Activation of cytokines as a mechanism of disease progression in heart failure

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The scientific quest for the basic mechanism(s) responsible for the development and progression of congestive heart failure in humans has been practically exhaustive; none the less, the mechanisms responsible for the decompensation of myocardial function after myocardial injury or haemodynamic overloading, or both, have remained elusive. Initially clinicians viewed heart failure as a problem of excessive salt and water retention that was caused by abnormalities of renal blood flow (the cardio-renal model). As clinicians began to perform careful haemodynamic measurements, it also became apparent that heart failure was accompanied by reduced cardiac output and excessive peripheral vasoconstriction. This led to the development of a cardiocirculatory or haemodynamic model for heart failure, in which heart failure was thought to arise from abnormalities of the pumping capacity of the heart. Neither of these models explained the progression of heart failure. Subsequently neurohormones were considered to lead to disease progression through myocyte loss, progressive myocardial fibrosis, as well as salt and water retention. More recently, it has become evident that another class of biologically active molecules, generally referred to as cytokines are also important in heart failure.¹⁻⁸

General background of cytokines

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. Classically, cytokines are thought to be secreted by neighbouring “producer cells” and to act in an autocrine, juxtacrine or paracrine manner to influence the biological behaviour of neighbouring “target cells” (see Mann and Young⁸ for a brief review). However, when cytokines are overproduced they can “spill over” into the peripheral circulation where they can exert endocrine-like effects. It should be recognised that although cytokines are similar in many respects to polypeptide hormones, cytokines can be produced by a variety of different cell types in a number of different tissues, in contrast with being produced by a specific cell type in a specific organ, as is the case for polypeptide hormones. Thus far two major classes of cytokines have been identified in heart failure: vasoconstrictor cytokines, such as endothelin, and vasodepressor proinflammatory cytokine, such as tumour necrosis factor (TNF) and interleukin 6 (IL6). The group of cytokines that are responsible both for initiating the primary host response to a bacterial infection, as well as initiating the repair of tissue after tissue

Table 1 The potential untoward effects of TNF in heart failure

- Produces left ventricular dysfunction in humans²¹
- Produces pulmonary oedema in humans²²
- Produces cardiomyopathy in humans²³
- Promotes left ventricular remodelling experimentally²⁴
- Produces abnormalities in myocardial metabolism experimentally²⁵
- Produces anorexia and cachexia experimentally²⁶
- Produces β -receptor uncoupling from adenylate cyclase experimentally²⁷
- Produces abnormalities of mitochondrial energetics²⁸
- Activation of fetal gene programme experimentally²⁹

injury have been termed “proinflammatory cytokines.”

Effects of cytokines on the heart

Many aspects of heart failure can be explained by the known biological effects of stress activated proinflammatory cytokines. When expressed at sufficiently high concentration, cytokines can mimic some aspects of heart failure phenotype including but not limited to progressive left ventricular dysfunction, pulmonary oedema, LV remodelling, fetal gene expression, and cardiomyopathy (table 1). The current literature suggests that TNF produces both immediate and delayed negative inotropic effect on myocardial contractility.

Thus the elaboration of cytokines may represent much like neurohormones, a biological mechanism that is responsible for producing symptoms in patients with heart failure.

Cytokines are thought to exert their effects by binding to specific receptors on the surface of the cell including the adult cardiac myocyte. In the case of TNF, this protein is known to bind to two types of TNF receptors: TNFR1 (p 55) and TNFR2 (p 75) receptor. Recent studies have shown that the adult human cardiac myocytes express both types of TNF receptors, and that the type 1 receptor is responsible for mediating the negative inotropic effects of TNF.^{9,17} Studies have also shown that when both TNF receptors are proteolytically cleaved from the cell membrane, 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” that are capable of rapidly neutralising the highly cytotoxic activities of TNF.¹³

Considerable interest in the potential role of IL6 in the heart has been generated by the observation that increased levels of IL6 are expressed in humans following acute myocardial infarction.¹⁴ The human IL6 receptor is a

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Table 2 Cytokines and cytokine receptors in heart failure

	Cytokines					Cytokine receptors		
	TNF	IL1	IL2	IL6	IFN γ	STNFR1	STNFR2	IL1RA
Levine ¹	+	nd	nd	nd	nd	nd	nd	nd
McMurray ²	+	nd	nd	nd	nd	nd	nd	nd
Dutka ³	+	nd	nd	nd	nd	nd	nd	nd
Wiederman ³⁰	+	—	nd	+	—	nd	nd	nd
Katz ⁴	+	—	+	nd	nd	nd	nd	nd
Matsumori ⁵	+	—	—	—	—	nd	nd	nd
Ferrari ³¹	+	nd	nd	nd	nd	+	+	nd
Pritchett ³²	—	nd	nd	nd	nd	nd	nd	nd
Torre ¹⁷	+	nd	nd	nd	nd	+	+	nd
Torre ⁷	+	nd	nd	+	nd	nd	nd	nd
Milani ³³	+	nd	nd	nd	nd	+	nd	+
Munger ³⁴	—	—	nd	+	nd	nd	nd	nd
Testa ³⁵	+	+	—	+	nd	nd	+	+
Anker ³⁶	+	nd	nd	nd	nd	nd	nd	nd
McGowan ³⁷	+	nd	nd	+	nd	nd	nd	nd
Mohler ³⁸	+	nd	nd	+	nd	nd	nd	nd
Nishigaki ³⁹	+	nd	nd	+	nd	nd	nd	nd
Anker ⁴⁰	+	nd	nd	nd	nd	+	+	nd

nd: not done, +: levels increased, —: levels not increased.

glycoprotein consisting of two functional chains: a 80 kDa IL6 binding protein, termed IL6R, and a 130 kDa “docking protein,” termed gp 130, which transmits the intracellular signal.¹⁰ Specific information on the presence or absence of the IL6 receptor in the heart is not yet available. Genetic mice that are deficient in gp130 are embryonic lethal because their hearts do not develop. For a more detailed discussion of the biology of these molecules and their receptors, the interested reader is referred to a recent review article on proinflammatory cytokines and the heart.⁹

Cytokines and heart failure

SITES AND SOURCES

Following the original description of increased cytokine levels in heart failure¹; there was speculation that activation of the immune system was responsible for the increased levels of cytokines in heart failure. Monocytes have traditionally been held as a major source of cytokine production. Neopterin levels, which are markers for cytokine production, are increased in patients with heart failure. However, it is important to recognise that TNF itself can stimulate monocytes to produce neopterin, accordingly, it is unclear whether increased neopterin levels represent primary activation of mononuclear cells with secondary production of TNF or whether TNF stimulates the mononuclear cell that subsequently releases neopterin.³⁰ The immune system being the sole source of cytokines was challenged by the observation that the heart is capable of producing TNF, IL1 and IL6. Haemodynamic overloading or myocardial stretch provokes de novo TNF mRNA and protein synthesis experimentally.¹⁶ Interestingly, neither TNF mRNA nor protein were expressed in myocytes subjected to normal haemodynamic loading conditions. Thus, conditions that are known to eventuate the development of heart failure also lead to TNF overproduction. The clinical importance of these experimental findings is further substantiated by the observation that TNF mRNA and protein are present in failing hearts whereas not detectable in non-failing human hearts. These data suggest that the proinflammatory cytokines are elaborated by

the heart in certain forms of stress, and that increased levels of TNF may represent spill over of cytokines that were produced within the myocardium. An intriguing possibility is that there may be “cross-talk” between cardiovascular and the immune systems. Other hypotheses are that a decreased cardiac output in the heart failure leads to elaboration of TNF by underperfused metabolic tissues or that increased levels of TNF in heart failure may result from altered distribution, degradation, or clearance of these molecules from plasma.

WHICH CYTOKINES ARE INCREASED IN HEART FAILURE?

Table 2 provides a summary of the studies that examined circulating cytokine levels in patients with symptomatic heart failure.

A number of studies consistently found increased levels of TNF in congestive heart failure, comparatively less is known about circulating levels of IL1, IL2, IL6 and IFN γ . Moreover, several studies suggest that there is an increasing cytokine production in direct relation to severity of disease.^{7 31 35 37} There is progressive increase in TNF levels in relation to deteriorating NYHA functional class. Moreover, there is relation to increased mortality with increasing levels of TNF in analysis of cytokine levels in SOLVD database⁷ and VEST (unpublished data). Similar to increased levels of neurohormones, TNF levels may be predictive of NYHA class and clinical outcome.

Most studies have found increased levels of IL6 in heart failure although this has not been true for all studies. Although the mechanism for increased levels of IL6 in heart failure is not known, it is interesting to note that TNF is sufficient to induce IL6 gene and protein expression in a variety of cell types¹⁰ suggesting there may be a cytokine cascade in the setting of heart failure.³⁷

At the time of writing, there is little clinical evidence that supports an important role for IL1, IL2 or IFN γ in heart failure. Thus in summary, on the basis of clinical material extant at this time, the preponderance of clinical data suggest that both TNF and IL6 constitute the major portfolio of stress activated cytokines in heart failure.

WHICH CYTOKINE RECEPTORS ARE INCREASED IN HEART FAILURE?

There are increased circulating levels of cytokine receptors and cytokine receptor antagonists (RA) in heart failure, including sTNFR1, sTNFR2, IL1RA and IL6R. Thus far two reports have demonstrated increased levels of sTNFR1 and sTNFR2 in patients with heart failure. In the report by Ferrari *et al*, circulating levels of sTNFR2 correlated independently with worse short-term prognosis. Although clinical significance of this is unclear, it has been suggested that sTNFRs may act as a biological reservoir for TNF that stabilises the molecule and slowly releases this cytokine into the circulation.

SUPPRESSION OF CYTOKINE PRODUCTION AS A THERAPEUTIC PARADIGM IN HEART FAILURE

One natural question arises from the above discussion whether modulation of cytokine production or cytokine bioactivity may be a method for treating patients with heart failure. There are several preliminary studies that may answer this question.

Parillo and colleagues⁴¹ randomly assigned 102 patients to either treatment with prednisone or placebo. After three months of treatment, they observed an increase in ejection fraction of >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% in the prednisone group, as compared with 2.1% in the control group ($p=0.054$). The patients were then further categorised respectively in two separately randomised subgroups: "reactive" patients, who had fibroblastic or lymphocytic infiltration or immunoglobulin deposition on endomyocardial biopsy, a positive gallium scan, or an increased erythrocyte sedimentation rate; or "non-reactive" patients, who had none of these features. At three 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, non-reactive patients did not improve with prednisone. Although specific cytokine levels were not measured in this study, their data suggest that patients with idiopathic dilated cardiomyopathy may have some improvement given a high dose of prednisone daily. This early study raises the possibility that suppression of cytokine production may be used as a therapeutic tool in treating patients with heart failure.

Another potentially important pharmacological method for suppressing cytokine production is through the use of agents that increase cAMP levels, such as dobutamine, which as noted above, will suppress TNF production.⁴² It is therefore tempting to speculate that one of the mechanisms for the sustained benefit of intravenous infusions of dobutamine may be through suppression of proinflammatory cytokines such as TNF. However, this point of view is not supported by a recent study, in which it was shown that treatment with either intravenous dobutamine or milrinone had no effect in terms of decreasing circulating TNF levels.³³

Mohler and colleagues³⁸ examined the effects of amlodipine on circulating levels of TNF and IL6 in a subset analysis of patients enrolled in the PRAISE trial. They observed that although treatment with amlodipine had no effect on TNF levels, there was a statistically significant decrease in IL6 levels after 24 weeks of treatment.

THERAPEUTIC EFFECT OF SOLUBLE TNF RECEPTOR IN HEART FAILURE PATIENTS

In a recent report using a soluble TNF receptor (etanercept, p75) that neutralises the biological effect of circulating TNF, Deswal *et al* showed that there was an improvement in

functional status and quality of life in 12 patients with advanced heart failure.⁴³

Subsequently a second multidose phase I trial was conducted with etanercept, p75 TNF receptor fusion protein in 47 patients with NYHA class III-IV heart failure and EF < 35% at three sites. This was a double blind, placebo controlled and randomised study, using placebo or etanercept 5 or 12 mg/m² as subcutaneous injections twice a week for three months. The primary objective was safety; secondary objectives were NYHA functional class, LVEF by MUGA, quality of life and clinical composite score. In this study etanercept was well tolerated, improvement in LVEF, quality of life, and clinical composite scores were greatest in the 12 mg/m² group.⁴⁴ There is also evidence of early reverse remodelling of the left ventricle in heart failure after treatment with etanercept, p 75 TNF receptor fusion protein. After three months of treatment, the LV end diastolic diameter, LV volumes and LV mass seem to decrease in the treated group compared with placebo. This was accompanied by improvement in LVEF and cardiac output measured by echocardiography.⁴⁵

A multicentre randomised phase III study, named Renaissance (Randomized Etanercept North American Strategy to Study Antagonism of Cytokines) is currently being conducted in more than 100 US, European and Australian centres to study the effects of etanercept in approximately 900 patients with advanced heart failure.

Summary

The cytokine hypothesis for heart failure suggests that heart failure progresses because cytokine cascades that are activated after myocardial injury exert deleterious effects on the heart and circulation. It bears emphasis that the cytokine hypothesis does not imply that cytokines cause heart failure in itself, but rather that the overexpression of cytokines contributes to the progression of heart failure once LV dysfunction ensues. The stress activated cytokines can exert autocrine/paracrine effects within the myocardium by binding to specific cytokine receptors. However, if cytokine expression is excessive these molecules may produce LV dysfunction and LV dilatation.

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