii) those that neutralize the effects of cytokines once they have been released into the circulation.

The credit for having been the first to test a specific anticytokine treatment (etanercept) in CHF patients goes to Mann's group (Houston, USA).<sup>63</sup> Etanercept consists of two soluble TNF- $\alpha$  p75 receptors linked to the Fc portion of a human immunoglobulin G-1 (IgG1) molecule (sTNFR:Fc) (Figure 4). Etanercept binds to TNF- $\alpha$  with very high affin-



**Figure 4.** Principal structure of soluble tumor necrosis factor  $\alpha$  receptor (p75) fusion protein (TNFR:Fc, etanercept).

ity (about 100-fold stronger than naturally occurring TNF- $\alpha$  receptors), inactivating circulating TNF- $\alpha$ . In this first study,<sup>63</sup> 12 patients received a single dose of etanercept (1, 4, or 10 mg/m<sup>2</sup>) and 6 patients received placebo to blind the assessments. Improvements in 6-min walking distance and left ventricular ejection fraction (in patients receiving 4 or 10 mg/m<sup>2</sup> etanercept were detected after 2 weeks. No untoward events were observed, and the provisional conclusion of this study was that this type of treatment is feasible in CHF patients.

Subsequently, a 3-center study was initiated to assess the effect of 2 doses of etanercept (injections of 5 [n=16] or 12 [n=15] mg/m<sup>2</sup> etanercept twice weekly) compared with placebo (n=16) for a period of 3 months. Initial results have been presented at recent conferences.<sup>64-66</sup> A total of 47 patients in NYHA class III and IV have been included, and etanercept was shown to reduce TNF- $\alpha$  bioactivity, improve left ventricular function, and have a significantly positive impact on a

composite clinical end point incorporating functional NYHA class, hospitalization events, and survival. Most importantly, this study showed that the anti-TNF- $\alpha$ -directed treatment was well tolerated.

On the basis of this experience of a total of only 43 CHF patients on active medication on the one hand, and that of many more patients with this treatment in rheumatoid arthritis (where etanercept now has Food and Drug Administration [FDA] approval for clinical use) and Crohn's disease on the other hand, two large multicenter studies of etanercept in CHF have been launched, one in North America (RENAISSANCE), the other in Europe (RECOVER). These studies are testing the effect of 25 mg/m<sup>2</sup> once to three times weekly compared with placebo. This is very similar to the dose approved for rheumatoid arthritis. Each study aims to recruit 900 patients. The studies are planned in such a way that a combined analysis of the morbidity/mortality effect will be possible. In contrast to the initial

studies, for these large-scale investigations it has been decided to recruit patients in NYHA class II as well as in class III and IV. If these trials are successful with regard to improving morbidity and mortality in CHF patients, a fundamentally new treatment principle will have been introduced for CHF. The results are expected to be available within the next 1 to 2 years.

### PROBLEMS AND OPEN QUESTIONS

TNF- $\alpha$  is just one (albeit important) inflammatory cytokine in the large network of immune factors. TNF- $\alpha$  and inflammatory cytokines in general also have some beneficial effects in CHF patients, at least early on in the disease process.<sup>67</sup> Imbalance of inflammatory and anti-inflammatory cytokines occurs in CHF,<sup>68</sup> and, to reestablish this balance, other cytokines could also be targeted. In the near future, the use of anti-TNF- $\alpha$  antibodies<sup>69,70</sup> or anti-IL-6 or anti-IL-1 treatment (now being tested in other conditions<sup>71</sup>) may reach a clinical test phase. Alternatively, anti-inflammatory recombinant cytokines, like recombinant IL-10<sup>72</sup> or IL-11,<sup>73</sup> could be beneficial in CHF patients.

A number of problems remain unanswered for the time being. Among them, the issue of introducing an injectable therapy appears to be a lesser issue—this has already been shown practicable in a trial using recombinant human growth hormone.<sup>74</sup> The two biggest issues may relate to the general problems of CHF therapy and the health care system. First, if anti-TNF- $\alpha$  therapy with etanercept were to be beneficial on top of current treatment, that would introduce just one more drug class for patients already taking an ACE inhibitor, a  $\beta$ -blocker, 1 to 3 diuretics (potentially plus



spironolactone), and possibly also digoxin, antiarrhythmic agents, angiotensin II-receptor antagonists, and other drugs. The future will show, which, if any, of these drugs are redundant, and in which subgroup of patients certain drugs are particularly valuable. Anticytokine therapy may well be only or particularly beneficial in patients with strongly upregulated inflammatory cytokines. This may be conceived to be a great economical risk when developing new drugs. However, the good news for the pharmaceutical industry is that some subgroups (like patients in NYHA class IV, with edematous decompensation and/or significant weight loss) are frequent enough to establish by themselves a big market. The good news for patients and their doctors is that this ensures therapeutic research progress, hopefully leading to improvement in patients' quality of life, symptomatology, and mortality.

The second problem is strongly linked to the first. The new anticytokine treatments will not be cheap. The price of a monthly dose of etanercept as used in rheumatoid arthritis is in the order of US \$ 1000. Clearly, the health care systems' funds are finite. It is a political problem to decide the allocation of health care funds. Cost-effectiveness research accompanying the clinical trials is a must for this new treatment approach in order to be accepted and used, even if clinical results appear very positive. Possibly, financial considerations will speed up the development of subgroup-specific therapeutic guidelines, rather than labeling all treatments "on top" of standard therapy for all patients.

Last, but not least, there is one other problem in the anticytokine approach. We don't know exactly why in a certain patient cytokine levels are raised. There are a num-

ber of theories.<sup>67,75-77</sup> The purist may say, first we need to know the cause of raised TNF- $\alpha$  levels, and then we should treat this cause. Research in this direction is certainly required. However, the chain of events leading to deterioration in CHF may be quite long. TNF- $\alpha$  and other cytokines are the cause of a number of important pathologic abnormalities in CHF. Counteracting them is now possible, and is both logical and potentially beneficial. In answer to the above question, counteracting cytokines in patients with CHF has to be tried now.

## CONCLUSIONS

If anti-TNF- $\alpha$  therapies could be shown to significantly modify the natural history of CHF, the highly suggestive etiological link between TNF- $\alpha$  and CHF deterioration could finally be considered proven. Many questions remain to be answered before we can make a final decision about the use of anticytokine treatment in patients with CHF. If one wants to keep up with research progress in this field, one needs to take a look beyond cardiology. The therapeutic progress made in chronic inflammatory diseases like rheumatoid arthritis and Crohn's disease appears most relevant to CHF doctors. Advanced CHF can be considered a state of chronic (low-grade) inflammation, and there are many reasons to think that anticytokine (and particularly anti-TNF- $\alpha$ ) therapy could be successful in these patients. However, it may well be that this will be limited to particular subgroups of patients with CHF. Whatever the outcome, we are experiencing the beginning of a new era of therapeutic research in heart failure.

SDA is supported with a postgraduate fellowship of the Max Delbrück Center for Molecular Medicine, Berlin, Germany.

## REFERENCES

- 1. Ceconi C, Curello S, Bachetti T, Corti A, Ferrari R.**  
*Tumor necrosis factor in congestive heart failure: a mechanism of disease for the new millennium?*  
*Prog Cardiovasc Dis.* 1998;41(suppl 1):25-30.

---

- 2. Muller-Werdan U, Engelmann H, Werdan K.**  
*Cardiodepression by tumor necrosis factor-alpha.*  
*Eur Cytokine Netw.* 1998;9:689-691.

---

- 3. Torre-Amione G, Bozkurt B, Deswal A, Mann DL.**  
*An overview of tumor necrosis factor  $\alpha$  and the failing human heart.*  
*Curr Opin Cardiol.* 1999;14:206-210.

---

- 4. Anker SD, Rauchhaus M.**  
*Insights into the pathogenesis of chronic heart failure: immune activation and cachexia.*  
*Curr Opin Cardiol.* 1999;14:211-216.

---

- 5. Moreland LW, Baumgartner SW, Schiff MH.**  
*Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein.*  
*N Engl J Med.* 1997;337:141-147.

---

- 6. Targan SR, Hanauer SB, van Deventer SJ, et al.**  
*A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group.*  
*N Engl J Med.* 1997;337:1029-1035.

---

- 7. Present DH, Rutgeerts P, Targan S, et al.**  
*Infliximab for the treatment of fistulas in patients with Crohn's disease.*  
*N Engl J Med.* 1999;340:1398-1405.

---

- 8. Fisher CJ Jr, Agosti JM, Opal SM, et al.**  
*Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group.*  
*N Engl J Med.* 1996;334:1697-1702.

Anticytokine therapy in heart failure? - Anker

**9. Cohen J, Carlet J.**

*INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group.*

*Crit Care Med.* 1996;24:1431-1440.

**10. Abraham E, Anzueto A, Gutierrez G, et al.**

*Double-blind randomised controlled trial of monoclonal antibody to human tumour necrosis factor in treatment of septic shock. NORASEPT II Study Group.*

*Lancet.* 1998;351:929-933.

**11. Levine B, Kalman J, Mayer L, Fillit HM, Packer M.**

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

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

**12. McMurray J, Abdullah I, Dargie HJ, Shapiro D.**

*Increased concentrations of tumour necrosis factor in "cachectic" patients with severe chronic heart failure.*

*Br Heart J.* 1991;66:356-358.

**13. Dutka DP, Elborn JS, Delamere F, Shale DJ, Morris, GK.**

*Tumour necrosis factor alpha in severe congestive cardiac failure.*

*Br Heart J.* 1993;70:141-143.

**14. Anker SD, Chua TP, Swan JW, et al.**

*Hormonal changes and catabolic/anabolic imbalance in chronic heart failure: the importance for cardiac cachexia.*

*Circulation.* 1997;96:526-534.

**15. Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL.**

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

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

**16. Niebauer J, Volk HD, Kemp M, et al.**

*Endotoxin and immune activation in chronic heart failure: a prospective cohort study*

*Lancet.* 1999;353:1838-1842.

**17. Vanderheyden M, Kersschot E, Paulus WJ.**

*Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm. Serial assessment in patients with congestive heart failure.*

*Eur Heart J.* 1998;19:747-752.

**18. Brunkhorst FM, Clark AL, Forycki ZF, Anker SD.**

*Pyrexia, procalcitonin, immune activation and survival in cardiogenic shock: the potential importance of bacterial translocation.*

*Int J Cardiol.* 1999;72:3-10.

**19. Kilbourn RG, Gross SS, Jubran A, et al.**

*N<sup>G</sup>-methyl-L-arginine inhibits tumor necrosis factor-induced hypotension: implications for the involvement of nitric oxide.*

*Proc Natl Acad Sci USA.* 1990;87:3629-3632.

**20. Matsubara T, Ziff M.**

*Increased superoxide anion release from human endothelial cells in response to cytokines.*

*J Immunol.* 1986;137:3295-3298.

**21. Krown KA, Page MT, Nguyen C, et al.**

*Tumor necrosis factor alpha-induced apoptosis in cardiac myocytes. Involvement of the sphingolipid signaling cascade in cardiac cell death.*

*J Clin Invest.* 1996;98:2854-2865.

**22. Adams V, Jiang H, Yu J, et al.**

*Apoptosis in skeletal myocytes of patients with chronic heart failure is associated with exercise intolerance.*

*J Am Coll Cardiol.* 1999;33:959-965.

**23. Muller-Werdan U, Schumann H, Fuchs R, et al.**

*Tumor necrosis factor alpha (TNF alpha) is cardiodepressant in pathophysiologically relevant concentrations without inducing inducible nitric oxide-(NO)-synthase (iNOS) or triggering serious cytotoxicity.*

*J Mol Cell Cardiol.* 1997;29:2915-2923.

**24. Bozkurt B, Kribbs SB, Clubb FJ Jr, et al.**

*Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive left ventricular dysfunction and remodeling in rats.*

*Circulation.* 1998;97:1382-1391.

**25. Katz SD, Rao R, Berman JW, et al.**

*Pathophysiological correlates of increased serum tumor necrosis factor in patients with congestive heart failure.*

*Circulation.* 1994;90:12-16.

**26. Hegewisch S, Weh HJ, Hossfeld DK.**

*TNF-induced cardiomyopathy.*

*Lancet.* 1990;2:294-295.

**27. Anker SD, Swan JW, Volterrani M, et al.**

*The influence of muscle mass, strength, fatigability and blood flow on exercise capacity in cachectic and non-cachectic patients with chronic heart failure.*

*Eur Heart J.* 1997;18:259-269.

**28. Anker SD, Volterrani M, Egerer KR, et al.**

*Tumor necrosis factor-alpha as a predictor of peak leg blood flow in patients with chronic heart failure.*

*Q J Med.* 1998;91:199-203.

**29. Mancini DM, Walter G, Reichek N, et al.**

*Contribution of skeletal muscle atrophy to exercise intolerance and altered muscle metabolism in heart failure.*

*Circulation.* 1992;85:1364-1373.

**30. Anker SD, Ponikowski PP, Clark AL, et al.**

*Cytokines and neurohormones relating to body composition alterations in the wasting syndrome of chronic heart failure.*

*Eur Heart J.* 1999;20:683-693.

**31. Mancini DM, Eisen H, Kusmaul W, Mull R, Edmunds Jr LH, Wilson JR.**

*Value of peak exercise oxygen consumption for optimal timing of cardiac transplantation in ambulatory patients with heart failure.*

*Circulation.* 1991;83:778-786.



**32. Anker SD, Ponikowski P, Varney S, et al.**

*Wasting as independent risk factor of survival in chronic heart failure.*

*Lancet.* 1997;349:1050-1053.

**33. Swan JW, Anker SD, Walton C, et al.**

*Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure.*

*J Am Coll Cardiol.* 1997;30:527-532.

**34. Paolisso G, Tagliamonte MR, Rizzo MR, et al.**

*Prognostic importance of insulin-mediated glucose uptake in aged patients with congestive heart failure secondary to mitral and/or aortic valve disease.*

*Am J Cardiol.* 1999;83:1338-1344.

**35. Anker SD, Clark AL, Kemp M, et al.**

*Tumor necrosis factor and steroid metabolism in chronic heart failure: possible relation to muscle wasting.*

*J Am Coll Cardiol.* 1997;30:997-1001.

**36. Torre-Amione G, Kapadia S, Lee J, et al.**

*Tumor necrosis factor- $\alpha$  and tumor necrosis factor receptors in the failing human heart.*

*Circulation.* 1996;93:704-711.

**37. Dibbs Z, Thornby J, White B, Mann DL.**

*Natural variability of circulating levels of cytokines and cytokine receptors in patients with heart failure: implications for clinical trials.*

*J Am Coll Cardiol.* 1999;33:1935-1942.

**38. Steele IC, Nugent AM, Maguire S, et al.**

*Cytokine profile in chronic cardiac failure.*

*Eur J Clin Invest.* 1996;26:1018-1022.

**39. Ferrari R, Bachetti T, Confortini R, et al.**

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

*Circulation.* 1995;92:1479-1486.

**40. Rauchhaus M, Doehner W, Francis D, et al.**

*Peripherally measured inflammatory cytokines and survival in chronic heart failure.*

*J Am Coll Cardiol.* 2000. Abstract in press.

**41. Sato Y, Takatsu Y, Kataoka K, et al.**

*Serial circulating concentrations of C-reactive protein, interleukin (IL)-4, and IL-6 in patients with acute left heart decompensation.*

*Clin Cardiol.* 1999;22:811-813.

**42. Leyva F, Anker SD, Godtsland I, et al.**

*Uric acid in chronic heart failure: a marker of chronic inflammation.*

*Eur Heart J.* 1998;19:1814-1822.

**43. Sharma R, Rauchhaus M, Ponikowski PP, et al.**

*The relationship of the erythrocyte sedimentation rate to inflammatory cytokines and survival in patients with chronic heart failure treated with angiotensin-converting enzyme inhibitors.*

*J Am Coll Cardiol.* 2000;36:523-528.

**44. Eriksson SV, Kjekshus J, Eneroth P, Swedberg K.**

*Neopterin, tumor necrosis factor, c-reactive protein and prostaglandin E<sub>2</sub> in patients with severe congestive heart failure treated with enalapril.*

*Circulation.* 1997;96(suppl):I322. Abstract.

**45. Gullestad L, Aukrust P, Ueland T, et al.**

*Effect of high- versus low-dose angiotensin converting enzyme inhibition on cytokine levels in chronic heart failure.*

*J Am Coll Cardiol.* 1999;34:2061-2067.

**46. Matsumori A, Shioi T, Yamada T, Matsui S, Sasayama S.**

*Vesnarinone, a new inotropic agent, inhibits cytokine production by stimulated human blood from patients with heart failure.*

*Circulation.* 1994;89:955-958.

**47. Endres S, Sinha B, Fulle HJ.**

*Amrinone suppresses the synthesis of tumor necrosis factor- $\alpha$  in human mononuclear cells.*

*Shock.* 1994;1:377-380.

**48. Matsumori A, Ono K, Nishio R, Nose Y, Sasayama S.**

*Amiodarone inhibits production of tumor necrosis factor- $\alpha$  by human mononuclear cells: a possible mechanism for its effect in heart failure.*

*Circulation.* 1997;96:1386-1389.

**49. Oral H, Fisher SG, Fay WP, Singh SN, Fletcher RD, Morady F.**

*Effects of amiodarone on tumor necrosis factor- $\alpha$  levels in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy.*

*Am J Cardiol.* 1999;83:388-391.

**50. Torre-Amione G, Stetson SJ, Youker KA, et al.**

*Decreased expression of tumor necrosis factor- $\alpha$  in failing human myocardium after mechanical circulatory support: a potential mechanism for cardiac recovery.*

*Circulation.* 1999;100:1189-1193.

**51. Hasper D, Hummel M, Kleber FX, Reindl I, Volk HD.**

*Systemic inflammation in patients with heart failure.*

*Eur Heart J.* 1998;19:761-765.

**52. Goldstein DJ, Moazami N, Seldomridge JA, et al.**

*Circulatory resuscitation with left ventricular assist device support reduces interleukins 6 and 8 levels.*

*Ann Thorac Surg.* 1997;63:971-974.

**53. McMurray J, Chopra M, Abdullah I, Smith WE, Dargie HJ.**

*Evidence of oxidative stress in chronic heart failure in humans.*

*Eur Heart J.* 1993;14:1493-1498.

**54. Keith M, Geranmayegan A, Sole MJ, et al.**

*Increased oxidative stress in patients with congestive heart failure.*

*J Am Coll Cardiol.* 1998;31:1352-1356.

**55. Dandona P, Karne R, Ghanim H, Hamouda W, Aljada A, Magsino CH Jr.**

*Carvedilol inhibits reactive oxygen species generation by leukocytes and oxidative damage to amino acids.*

*Circulation.* 2000;101:122-124.

Anticytokine therapy in heart failure? - Anker

**56. Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ.**

*Activation of mitogen-activated protein kinase by H<sub>2</sub>O<sub>2</sub>. Role in cell survival following oxidant injury.*

*J Biol Chem.* 1996;271:4138-4142.

**57. Huot J, Houle F, Marceau F, Landry J.**

*Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock protein 27 pathway in vascular endothelial cells.*

*Circ Res.* 1997;80:383-392.

**58. Sung CP, Arleth AJ, Eichman C, Truneh A, Ohlstein EH.**

*Carvedilol, a multiple-action neurohumoral antagonist, inhibits mitogen-activated protein kinase and cell cycle progression in vascular smooth muscle cells.*

*J Pharmacol Exp Ther.* 1997;283:910-917.

**59. Vanderheyden M, Kersschot E, Paulus WJ.**

*Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm. Serial assessment in patients with congestive heart failure.*

*Eur Heart J.* 1998;19:747-752.

**60. Sliwa K, Skudicky D, Candy G, Wisenbaugh T, Sareli P.**

*Randomised trial of effects of pentoxifylline on left-ventricular performance in idiopathic dilated cardiomyopathy.*

*Lancet.* 1998;351:1091-1093.

**61. Skudicky D, Sliwa K, Bergemann A, Candy G, Sareli P.**

*Long-term effects of pentoxifylline in patients with idiopathic dilated cardiomyopathy: one-year results.*

*Eur Heart J.* 1999;20(suppl):94. Abstract.

**62. Dubost JJ, Soubrier M, Ristori JM, et al.**

*An open study of the anti-TNF alpha agent pentoxifylline in the treatment of rheumatoid arthritis.*

*Rev Rhum Engl Ed.* 1997;64:789-793.

**63. Deswal A, Bozkurt B, Seta Y, et al.**

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

*Circulation.* 1999;99:3224-3226.

**64. Bozkurt B, Torre-Amione G, Soran OZ, et al.**

*Results of a multidose phase I trial with tumor necrosis factor receptor (p75) fusion protein (etanercept) in patients with heart failure.*

*J Am Coll Cardiol.* 1999;33(suppl A):184A. Abstract.

**65. Bozkurt B, Torre-Amione G, Soran OZ, Whitmore J, Warren M, Mann DL.**

*Suppression of cardioinflammatory cytokines by Enbrel (etanercept, p75 TNF receptor fusion protein) correlates with clinical improvement in heart failure patients.*

*Circulation.* 1999;100(suppl I):I21. Abstract.

**66. Bozkurt B, Torre-Amione G, Deswal A, et al.**

*Regression of left ventricular remodeling in chronic heart failure after treatment with Enbrel (etanercept, p75 TNF receptor Fc fusion protein).*

*Circulation.* 1999;100(suppl I):I645. Abstract.

**67. Seta Y, Shan K, Bozkurt B, Oral H, Mann DL.**

*Basic mechanisms in heart failure: the cytokine hypothesis.*

*J Card Fail.* 1996;2:243-249.

**68. Aukrust P, Ueland T, Lien E, et al.**

*Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy.*

*Am J Cardiol.* 1999;83:376-382.

**69. Stack WA, Mann SD, Roy AJ, et al.**

*Randomised controlled trial of CDP571 antibody to tumour necrosis factor-alpha in Crohn's disease.*

*Lancet.* 1997;349:521-524.

**70. Maini R, St Clair EW, Breedveld F, et al.**

*Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group.*

*Lancet.* 1999;354:1932-1939.

**71. Breedveld FC.**

*Future trends in the treatment of rheumatoid arthritis: cytokine targets.*

*Rheumatology (Oxford).* 1999;38(suppl 2):11-13.

**72. Asadullah K, Sterry W, Stephanek K, et al.**

*IL-10 is a key cytokine in psoriasis. Proof of principle by IL-10 therapy: a new therapeutic approach.*

*J Clin Invest.* 1998;101:783-794.

**73. Trepicchio WL, Ozawa M, Walters IB, et al.**

*Interleukin-11 therapy selectively down-regulates type I cytokine proinflammatory pathways in psoriasis lesions.*

*J Clin Invest.* 1999;104:1527-1537.

**74. Osterziel KJ, Strohm O, Schuler J, et al.**

*Randomised, double-blind, placebo-controlled trial of human recombinant growth hormone in patients with chronic heart failure due to dilated cardiomyopathy.*

*Lancet.* 1998;351:1233-1237.

**75. Anker SD, Egerer KR, Volk HD, Kox WJ, Poole-Wilson PA, Coats AJ.**

*Elevated soluble CD14 receptors and altered cytokines in chronic heart failure.*

*Am J Cardiol.* 1997;79:1426-1430.

**76. Hasper D, Hummel M, Kleber FX, Reindl I, Volk HD.**

*Systemic inflammation in patients with heart failure.*

*Eur Heart J.* 1998;19:761-765.

**77. Paulus WJ.**

*How are cytokines activated in heart failure?*

*Eur J Heart Fail.* 1999;1:309-312.



# Surfing the Heart

*International Society for Heart Research – The Cochrane Library  
Annals of Improbable Research – European Society of Cardiology*

Claudio Ceconi, MD

e-mail: [cceconi@libero.it](mailto:cceconi@libero.it) – Cardiovascular Research Center – S. Maugeri Foundation – Gussago – ITALY



The International Society for Heart Research (ISHR) aims to promote discovery in cardiovascular sciences and the dissemination of knowledge in the field through publications, conferences, and other media. The web site of the ISHR is an excellent example of how a web site should be designed in order to effectively disseminate information about a scientific society and supply useful news to its members. The information relevant to the society and its publications is clearly organized. The web site includes features such as the Research Topic Message Board for on-line discussion and members' questions, the ISHR Employment Service, which is a database of job offers and seekers of employment, and the searchable ISHR World Wide Member Directory. Web sites also exist for other branches, including the European Section ([www.biomed.cas.cz/fgu/ishr\\_es/](http://www.biomed.cas.cz/fgu/ishr_es/)).



[www.cochrane.org/cochrane/cdsr.htm](http://www.cochrane.org/cochrane/cdsr.htm)

The Cochrane Library, an off-shoot of the Cochrane Collaboration, supplies high-quality data to people both providing and receiving care, and to those responsible for research, teaching, funding, and administration at all levels. The Cochrane Library's huge database is routinely updated by the 7000 collaborating members of the foundation. The Cochrane Library web sites are primarily addressed to institutions, and charge for a large number of their services. However, the services can be distributed with a single license on intranet networks of universities and institutes. The Cochrane Collaboration has two web sites specific to cardiology: [www.epi.bris.ac.uk/cochrane/heart.htm](http://www.epi.bris.ac.uk/cochrane/heart.htm) and [www.epi.bris.ac.uk/cochrane/cardi.html](http://www.epi.bris.ac.uk/cochrane/cardi.html).



The *Annals of Improbable Research* is a satirical journal of science, medicine, and technology. The editors claim that they explore the question: "How do you tell what's important and what's not, and what's real and what's not—in science and everywhere else?" Their web site highlights just how difficult it can be to answer this! The web site reproduces sections of the magazine, including the *Ig™ Nobel Prizes*, which are awards for achievements that cannot, or should not, be reproduced, and *Breaking News* and *Broken Features*, which is based on the standard research literature, in which sometimes the most astonishing scientific questions are taken very seriously.



The next frontier of the Internet is *interactivity*. Webcasts, ie, Internet broadcasts, are becoming more common, and advanced person-to-person communication is being developed. You can experience the Amsterdam 2000 European Society of Cardiology Congress in the form of webcast presentations at [www.escardio.org/scinfo/WebCasts/webcasts\\_introduction.htm](http://www.escardio.org/scinfo/WebCasts/webcasts_introduction.htm). The topics covered are at the cutting edge of medical research, and range from the *Role of inflammation in cellular remodeling to Using the Internet in clinical practice and in trials*. A further step in the direction of using web communication is [www.escardio.org/zeditorialteam/editorials.htm](http://www.escardio.org/zeditorialteam/editorials.htm), where Prof Maarten Simoons, President of European Society of Cardiology, speaks directly to the community of European professionals in cardiology, and also to patients.

All sites accessed 1st December 2000



# Trails of Discovery

## *The importance of chance and the prepared mind in the discovery of the $\beta$ -blockers*

Desmond Fitzgerald, BSc, FRCP, FFPM

Visiting Professor - Department of Physiology and Pharmacology - University of Strathclyde - Glasgow - UK

The purpose of this essay is to illustrate the exciting, frustrating, and sometimes treacherous nature of innovative drug discovery by recounting some of the lesser known incidents that led to the discovery of  $\beta$ -blockers, which are now so widely used in cardiovascular therapy.

The story starts with a chemist, Jack Mills, in the Eli Lilly research laboratories in Indianapolis, USA, who was trying to make compounds that might be better than the bronchodilators used clinically (isoprenaline and adrenaline). The laboratory technician, Mr Le Compte, observed that the dichloro analog of isoprenaline (dichloroisoprenaline [DCI]; 2 chlorine substituents instead of 2 hydroxyls on the benzene ring) prevented the relaxing action of adrenaline on tracheal rings in the organ bath. His superior, Mr Powell, asked a colleague, Dr Slater, to help him analyze these unexplained effects, which, at the time, were of little interest to the cardiovascular research group. At that time, Slater was working on centrally acting adrenergic blockers to treat anxiety.<sup>1</sup> Subsequent experiments in cats and dogs showed that DCI blocked the vasodilator actions of isoprenaline (now called  $\beta_2$ -mediated vasodilatation). The findings were presented at the Federation Scientific meeting in the USA in 1957 and published in full

in 1958.<sup>2</sup> By chance, Neil Moran (Emory University, Atlanta) heard about the paper. He had been studying the cardiovascular effects of different catecholamines in anesthetized dogs.



Figure 1. Professor Sir James Black.

Within 2 weeks, he obtained a supply of DCI from Slater and found that it blocked the actions of catecholamines both on the heart and blood vessels.<sup>3</sup> In the discussion section of the paper, published in 1958 with his colleague Perkins, he coined the term " $\beta$ -adren-ergic blocking drug." The Eli Lilly researchers could not think of a clinical application for DCI, though it was used to control perioperative arrhythmias in a few patients undergoing removal of a pheochromocytoma in the USA and in Italy.<sup>4,5</sup>

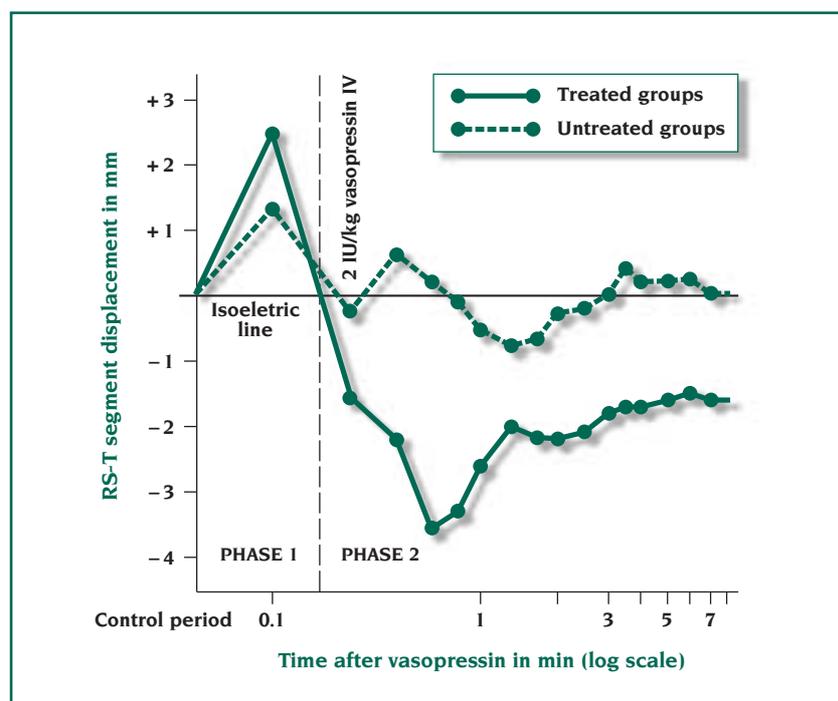
During this period (1955-1958), Jim Black (now Professor Sir James Black), a senior lecturer in the Department of Veterinary Physiology in the University of Glasgow (Figure 1), was studying the effects of myocardial ischemia on

cardiac function, especially the induction of lethal cardiac arrhythmias. In collaboration with other colleagues, he observed that small improvements in cardiac oxygenation, eg, by applying hyperbaric oxygen, prevented ventricular arrhythmias. He studied the effects of a water-soluble protein-free extract of cardiac muscle that had been used in Switzerland and Germany to treat patients with myocardial ischemia. It was called Recosen<sup>®</sup>, and was marketed by Robapharm, Basel, Switzerland. Black administered 1 mL IM for 10 days to rabbits. He induced reversible myocardial ischemia by giving vaso-pressin (pitressin), which caused marked generalized vasoconstriction accompanied by ECG changes. Rabbits treated with Recosen<sup>®</sup> showed a significant diminution in the marked RS-T depression induced by vasopressin in control rabbits (Figure 2).<sup>6</sup>

Thus, in 1958, Moran's prepared mind was able to identify DCI as the first  $\beta$ -blocker, while Black's prepared mind was seeking novel ways of inhibiting the work of the ischemic heart, while Eli Lilly, which had the  $\beta$ -blocker, could think of no medical use for it. In July 1958, Black joined ICI Pharmaceuticals Division to work on novel treatments for coronary artery disease. Jeff Thorpe, who was already working on other approaches to coronary artery disease, had just discovered the hypolipidemic actions of clofibrate.<sup>7</sup> Black felt that the sympathetic nervous system played a major role in the ECG changes of acute coronary insufficiency, emphasizing that injections

### Address for correspondence:

Dr Desmond Fitzgerald, Materia Medica, Mere Croft, Chester Road, Mere, Nr Knutsford WA16 6LG, UK (e-mail: awhite@man.ac.uk)



**Figure 2.** Time course of electrocardiographic changes following vasopressin in untreated rabbits and in rabbits treated with heart extract. The RS-T segments in phase 2 of treated animals were significantly less depressed.

of adrenaline can mimic this by stimulating the myocardial oxygen consumption beyond the available substrate supply, ie, the “anoxiating” action of adrenaline (Internal research report: 22 January 1959). In this report, he went on to speculate that the action of Recosen® in his previous rabbits experiments might be due to an “anti-adrenaline-like action.” He obtained a supply of DCI from Eli Lilly and studied its properties in detail. Though it was a potent antagonist at cardiac adrenoceptors, it had too much stimulant activity (intrinsic sympathomimetic activity [ISA]). An analog, pronethalol, was made in February 1960, and, by June of that year, the research team felt that it met the criteria suitable for evaluation in angina pectoris and cardiac arrhythmias. Clinical trials started in September 1961.

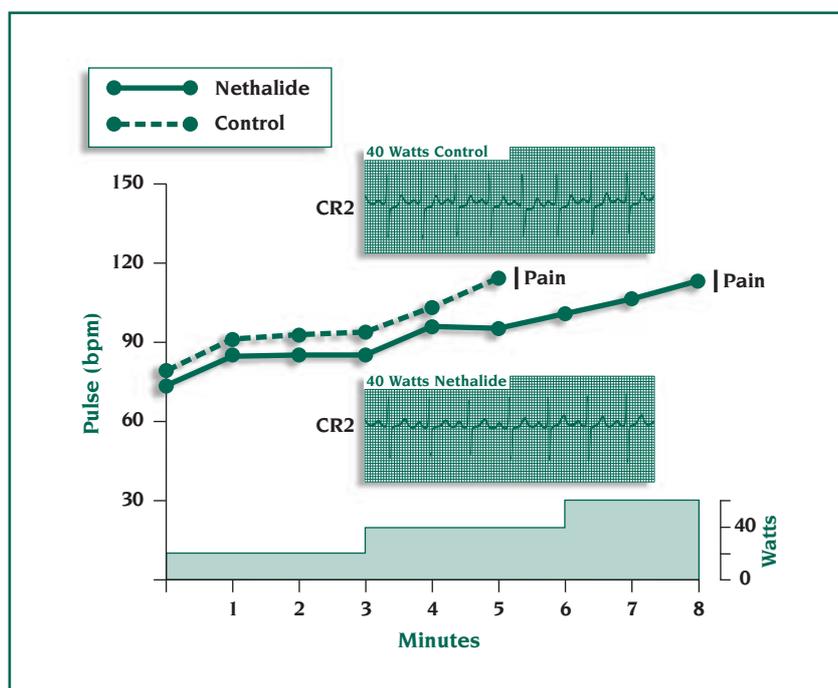
While this research was progressing in the ICI laboratories, Lish and colleagues, in the Mead Johnson laboratories in Evansville, Indiana ( USA ), was

seeking novel sympathomimetic compounds in order to inhibit intestinal, tracheal, and uterine smooth muscle.<sup>8</sup> They found that putting a novel substituent on the benzene ring of catecholamines produced compounds with a range of adrenergic activities. One of these compounds was the  $\beta$ -antagonist sotalol. This was synthesized in October 1960, 8 months after pronethalol had been made in the ICI laboratories and 18 months before its structure was published in August 1962. Their first patent, submitted to the USA in 1962, contained many claims, including  $\beta$ -blockade, but the exemplified compound was a potent vasoconstrictor, presumably for use topically to treat rhinitis, or systemically to raise blood pressure in shock, including cardiogenic shock, which was advocated at that time. At the same time, workers at Boehringer Ingelheim (Germany) were seeking improved anorectic agents to treat obesity. Some of their compounds resembled DCI in structure and were shown to

specifically block the actions of isoprenaline. In July 1960, propranolol (Inderal®) was made by their chemists even before ICI had synthesized it. Despite repeated discussions, their senior research management saw no utility for such agents.<sup>9</sup>

Also in the same period, Bengt Ablad, working in the cardiovascular group in Hässle, Gothenburg, initiated a research program to find a new antiarrhythmic drug, which, by preventing catecholamine stimulation of the heart, would reduce cardiac arrhythmias. In May 1961, a chemical program to find compounds with activities like DCI was started.<sup>10</sup> It was only when the Dornhorst and Robinson paper on pronethalol (nethalide)<sup>11</sup> was published in August 1962 that the Hässle researchers found that ICI was working on the same idea and was well ahead of them. Their challenge was to develop their own patentable compound since it was clear by August 1962 that blockade of cardiac  $\beta$ -receptors in patients with angina was efficacious (see Figure 3 next page):

*“The heart rate is also slowed in patients with ischemic heart disease, and this action may prove beneficial. The pulse rate is shown during two exercise tests in a patient with angina pectoris, whose rate of work was increased in steps. Nethalide (250 mg orally) reduced the heart rate at rest and during work, and the patient was able to achieve a further step in the test before pain developed. The electrocardiogram showed less abnormality during exercise at 40 watts after taking the drug than it had done in the control test. Fourteen patients with ischemic heart disease were studied after a placebo and after nethalide. The average reduction of heart rate at rest was 14% and on exercise 18%. Nine patients were able to perform more work before pain developed, and, in 5, the exercising electrocardiogram was improved.”*



**Figure 3.** Effect of nethalide on pulse rate. (Based on a poster by Dornhorst and Robinson, 1962.)

In contrast to Jim Black's views, the Hässle researchers' objective was: "To develop a potent  $\beta$ -receptor antagonist... which should include a moderate  $\beta$ -receptor stimulation."<sup>12</sup> Ironically, their most successful  $\beta$ -blocker is the  $\beta_1$ -selective agent metoprolol, which is devoid of any  $\beta$ -receptor stimulant action.

In 1960, Graham Hayward's group in the Cardiology Department in St Bartholomew's Hospital, London, showed that cervical sympathectomy in patients with angina pectoris was beneficial, but the mechanism of action was not clear. Douglas Chamberlain, working as a research fellow in that group, thought that the beneficial effects were due to a reduction in sympathetic nerve stimulation of the heart. He thought that a drug blocking the cardiac actions of neuronal catecholamines would be of similar benefit in angina pectoris, so he wrote to Lilly for supplies of DCI in order to test its effects in anginal patients. The supply of DCI arrived

just as the *Lancet* paper<sup>11</sup> describing pronethalol's properties and efficacy in anginal patients was published. He therefore used pronethalol to compare its effects with that of sympathectomy in anginal patients.<sup>13</sup>

This early history of the discovery of the  $\beta$ -blockers provides a good illustration of the role of the prepared mind in discovery. Published and unpublished papers relating to Black's approach clearly indicate that reducing myocardial ischemia and the associated arrhythmias was his final objective. He considered a range of approaches such as heart extracts and androgenic steroids to achieve this, but quickly saw the potential of DCI. In addition, he rejected DCI because it had too much  $\beta$ -stimulant activity, and, therefore, sought a compound without ISA. Chamberlain also saw the need to reduce cardiac stimulation in angina pectoris and did not accept that the benefit of sympathectomy resulted from analgesia secondary to section of cardiac afferent nerves. The Hässle

group also wished to reduce catecholamine arrhythmias in diseased hearts, and thus were attracted to part of the Black hypothesis. The Mead Johnson management remained indecisive about what to do with sotalol, as evidenced by their patent withdrawals and reapplications between 1962 and 1965. The final US patent was approved in 1967, 5 years after the proof of concept of  $\beta$ -blockade in angina was published. Lilly, despite the fact that Jack Mills synthesized not only DCI, but also pronethalol, did not exploit these opportunities. Boehringer Ingelheim eventually tried to license a close analog of Inderal®, which was dominated by the ICI patents even though it had been synthesized, but not patented, prior to ICI making the same compound. ICI had tested it in man, in comparison with propranolol, but did not develop it because it had ISA.

A further historical irony is that Fourneau described the synthesis of several aminoalcohols including phenoxypropanolamines in 1915.<sup>14</sup> His long paper ends with the comment that "MM. Billou and Launoy tested such compounds, and their effect on the heart would not permit use in therapeutics even though certain analogs had antipyretic and analgesic properties." One of the compounds described in his paper was subsequently synthesized by ICI and shown to be a nonselective  $\beta$ -blocker. The path to new effective treatments is rarely smooth and straight.

Perhaps one valuable asset in drug discovery is a clinical background. Douglas Chamberlain's thought processes depended upon an ability to link cardiovascular physiology to clinical diseases. Similarly, the serendipitous observation of the hypotensive actions of pronethalol by Pritchard, which had not been predicted from animal experiments, depended upon both a knowledge of physiology combined with excellent clinical skills.



These talents are as much in demand today as they were 30 years ago, despite the burgeoning technology platforms so popular with current pharmaceutical research.

In a private conversation with Jim Black, his answer to my question: "Would you have thought of  $\beta$ -blockade for treating myocardial ischemia if you hadn't studied medicine?" he replied: "No." The brevity of this response reflects an important aspect of his character. He is an intensely private person, and though he is lively and a great raconteur, he resists speculating in public about his research. Perhaps one can get some feel for his personal and formidable intellectual attributes by reading selected published interviews and some of his papers. His intellectual stature can be gauged in his essay entitled *A Personal View of Pharmacology*,<sup>15</sup> which he describes as a "family album, not a work of reference." It describes his progress over 30 years in the complex mathematical modeling designed to assist in the interpretation of drug action. In the 33-page essay, less than one page is devoted to his original work on  $\beta$ -adrenoceptor and H<sub>2</sub> receptor antagonists. There is no mention of his discovery, either of propranolol or cimetidine (H<sub>2</sub> blocker), which have transformed the treatment of cardiovascular and peptic ulcer diseases. In this context, his response to an interviewer for the journal *Omni*,<sup>16</sup> shortly after he was awarded the Nobel Prize, gives some insight to his personal value systems:

**Omni:** "You have earned more money for the drug companies—perhaps unwittingly—than any other man on earth!"

**Black:** "...I refuse to take the blame for that. For the same science, it could have been a much more limited disease. I didn't set out to make these companies vast fortunes, but to solve the problem I saw in front of me." (p128).

Earlier in the same article the interviewer writes:

"His only regret about the billions he made for his former employers is that they spent most of it to build corporate hierarchies having nothing to do with creative thinking." (p80).

This critical view contrasts somewhat with his opinion when he first joined ICI in 1958. In his closing remarks in a symposium to mark 20 years of propranolol (Kings College, Cambridge, 1985) he commented:

"To go there (ICI) when it was brand new, as I did, was really a tremendous experience. It was like joining some great club, there was a marvelous attitude among the people there at that time."

In a discussion with Professor Alice Sapienza (Simmons College, Boston) he summarized his research philosophy as:

"(i) To avoid wishful thinking; (ii) to start with molecules that have some selective action; (iii) to have a tissue assay that measures precisely the effect you want to have. I look for goals, constraints, and restrictions because I think that is how you win. The purpose of the drug industry is to supply the society in which it is imbedded with new drugs, better drugs. It is there to fulfill a need."

Few could argue that Jim Black has practiced what he preaches.

## REFERENCES

### 1. Slater IH, Jones GT.

Pharmacological properties of ethoxy butamoxane and related compounds.

*J Pharmacol.* 1957;122:69A.

### 2. Powell CE, Slater IH.

Blocking of inhibitory adrenergic receptors by a dichloro analogue of isoproterenol.

*J Pharmacol.* 1958;122:480-488.

### 3. Moran NC, Perkins ME.

Adrenergic blockade of the mammalian heart by a dichloro analogue of isoproterenol.

*J Pharmacol.* 1958;124:223-237.

### 4. Riddell DH, Schull LG, Frist TF, Baker TD.

Experience with pheochromocytoma in 21 patients: use of dichloroisoproterenol hydrochloride for cardiac arrhythmia.

*Ann Surg.* 1963;157:980-988.

### 5. De Blasi S.

Uso del dicloroisoproterenolo, adrenolitico "beta" in due casi di feocromocitoma.

*Minerva Anestesiol.* 1964;30:157-181.

### 6. Black JW.

Electrocardiographic changes produced in rabbits by vasopressin (pitressin) and their alteration by prolonged treatment with a commercial heart extract.

*J Pharm Pharmacol.* 1960;12:87-94.

### 7. Thorp JM.

An experimental approach to the problem of disordered lipid metabolism.

*J Atheroscler Res.* 1963;3:351-360.

### 8. Lish PM, Duncan KW, Peters EI.

A survey of the effects of isoxuprine on non-vascular smooth muscle.

*J Pharmacol.* 1960;129:191-199.

### 9. Shanks RG.

The discovery of  $\beta$ -adrenoceptor blocking drugs.

*Trends Pharmacol Sci.* 1984;5:405-409.

**The discovery of the  $\beta$ -blockers - Fitzgerald**

**10. Corrodi H, Persson H, Carlsson A, Roberts J.**

*A new series of substances which block the adrenergic  $\beta$ -receptors.*

*J Med Chem.* 1963;6:751-755.

---

**11. Dornhorst AC, Robinson BF.**

*Clinical pharmacology of a  $\beta$ -adrenergic blocking agent (nethalide).*

*Lancet.* 1962;2:314-316.

---

**12. Abläd B.**

*Introduction. Pharmacological studies on H 56/28—a new  $\beta$ -adrenergic receptor antagonist.*

*Acta Pharmacol Toxicol.* 1967;25(suppl 2):5-8.

---

**13. Apthorp GH, Chamberlain DA, Hayward GW.**

*The effects of sympathectomy on the electrocardiogram and effort tolerance in angina pectoris.*

*Br Heart J.* 1964;26:218-226.

---

**14. Fourneau E.**

*Aminoalcools. Dérivés des éthers oxydes de la glycérine et des phénols.*

*J Pharm Chim.* 1915;7:7-103.

---

**15. Black J.**

*A personal view of pharmacology.*

*Annu Rev Pharmacol Toxicol.* 1996;36:1-33.

---

**16. Black J.**

*Interview: Sir James Black.*

*Omni.* 1990;12:78-92, 126-134.

---

# Cytokines

## Summaries of Ten Seminal Papers

**Biykem Bozkurt, MD, FACC**

Assistant Chief - Medical Services - Veterans Affairs Medical Center and Assistant Professor of Medicine -  
Section of Cardiology - Baylor College of Medicine - 2002 Holcombe Blvd - Houston - TX 77030 - USA

①

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

**B. Levine and others.** *N Engl J Med.* 1990

②

Increased circulating cytokines in patients with myocarditis and cardiomyopathy

**A. Matsumori and others.** *Br Heart J.* 1994

③

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

**R. Ferrari and others.** *Circulation.* 1995

④

Tumor necrosis factor- $\alpha$  and tumor necrosis factor receptors in the failing human heart

**G. Torre-Amione and others.** *Circulation.* 1996

⑤

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

**G. Torre-Amione and others.** *J Am Coll Cardiol.* 1996

⑥

Cytokines and cardiac contractile function

**R.A. Kelly, T.W. Smith.** *Circulation.* 1997

⑦

Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor- $\alpha$

**T. Kubota and others.** *Circ Res.* 1997

⑧

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

**K. Sliwa and others.** *Lancet.* 1998

⑨

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

**A. Deswal and others.** *Circulation.* 1999

⑩

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

**L. Agnoletti and others.** *Circulation.* 1999

Selection of seminal papers by

**D.C. Mann, MD; P. Knueferman, MD; Georg Baumgarten, MD**  
Winters Center for Heart Failure Research - Dept of Medicine -  
VAMC and Baylor College of Medicine - Houston - Texas - USA

Highlights of the years by **P.B. Garlick**

Division of Radiological Sciences - Guy's Hospital -  
London SE1 9RT - UK

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

B. Levine, J. Kalman, L. Mayer, H.M. Fillit, M. Packer

*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- $\alpha$ ) in patients with advanced heart failure. TNF- $\alpha$  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- $\alpha$ . 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- $\alpha$  and revealed its role as a central mediator of endotoxic shock. Therefore, TNF- $\alpha$  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- $\alpha$  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- $\alpha$  greater than 2 SD above the mean value for the control group. The patients with high levels of TNF- $\alpha$  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- $\alpha$  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- $\alpha$  in cardiac cachexia seen with heart failure, subsequently, other investigators noted that the TNF- $\alpha$  levels were elevated not only in cachectic, but also in non-cachectic advanced heart failure patients as well. TNF- $\alpha$  levels actually correlate with severity of heart failure rather than cachexia alone, and TNF- $\alpha$  can be associated with

many aspects of heart failure other than cachexia. TNF- $\alpha$  produces both immediate and delayed negative inotropic effect on myocardial contractility. When expressed at sufficiently high concentration, TNF- $\alpha$  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- $\alpha$  in patients with advanced heart failure. There is a progressive increase in TNF- $\alpha$  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- $\alpha$ . Much like elevated neurohormones, TNF- $\alpha$  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

A. Matsumori, T. Yamada, H. Suzuki, Y. Matoba, S. Sasayama

*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- $\alpha$ ) might participate in the development of congestive heart failure came in 1990 when Levine et al demonstrated that circulating levels of TNF- $\alpha$  were elevated in patients with end-stage heart failure and cachexia. Subsequent studies demonstrated comparable elevations in interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ ), and a direct relationship between TNF- $\alpha$  levels and functional heart failure classification (New York Heart Association [NYHA]). Furthermore, direct relationships were identified between circulating levels of TNF- $\alpha$  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 $\alpha$ , IL-1 $\beta$ , IL-2, IL-6, TNF- $\alpha$  and TNF- $\beta$ , granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, macrophage colony-stimulating factor, interferon- $\alpha$ , and interferon- $\gamma$  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 $\alpha$ , TNF- $\alpha$ , 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

R. Ferrari, T. Bachetti, R. Confortini, C. Opasich, O. Febo, A. Corti, G. Cassani, O. Visioli

*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- $\alpha$  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- $\alpha$  mRNA and TNF- $\alpha$  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)

G. Torre-Amione, S. Kapadia, C. Benedict, H. Oral, J.B. Young, D.L. Mann

*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  $\alpha$  (TNF- $\alpha$ ) 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- $\alpha$ . 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- $\alpha$  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- $\alpha$  were elevated in patients in NYHA functional classes I to III, with values of  $1.95 \pm 0.54$ ,  $2.63 \pm 0.48$ , and  $6.4 \pm 1.9$  pg/mL, respectively compared with age-matched control subjects ( $0.75 \pm 0.05$  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 ( $3.3 \pm 0.55$ ,  $6.2 \pm 1.1$ , and  $5.22 \pm 0.9$  pg/mL, respectively) compared with age-matched control subjects ( $1.8 \pm 0.5$  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- $\alpha$  and survival, whereas there was no significant relation for plasma IL-6. Except for atrial natriuretic factor, which correlated weakly with circulating TNF- $\alpha$  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- $\alpha$ .

Thus, analogous to elevated levels of neurohormones, TNF- $\alpha$  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 $\beta$  (IL-1 $\beta$ ) 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- $\alpha$  and IL-1 $\beta$  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 $\beta$  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  $\beta$ -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- $\alpha$

T. Kubota, C.F. McTiernan, C.S. Frye, S.E. Slawson, B.H. Lemster, A.P. Koretsky, A.J. Demetris, A.M. Feldman

*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- $\alpha$  (TNF- $\alpha$ ). The failing human heart expresses TNF- $\alpha$ . Its cardiac genetic overexpression was long awaited to support the pathophysiological significance of TNF- $\alpha$  for the heart.

Kubota and his colleagues had previously reported that robust overexpression of TNF- $\alpha$  in the murine heart causes lethal myocarditis. In this earlier report, a transgene construct was made containing the murine  $\alpha$ -myosin heavy chain promoter and the coding sequence of murine TNF- $\alpha$ . 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- $\alpha$  protein in the transgenic heart.

In this study, Kubota et al modified the transgene to reduce the production of TNF- $\alpha$  by preserving the destabilizing sequence in TNF- $\alpha$  cDNA. Expression was driven by the murine  $\alpha$ -myosin heavy chain promoter. Use of this modified construct allowed the establishment of a murine transgenic line with more modest TNF- $\alpha$  overexpression rather than lethally toxic high levels, and most mice survived the neonatal period. These mice with modest cardiac TNF- $\alpha$  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- $\alpha$  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- $\alpha$  recapitulated the phenotype of congestive heart failure. This provides a novel model to elucidate the role of TNF- $\alpha$  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

K. Sliwa, D. Skudicky, G. Candy, T. Wisenbaugh, P. Sareli

*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- $\alpha$  responses during septic shock. The inhibition of TNF- $\alpha$  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  $22.3 \pm 9.0$  to  $38.7 \pm 15.0$ ) 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  $6.5 \pm 5.0$  pg/mL to  $2.1 \pm 1.0$  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

A. Deswal, B. Bozkurt, Y. Seta, S. Parilti-Eiswirth, F.A. Hayes, C. Blosch, D.L. Mann

*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 IgG<sub>1</sub> 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/m<sup>2</sup>). 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/m<sup>2</sup> 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/m<sup>2</sup>, 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/m<sup>2</sup> 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- $\alpha$

L. Agnoletti, S. Curello, T. Bachetti, F. Malacarne, G. Gaia, L. Comini, M. Volterrani, P. Bonetti, G. Parrinello, M. Cadei, P.G. Grigolato, R. Ferrari

*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- $\gamma$  and interleukin- $1\beta$ , 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.

1999

Microsoft ruled to be a monopoly by the US Federal Court; the World Trade Organization meeting in Seattle is disrupted by environmental, labor, and human rights protestors; and the People's Republic of China announces its first successful unmanned space mission

# Cytokines

## Bibliography of One Hundred Key Papers

- 
- Agnoletti L, Curello S, Bachetti T, et al.** *Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: role of tumor necrosis factor-alpha.*  
**Circulation.** 1999;100:1983-1991.
- 
- Anker SD, Clark AL, Kemp M, et al.** *Tumor necrosis factor and steroid metabolism in chronic heart failure: possible relation to muscle wasting.*  
**J Am Coll Cardiol.** 1997;30:997-1001.
- 
- Anker SD, Egerer KR, Volk HD, et al.** *Elevated soluble CD14 receptors and altered cytokines in chronic heart failure.*  
**Am J Cardiol.** 1997;79:1426-1429.
- 
- Anker SD, Volterrani M, Egerer KR, et al.** *TNF- $\alpha$  as predictor of peak leg blood flow in chronic heart failure.*  
**Q J Med.** 1998;91:199-203.
- 
- Aukrust P, Ueland T, Lien E, et al.** *Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy.*  
**Am J Cardiol.** 1999;83:376-382.
- 
- Bachetti T, Comini L, Agnoletti L, et al.** *Attivazione e ruolo del fattore di necrosi tumorale alfa nello scompenso cardiaco congestizio.*  
**Cardiologia.** 1996;41:343-347.
- 
- Balligand JL, Ungureanu D, Kelly RA, et al.** *Abnormal contractile function due to induction of nitric oxide synthesis in rat cardiac myocytes follows exposure to activated macrophage-conditioned medium.*  
**J Clin Invest.** 1993;91:2314-2319.
- 
- Bozkurt B, Kribbs S, Clubb FJ Jr, et al.** *Pathophysiologically relevant concentrations of tumor necrosis factor- $\alpha$  promote progressive left ventricular dysfunction and remodeling in rats.*  
**Circulation.** 1998;97:1382-1391.
- 
- Bozkurt B, Shan K, Seta Y, Oral H, Mann DL.** *Tumor necrosis factor- $\alpha$  and tumor necrosis factor receptors in human heart failure.*  
**Heart Fail Rev.** 1996;1:211-219.
- 
- Cain BS, Meldrum DR, Dinarello CA, et al.** *Tumor necrosis factor-alpha and interleukin-1 beta synergistically depress human myocardial function.*  
**Crit Care Med.** 1999;27:1309-1318.
- 
- Cecconi C, Curello S, Bachetti T, Corti A, Ferrari R.** *Tumor necrosis factor in congestive heart failure: a mechanism of disease for the new millennium?*  
**Prog Cardiovasc Dis.** 1998;41(suppl 1):25-30.
- 
- Chandrasekar B, Colston JT, Freeman GL.** *Induction of proinflammatory cytokine and antioxidant enzyme gene expression following brief myocardial ischaemia.*  
**Clin Exp Immunol.** 1997;108:346-351.
-

**Bibliography of One Hundred Key Papers**

---

- Colucci WS.** *Molecular and cellular mechanisms of myocardial failure.*  
**Am J Cardiol.** 1997;80:15L-25L.
- 
- Deng MC, Erren M, Kammerling L, et al.** *The relation of interleukin-6, tumor necrosis factor-alpha, IL-2, and IL-2 receptor levels to cellular rejection, allograft dysfunction, and clinical events early after cardiac transplantation.*  
**Transplantation.** 1995;60:1118-1124.
- 
- Deswal A, Bozkurt B, Seta Y, et al.** *A phase I trial of tumor necrosis factor receptor (p75) fusion protein (TNFR:Fc) in patients with advanced heart failure.*  
**Circulation.** 1999;99:3224-3226.
- 
- Dibbs Z, Thornby J, White BG, Mann DL.** *Natural variability of circulating levels of cytokines and cytokine receptors in patients with heart failure: implications for clinical trials.*  
**J Am Coll Cardiol.** 1999;33:1935-1942.
- 
- Ferrari R, Bachetti T, Confortini R, et al.** *Tumor necrosis factor soluble receptors in patients with various degrees of congestive failure.*  
**Circulation.** 1995;92:1479-1486.
- 
- Ferrari R, Corti A, Bachetti T.** *Tumor necrosis factor alpha in heart failure.*  
**Heart Fail.** 1995;(August/September):142-148.
- 
- Finkel MS.** *Effects of proinflammatory cytokines on the contractility of mammalian heart.*  
**Heart Fail Rev.** 1996;1:203-210.
- 
- Finkel MS, Hoffman RA, Shen L, Oddis CV, Simmons RL, Hattler BG.** *Interleukin-6 (IL-6) as a mediator of stunned myocardium.*  
**Am J Cardiol.** 1993;71:1231-1232.
- 
- Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL.** *Negative inotropic effects of cytokines on the heart mediated by nitric oxide.*  
**Science.** 1992;257:387-389.
- 
- Frangogiannis NG, Lindsey ML, Michael LH, et al.** *Resident cardiac mast cells degranulate and release preformed TNF-alpha, initiating the cytokine cascade in experimental canine myocardial ischemia/reperfusion.*  
**Circulation.** 1998;98:699-710.
- 
- Givertz MM, Colucci WS.** *New targets for heart-failure therapy: endothelin, inflammatory cytokines, and oxidative stress.*  
**Lancet.** 1998;352(suppl 1):S134-S138.
- 
- Gulick T, Chung MK, Pieper SJ, Lange LG, Schreiner GF.** *Interleukin 1 and tumor necrosis factor inhibit cardiac myocyte beta-adrenergic responsiveness.*  
**Proc Natl Acad Sci USA.** 1989;86:6753-6757.
- 
- Gwechenberger M, Mendoza LH, Youker KA, et al.** *Cardiac myocytes produce interleukin-6 in culture and in viable border zone of reperfused infarctions.*  
**Circulation.** 1999;99:546-551.
- 
- Han RO, Ray PE, Baughman KL, Feldman AM.** *Detection of interleukin and interleukin-receptor mRNA in human heart by polymerase chain reaction.*  
**Biochem Biophys Res Commun.** 1991;181:520-523.
-



- 
- Hasper D, Hummel M, Docke WD, et al.** *Inflammatory mediators in patients with biventricular assist device systems.*  
**Z Kardiol.** 1996;85:820-827.
- 
- Hattler BG, Zeevi A, Oddis CV, Finkel MS.** *Cytokine induction during cardiac surgery: Analysis of TNF- $\alpha$  expression pre- and postcardiopulmonary bypass.*  
**J Card Surg.** 1995;10:418-422.
- 
- Hirota H, Chen J, Betz UA, et al.** *Loss of a gp130 cardiac muscle cell survival pathway is a critical event in the onset of heart failure during biomechanical stress.*  
**Cell.** 1999;97:189-198.
- 
- Hirota H, Yoshida K, Kishimoto T, Taga T.** *Continuous activation of gp130, a signal-transducing receptor component for interleukin 6-related cytokines, causes myocardial hypertrophy in mice.*  
**Proc Natl Acad Sci USA.** 1995;92:4862-4866.
- 
- Iwasaki A, Matsumori A, Yamada T, Shioi T, Wang W, Ono K, et al.** *Pimobendan inhibits the production of proinflammatory cytokines and gene expression of inducible nitric oxide synthase in a murine model of viral myocarditis.*  
**J Am Coll Cardiol.** 1999;33:1400-1407.
- 
- Kapadia SR.** *Cytokines and heart failure.*  
**Cardiol Rev.** 1999;7:196-206.
- 
- Kapadia S, Dibbs Z, Kurrelmeyer K, et al.** *The role of cytokines in the failing human heart. In: Crawford M, ed. Cardiology Clinics.* Philadelphia, Pa: W.B. Saunders; 1998:645-656.
- 
- Kapadia S, Lee JR, Torre-Amione G, Birdsall HH, Ma TS, Mann DL.** *Tumor necrosis factor gene and protein expression in adult feline myocardium after endotoxin administration.*  
**J Clin Invest.** 1995;96:1042-1052.
- 
- Kapadia S, Oral H, Lee J, Nakano M, Taffet GE, Mann DL.** *Hemodynamic regulation of tumor necrosis factor- $\alpha$  gene and protein expression in adult feline myocardium.*  
**Circ Res.** 1997;81:187-195.
- 
- Kapadia S, Torre-Amione G, Mann DL.** *Pitfalls in measuring cytokines.*  
**Ann Intern Med.** 1994;121:149-150.
- 
- Kapadia S, Torre-Amione G, Yokoyama T, Mann DL.** *Soluble tumor necrosis factor binding proteins modulate the negative inotropic effects of TNF- $\alpha$  in vitro.*  
**Am J Physiol.** 1995;37:H517-H525.
- 
- Katz SD, Rao R, Berman JW, et al.** *Pathophysiological correlates of increased serum tumor necrosis factor in patients with congestive heart failure: relation to nitric oxide-dependent vasodilation in the forearm circulation.*  
**Circulation.** 1994; 90:12-16.
- 
- Kelly RA, Ballingand JL, Smith TW.** *Nitric oxide and cardiac function.*  
**Circ Res.** 1996;79:363-380.
- 
- Kelly RA, Smith TA.** *Cytokines and cardiac contractile function.*  
**Circulation.** 1997; 95:778-781.
- 
- Kishimoto T, Akira S, Narazaki M, Taga T.** *Interleukin-6 family of cytokines and gp130.*  
**J Am Soc Hematology.** 1995;86:1243-1254.
-

**Bibliography of One Hundred Key Papers**

---

- Koller-Strametz J, Pacher R, Frey B, Kos T, Woloszczuk W, Stanek B.** *Circulating tumor necrosis factor-alpha levels in chronic heart failure: relation to its soluble receptor II, interleukin-6, and neurohumoral variables.* **J Heart Lung Transplant.** 1998;17:356-362.
- 
- Kubota T, Bounoutas GS, Miyagishima M, et al.** *Soluble tumor necrosis factor receptor abrogates myocardial inflammation, but not hypertrophy in cytokine-induced cardiomyopathy.* **Circulation.** 2000;101:2518-2525.
- 
- Kubota T, McNamara DM, Wang JJ, et al.** *Effects of tumor necrosis factor gene polymorphisms on patients with congestive heart failure.* **Circulation.** 1998;97:2499-2501.
- 
- Kubota T, McTiernan CF, Frye CS, Demetris AJ, Feldman AM.** *Cardiac-specific overexpression of tumor necrosis factor-alpha causes lethal myocarditis in transgenic mice.* **J Card Fail.** 1997;3:117-124.
- 
- Kubota T, McTiernan CF, Frye CS, et al.** *Dilated cardiomyopathy in transgenic mice with cardiac specific overexpression of tumor necrosis factor-alpha.* **Circ Res.** 1997;81:627-635.
- 
- Levine B, Kalman J, Mayer L, Fillit HM, Packer M.** *Elevated circulating levels of tumor necrosis factor in severe chronic heart failure.* **N Engl J Med.** 1990;223:236-241.
- 
- Li YY, Feldman AM, Sun Y, McTiernan CF.** *Differential expression of tissue inhibitors of metalloproteinases in the failing human heart.* **Circulation.** 1998;98:1728-1734.
- 
- Li YY, McTiernan CF, Feldman AM.** *Proinflammatory cytokines regulate tissue inhibitors of metalloproteinases and disintegrin metalloproteinase in cardiac cells.* **Cardiovasc Res.** 1999;42:162-172.
- 
- MacGowan GA, Kormos RL, McNamara DM, Alvarez RJ, Rosenblum WD, Pham S, et al.** *Predicting short-term outcome in severely ill heart failure patients: implications regarding listing for urgent cardiac transplantation and patient selection for temporary ventricular assist device support.* **J Card Fail.** 1998;4:169-175.
- 
- MacGowan GA, Mann DL, Kormas RL, Feldman AM, Murali S.** *Circulating interleukin-6 in severe heart failure.* **Am J Cardiol.** 1997;79:1128-1131.
- 
- Mann DL.** *Stress activated cytokines and the heart.* **Cytokine Growth Factor Rev.** 1996;7:341-354.
- 
- Mann DL.** *Cytokines as mediators of disease progression in the failing heart. In: Hosenpud JD, Greenberg BH, eds. Congestive Heart Failure.* Philadelphia, Pa: Lippincott-Williams & Wilkins; 1999:213-232.
- 
- Mann DL.** *Inflammatory mediators in heart failure: homogeneity through heterogeneity.* **Lancet.** 1999; 353:1812-1813.
- 
- Mann DL, Lee-Jackson D, Yokoyama T.** *Tumor necrosis factor-alpha and cardiac remodeling.* **Heart Fail.** 1995;(September/October):166-175.
-



- Mann DL, Young JB.** *Basic mechanisms in congestive heart failure: recognizing the role of proinflammatory cytokines.*  
**Chest.** 1994;105:897-904.
- 
- Matsui S, Matsumori A, Matoba Y, Uchida A, Sasayama S.** *Treatment of virus-induced myocardial injury with a novel immunomodulating agent, vesnarinone.*  
**J Clin Invest.** 1994;94:1212-1217.
- 
- Matsumori A, Ono K, Nishio R, Nose Y, Sasayama S.** *Amiodarone inhibits production of tumor necrosis factor- $\alpha$  by human mononuclear cells. A possible mechanism for its effect in heart failure.*  
**Circulation.** 1997;96:1386-1389.
- 
- Matsumori A, Shioi T, Yamada T, Matsui S, Sasayama S.** *Vesnarinone, a new inotropic agent, inhibits cytokine production by stimulated human blood from patients with heart failure.*  
**Circulation.** 1994;89:955-958.
- 
- Matsumori A, Yamada T, Kawai C.** *Immunomodulating therapy in viral myocarditis: effects of tumour necrosis factor, interleukin 2 and anti-interleukin-2 receptor antibody in an animal model.*  
**Eur Heart J.** 1991;12:203-205.
- 
- Matsumori A, Yamada T, Matoba Y, Sasayama S.** *Tumor necrosis factor in cardiomyopathy and myocarditis.*  
**Circulation.** 1992;86(suppl I):1439. Abstract.
- 
- Matsumori A, Yamada T, Suzuki H, Matoba Y, Sasayama S.** *Increased circulating cytokines in patients with myocarditis and cardiomyopathy.*  
**Hypertension.** 1989;13:706-711.
- 
- McTiernan CF, Lemster BH, Frye C, Brooks S, Combes A, Feldman AM.** *Interleukin-1 $\beta$  inhibits phospholamban gene expression in cultured cardiomyocytes.*  
**Circ Res.** 1997;81:493-503.
- 
- Meldrum DR.** *Tumor necrosis factor in the heart.*  
**Am J Physiol.** 1998;274:R577-R595.
- 
- Meldrum DR, Cain BS, Cleveland JC Jr, et al.** *Adenosine decreases post-ischemic cardiac TNF- $\alpha$  production: anti-inflammatory implications for preconditioning and transplantation.*  
**Immunology.** 1997;92:472-477.
- 
- Muller-Werdan U, Engelmann H, Werdan K.** *Cardiodepression by tumor necrosis factor-alpha.*  
**Eur Cytokine Netw.** 1998;9:689-691.
- 
- Muller-Werdan U, Schumann H, Loppnow H, et al.** *Endotoxin and tumor necrosis factor alpha exert a similar proinflammatory effect in neonatal rat cardiomyocytes, but have different cardiodepressant profiles.*  
**J Mol Cell Cardiol.** 1998;30:1027-1036.
- 
- Munger MA, Johnson B, Amber IJ, Callahan KS, Gilbert EM.** *Circulating concentrations of proinflammatory cytokines in mild or moderate heart failure secondary to ischemic or idiopathic dilated cardiomyopathy.*  
**Am J Cardiol.** 1996;77:723-727.
- 
- Nakano M, Knowlton AA, Dibbs Z, Mann DL.** *Tumor necrosis factor- $\alpha$  confers resistance to injury induced by hypoxic injury in the adult mammalian cardiac myocyte.*  
**Circulation.** 1998;97:1392-1400.
- 
- Nakano M, Knowlton AA, Yokoyama T, Lesslauer W.** *Tumor necrosis factor- $\alpha$  induced expression of heat shock protein 72*

**Bibliography of One Hundred Key Papers**

---

- Mann DL.** *in adult feline cardiac myocytes.*  
**Am J Physiol.** 1996;270:H1231-H1239.
- 
- Niebauer J, Pflaum CD, Clark AL, et al.** *Deficient insulin-like growth factor I in chronic heart failure predicts altered body composition, anabolic deficiency, cytokine and neurohormonal activation.*  
**J Am Coll Cardiol.** 1998;32:393-397.
- 
- Niebauer J, Volk HD, Kemp M, et al.** *Endotoxin and immune activation in chronic heart failure: a prospective cohort study.*  
**Lancet.** 1999;353:1838-1842.
- 
- Oddis CV, Finkel MS.** *Cytokine-stimulated nitric oxide production inhibits mitochondrial activity in cardiac myocytes.*  
**Biochem Biophys Res Commun.** 1995;213:1002-1009.
- 
- Oddis CV, Simmons RL, Hattler BG, Finkel MS.** *Chronotropic effects of cytokines and the nitric oxide synthase inhibitor, L-NMMA, on cardiac myocytes.*  
**Biochem Biophys Res Commun.** 1994;205:992-997.
- 
- Oddis CV, Simmons RL, Hattler BG, Finkel MS.** *cAMP enhances inducible nitric oxide synthase mRNA stability in cardiac myocytes.*  
**Am J Physiol.** 1995;269:H2044-H2050.
- 
- Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S.** *Cytokine gene expression after myocardial infarction in rat hearts: possible implication in left ventricular remodeling.*  
**Circulation.** 1998;98:149-156.
- 
- Oral H, Dorn GW, II, Mann DL.** *Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor- $\alpha$  in the adult mammalian cardiac myocyte.*  
**J Biol Chem.** 1997;272:4836-4842.
- 
- Oral H, Kapadia S, Nakano M, et al.** *Tumor necrosis factor- $\alpha$  and the failing human heart.*  
**Clin Cardiol.** 1995;18(suppl IV):IV20-IV27.
- 
- Packer M.** *Is tumor necrosis factor an important neurohormonal mechanism in chronic heart failure?*  
**Circulation.** 1995;92:1379-1382.
- 
- Paulus WJ.** *How are cytokines activated in heart failure?*  
**Eur J Heart Fail.** 1999;1:309-312.
- 
- Pulkki KJ.** *Cytokines and cardiomyocyte death.*  
**Ann Med.** 1997;29:339-343.
- 
- Sato Y, Matsumori A, Sasayama S.** *Inotropic agent vesnarinone inhibits cytokine production and E-selectin expression in human umbilical vein endothelial cells.*  
**J Mol Cell Cardiol.** 1995;27:2265-2273.
- 
- Schreiner GF.** *Immune modulation of cardiac cell function.*  
**Trans Am Clin Climatol Assoc.** 1998;109:39-49; discussion 49-50.
- 
- Seta Y, Shan K, Bozkurt B, Oral H, Mann DL.** *Basic mechanisms in heart failure: the cytokine hypothesis.*



- 
- J Card Fail.* 1996;2:243-249.
- 
- Shan K, Kurrelmeyer K, Seta Y, et al.** *The role of cytokines in disease progression in heart failure.*  
*Curr Opin Cardiol.* 1997;12:218-223.
- 
- Sheng Z, Knowlton K, Chen J, Hoshijima M, Brown JH, Chien KR.** *Cardiotrophin 1 (CT-1) inhibition of cardiac myocyte apoptosis via a mitogen-activated protein kinase-dependent pathway.*  
*J Biol Chem.* 1997;272:5783-5791.
- 
- Shimokawa H, Kuroiwa-Matsumoto M, Takeshita A.** *Cytokine generation capacities of monocytes are reduced in patients with severe heart failure.*  
*Am Heart J.* 1998;136:991-1002.
- 
- Shioi T, Matsumori A, Sasayama S.** *Persistent expression of cytokine in the chronic stage of viral myocarditis in mice.*  
*Circulation.* 1996;94:2930-2937.
- 
- Sliwa K, Skudicky D, Candy G, Wisenbaugh T, Sareli P.** *Randomized investigation of effects of pentoxifylline on left ventricular performance in idiopathic dilated cardiomyopathy.*  
*Lancet.* 1998;351:1091-1093.
- 
- Testa M, Yeh M, Lee P, et al.** *Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension.*  
*J Am Coll Cardiol.* 1996;28:964-971.
- 
- Torre-Amione G, Kapadia S, Benedict CR, Oral H, Young JB, Mann DL.** *Proinflammatory cytokine levels in patients with depressed left left ventricular ejection fraction: a report from the studies of left ventricular dysfunction (SOLVD).*  
*J Am Coll Cardiol.* 1996;27:1201-1206.
- 
- Torre-Amione G, Kapadia S, Lee J, Bies RD, Lebovitz R, Mann DL.** *Expression and functional significance of tumor necrosis factor receptors in human myocardium.*  
*Circulation.* 1995;92:1487-1493.
- 
- Torre-Amione G, Kapadia S, Lee J, et al.** *Tumor necrosis factor- $\alpha$  and tumor necrosis factor receptors in the failing human heart.*  
*Circulation.* 1996;93:704-711.
- 
- Torre-Amione G, MacLellan WR, Kapadia S, et al.** *Tumor necrosis factor- $\alpha$  is persistently expressed in cardiac allografts in the absence of histological or clinical evidence of rejection.*  
*Transplant Proc.* 1998;30:875-877.
- 
- Torre-Amione G, Stetson SJ, Youker KA, et al.** *Decreased expression of tumor necrosis factor-alpha in failing human myocardium after mechanical circulatory support: a potential mechanism for cardiac recovery.*  
*Circulation.* 1999;100:1189-1193.
- 
- Tsutamoto T, Hisanaga T, Wada A, et al.** *Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure.*  
*J Am Coll Cardiol.* 1998;31:391-398.
- 
- Turner D, Grant SC, Yonan N, et al.** *Cytokine gene polymorphism and heart transplant rejection.*

**Bibliography of One Hundred Key Papers**

---

- Ungunreanu-Longrois D, Balligand JL, Okada I, et al.** *Contractile responsiveness of ventricular myocytes to isoproterenol is regulated by induction of nitric oxide synthase activity in cardiac microvascular endothelial cells in heterotypic primary culture.* **Circ Res.** 1995;77:486-493.
- 
- Vanderheyden M, Kersschot E, Paulus WJ.** *Pro-inflammatory cytokines and endothelium-dependent vasodilation in the forearm. Serial assessment in patients with congestive heart failure.* **Eur Heart J.** 1998;19:747-752.
- 
- Wagner DR, Combes A, McTiernan CF, Sanders VJ, Lemster B, Feldman AM.** *Adenosine inhibits lipopolysaccharide-induced cardiac expression of tumor necrosis factor- $\alpha$ .* **Circ Res.** 1998;82:47-56.
- 
- Wagner DR, Kubota T, Sanders VJ, McTiernan CF, Feldman AM.** *Differential regulation of cardiac expression of IL-6 and TNF- $\alpha$  by A<sub>2</sub>- and A<sub>3</sub>-adenosine receptors.* **Am J Physiol.** 1999;276(6 pt 2):H2141-H2147.
- 
- Wagner DR, McTiernan CF, Sanders VJ, Feldman AM.** *Adenosine inhibits lipopolysaccharide-induced secretion of tumor necrosis factor- $\alpha$  in the failing human heart.* **Circulation.** 1998;97:521-524.
- 
- Wollert KC, Taga T, Saito M, et al.** *Cardiotrophin-1 activates a distinct form of cardiac muscle cell hypertrophy.* **J Biol Chem.** 1996;271:9535-9545.
- 
- Yamauchi-Takahara K, Ihara Y, Ogata A, Yoshizaki K, Azuma J, Kishimoto T.** *Hypoxic stress induces cardiac myocyte-derived interleukin-6.* **Circulation.** 1995;91:1520-1524.
- 
- Yokoyama T, Nakano M, Bednarczyk JL, McIntyre BW, Entman ML, Mann DL.** *Tumor necrosis factor- $\alpha$  provokes a hypertrophic growth response in adult cardiac myocytes.* **Circulation.** 1997;95:1247-1252.
- 
- Yokoyama T, Vaca L, Rossen RD, Durante W, Hazarika P, Mann DL.** *Cellular basis for the negative inotropic effects of tumor necrosis factor- $\alpha$  in the adult mammalian heart.* **J Clin Invest.** 1993;92:2303-2312.
- 
- Yoshida K, Taga T, Saito M, et al.** *Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders.* **Proc Natl Acad Sci USA.** 1996;93:407-411.
- 
- Yue P, Massie BM, Simpson PC, Long CS.** *Cytokine expression increases in nonmyocytes from rats with postinfarction heart failure.* **Am J Physiol.** 1998;275:H250-H258.
-