Basic science for the clinician

When are pro-inflammatory cytokines SAFE in heart failure?

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The cytokine hypothesis presently suggests that an excessive production of pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF) and interleukin 6 (IL6), contributes to the pathogenesis of heart failure. The concept, successfully proved in genetically modified animal models, failed to translate to humans. Recently, accumulation of apparently paradoxical experimental data demonstrates that, under certain conditions, production of pro-inflammatory cytokines can initiate the activation of a pro-survival cardioprotective signalling pathway. This novel path that involves the activation of a transcription factor, signal transducer and activator of transcription 3 (STAT3), has been termed the survival activating factor enhancement (SAFE) pathway. In this review, we will discuss whether targeting the SAFE pathway may be considered as a preventive and/or therapeutic measure for the treatment of heart failure.

Introduction

In the 1990s, the introduction of the cytokine hypothesis proposed that an excessive production of pro-inflammatory cytokines contributes to heart failure (HF).¹² Despite encouraging animal studies and small clinical trials, larger clinical trials targeting the cytokines in HF have failed. The question as to whether the increased levels of inflammatory cytokines observed with HF are a cause or consequence of the disease still remains unresolved. In the new millennium, data from numerous animal studies have lead to a better understanding of the beneficial vs. deleterious effect of pro-inflammatory cytokines in pathophysiological conditions. Recent experimental work currently suggests that activation of these cytokines, including tumour necrosis factor alpha (TNF) and the interleukin 6 family, can promote a pro-survival signalling pathway termed the SAFE (survivor activating factor enhancement) pathway to protect against myocardial infarction (MI).³⁴ In this review, we will summarize evidence for and against the cytokine hypothesis in both experimental models and clinical conditions and we will discuss whether targeting the SAFE pathway may be considered as a preventive and/or therapeutic measure for the treatment of HF.

Pro- and anti-inflammatory cytokines imbalance in heart failure

Pro-inflammatory cytokines in heart failure

The interest of TNF (also known as cachectin) in HF evolved from the observation that cachexia is a common phenomenon associated with severe HF.⁵ In the 1990s, numerous studies evidenced an increase in circulating TNF, function to the severity and the outcome of HF.⁶,⁷ The production of TNF in HF may originate from the periphery (liver) or the myocardium (see review⁸). In HF patients, cachexia was associated with a further increase in circulating TNF receptors type I and type II (TNFR1 and TNR2), through which TNF is believed to exert its function.⁹ Tumour necrosis factor receptor type I is

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Anti-inflammatory cytokines SAFE in heart failure?

Overall, the production of anti-inflammatory cytokines has been much less studied than the pro-inflammatory cytokines in the human heart. The main anti-inflammatory cytokine studied in HF is interleukin 10 (IL10). Produced in mononuclear cells (essentially macrophages and T cells), IL10 inhibits the production of pro-inflammatory cytokines as well as the matrix metalloproteinases by activated monocytes.\textsuperscript{15} Compared with healthy control subjects, plasma levels of IL10 are decreased in patients with HF, in particular, in patients suffering from advanced HF NYHA class III and IV.\textsuperscript{16}

Circulating thrombospondin-1, another anti-inflammatory cytokine released upon activation of platelets, is also reduced in HF, together with tumour growth factor β1.\textsuperscript{17,18}

The cytokine hypothesis: evidence for and against

Evidence for a contribution of cytokines in the development and pathogenesis of the disease

The adverse effect of pro-inflammatory cytokines in the heart has been widely demonstrated in animal models, using mainly cytokine depleted or overexpressing mouse models. In experimental models, inflammatory cytokines promote left ventricular remodelling, acute reversible contractile dysfunction, and uncouple myocardial β-adrenergic receptors (see review\textsuperscript{19}). Cardiac specific overexpression of TNF causes cardiac failure,\textsuperscript{20} while pathophysiological relevant concentrations of TNF promote progressive left ventricular dysfunction and remodelling in rats.\textsuperscript{21} Interleukin 10 knockout animals exhibit increased mortality and larger infarct size compared with their littermate controls.\textsuperscript{22} All these data suggest that limiting the cytokine imbalance observed in pathophysiological conditions, such as HF may limit the development of the disease and improve its outcome. Such modulation of the cytokine balance in HF can be approached in two ways: (i) by enhancing the level of anti-inflammatory cytokines and (ii) by targeting a decrease in pro-inflammatory cytokines. These two approaches have been considered, to varying degrees, in both experimental models and clinical conditions.

Increase of anti-inflammatory cytokines

The concept of raising the anti-inflammatory cytokines in HF has been poorly studied. In animal studies, treatment with recombinant IL10 limits myocardial lesions in viral myocarditis in rats\textsuperscript{23} and improves left ventricular function in rats with HF after experimental MI.\textsuperscript{24} This effect was associated with a decrease in circulating inflammatory cytokines TNF and IL6 and a reduction in myocardial macrophage infiltration.\textsuperscript{24} Similarly, chronic treatment with atorvastatin increases plasma levels of IL10, decreases the TNF/IL10 ratio, ameliorates left ventricular remodelling, and improves left ventricular function in rats with HF subsequent to MI.\textsuperscript{25} In fact, the simplest and safest way to increase IL10 is with physical exercise. In a rat model of chronic HF, regular swimming improves
plasma levels of IL10 and cardiac function and reduces muscle cellular damage. In human studies, specifically targeting an increase of IL10 in HF has been essentially ignored. Intense treatment with atorvastatin in patients with stable coronary disease reduces subsequent hospitalization for HF. Immunomodulation therapy with intravenous immunoglobulin in patients with chronic HF improved the left ventricular ejection fraction and increased plasma IL10 levels.

A recent Cochrane systematic review and meta-analysis shows that exercise training, known to increase IL10 levels, reduces HF-related hospitalization and results in clinical improvement in health-related quality-of-life. Similarly, the HF-ACTION study demonstrates that participation in an exercise training programme provides improvement in HF patients-reported health status compared with usual care. Targeting the anti-inflammatory cytokines to limit the development of the pathology in HF certainly merits further investigation.

Decrease of pro-inflammatory cytokines

The concept of decreasing the pro-inflammatory cytokines has been more readily explored in both clinical and experimental settings but this has led to conflicting and confusing data.

In a viral myocarditis mouse model, anti-TNF improved survival and myocardial lesions. Tumour necrosis factor neutralization attenuated the impairment of left ventricular function 10 weeks after MI in rats. Similarly, contractile dysfunction was attenuated in TNF R1 knockout mice with MI or with TNF antibodies after microembolization. Conversely, adenoviral transfection of TNFR1 increased contractile dysfunction following MI.

In a small randomized preclinical trial with 18 patients of NYHA class III, a single intravenous infusion of the TNF antagonist Etanercept resulted in a decrease in TNF bioactivity and a significant overall increase in quality-of-life scores. Similarly, pentoxyfilline, a putative TNF inhibitor, was beneficial in small clinical trials with HF patients. Pentoxyfilline therapy of ischaemic cardiomyopathy for 6 months improved the ejection fraction. The benefit with regard to symptoms of HF and cardiac function was seen in all grades of severity of HF (class I–IV) and in patients with ischaemic and idiopathic dilated cardiomyopathy. However, the beneficial effect has not consistently been associated with a reduction in pro-inflammatory cytokines, therefore suggesting that pentoxyfilline, which is a phosphodiesterase inhibitor, may protect independently of an immunomodulatory effect (see review).

Diet enriched with omega-3 polyunsaturated fatty acids (ω-3 PUFA) may decrease pro-inflammatory cytokines in patients with HF but further studies are needed to determine the optimal source and dosing of ω-3 PUFA.

Evidence against a contribution of cytokines in the disease

In contrast to the preclinical trials, large randomized placebo-controlled clinical trials with anti-TNF therapies were disappointing. No beneficial effect was observed with Etanercept in patients with chronic HF (NYHA class II–IV) as reported in RENEWAL, RENAISSANCE, and RECOVER (see review). The ACCLAIM study tested the effect of immunomodulation therapy in patients with chronic HF. The rationale of the study was to have a non-specific but broad immunomodulatory effect by reducing pro-inflammatory cytokines and increasing anti-inflammatory cytokines. The trial did not show any significant reduction in mortality or cardiovascular-related hospitalization. Unfortunately, the levels of cytokine activation were not assessed in the study. Of note, two specific sub-group of patients, those without a previous history of MI and those with NYHA II had significant reduction in their primary endpoint therefore suggesting that this therapy may benefit certain subgroups with HF.

The beneficial effect of several drugs in HF also brings into question the contribution of pro-inflammatory cytokines in the disease. In patients hospitalized with advanced acutely decompensated congestive HF, traditional therapy leads to clinical improvement but no reduction in pro-inflammatory cytokine levels. Similarly, treatment with amiodarone in patients with ischaemic cardiomyopathy is associated with an increase in circulating TNF levels but this increase is not associated with an adverse effect on survival.

Although the severity of HF correlates with an increase in plasma inflammatory cytokines levels in patients, it is important to mention that these levels are much lower compared with inflammatory diseases, such as septic conditions (Table 1). Does this mean that their contribution to the disease, if any, may be relatively modest? Or are there other explanations?

Pro-inflammatory cytokines initiate a cardioprotective signalling cascade in the heart: the survival activating factor enhancement pathway

With the new millennium, a large body of experimental work conducted in various animal models has brought more insights with respect to the disappointment of multiple clinical trials targeting the inflammatory cytokines in HF. In fact, activation of the immune system (with TNF and/or IL6) can promote the activation of an intrinsic cell survival signalling pathway that involves activation of a transcription factor, the signal transducer and activator of transcription 3 (STAT3). This pathway, recently discovered in MI, has been termed the SAFE (survivor activating factor enhancement) pathway.

Tumour necrosis factor and interleukin 6 cytokines can protect against ischaemia-reperfusion injury

As observed in HF, an increase in pro-inflammatory cytokines occurs in patients with MI and circulating IL6, TNF, and their respective receptors are increased further after reperfusion (see review). Both cytokines are thought to contribute to contractile dysfunction, most likely as a result of perturbation in calcium homeostasis and formation of free radicals.

However, as early as 1998, there was experimental data disputing the contribution of TNF or IL6 to the damage associated with MI. Hence, exogenous addition of TNF protects against hypoxic injury in cardiomyocytes and mice lacking both TNFR1 and TNFR2 are more susceptible to MI than their littermate controls.
Similarly, expression of IL6 occurs in the viable border zone of a myocardial infarct.\textsuperscript{55}

In fact, the cardioprotective effect of TNF is influenced by several factors including dose, sex, time, and type of receptors activated. Hence, TNF protects against ischaemia-reperfusion in a dose-dependent manner with small amounts of exogenous TNF (0.5 ng/mL, \textit{in vitro}) given prior to ischaemia-reperfusion enhancing cell survival while higher concentrations (10–20 ng/mL, \textit{in vitro}) are cytotoxic.\textsuperscript{3,56,57} Deficiency of TNFR1 protects the myocardium through IL6 following TNF infusion,\textsuperscript{58} and the activation of the TNFR2 seems to convey the protective effect of TNF.\textsuperscript{59–61}

Both cytokines are also important components in the powerful protective effect of preconditioning, which fails in mice in the absence of either TNF or IL6.\textsuperscript{56,62–65} Tumour necrosis factor can mimic pre- or post-conditioning \textit{in vivo}.\textsuperscript{67,68}

**Signal transducer and activator of transcription 3 is a downstream target of tumour necrosis factor and interleukin 6**

Once TNF and IL6 bind to their specific receptors (TNFR and gp130), a common signalling path, called the Janus kinase (JAK)/STAT3 pathway can be activated (Figure 1). Janus kinases are a family of tyrosine kinases that are associated with the cytoplasmic domain of cytokine and growth factor receptors (including TNFR and gp130) and play a major role in transducing signals from the cytosol to the nucleus (see reviews\textsuperscript{69–71}). Upon activation of the receptors, JAK proteins phosphorylate and create a docking site for STAT proteins, which in turn are activated by phosphorylation (Figure 1). While the mechanisms involved in JAK activation by the gp130 and TNFR1 have been clearly characterized, the activation of JAK 2 by TNFR2 still remains to be elucidated. Tumour necrosis factor receptor II does not contain an intrinsic protein tyrosine kinase but the phosphorylation of JAK2 by this receptor may occur via TNF receptor-associated factor 2 (TRAF2) or nuclear factor-kappa\textit{B} activation, both components previously implicated in TNF-mediated cardioprotection.\textsuperscript{72,73} The STAT family of transcription factor proteins consists of seven identified members: only STAT3 will be considered in this review. Tyrosine phosphorylation of STAT3 enables it to homodimerize and translocate to the nucleus. Both tyrosine and serine phosphorylated STAT3 are also present within the mitochondria.\textsuperscript{74,75} Under physiological conditions, this pathway can be regulated by the suppressor of cytokine signalling (SOCS) proteins that serve as a negative regulator of JAK/STAT signalling. Activation of STAT3 induces the expression of SOCS which in turn binds to the tyrosine 757 residue of membrane receptor which is necessary for the docking of JAK and STAT3 at the receptors to activate the JAK/STAT3 pathway (see reviews\textsuperscript{71,76}).

Activation of the SAFE pathway with TNF/JAK/STAT3 or IL6/JAK/STAT3 signalling is required for the cardioprotective effect of ischaemic pre- and post-conditioning as neither TNF knockout, IL6 knockout or cardiomyocyte STAT3 knockout mice could be protected with a conditioning stimulus.\textsuperscript{62,63,77–79} Activation of

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**Figure 1** Survivor activating factor enhancement (SAFE) pathway activated in the heart. After binding onto their specific receptors, tumour necrosis factor and interleukin 6 family cytokines activate the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signalling path. After phosphorylation, signal transducer and activator of transcription 3 translocates either in the nucleus or in the mitochondria. The activation of the survivor activating factor enhancement pathway is regulated by the activation of the suppressor of cytokine signalling (SOCS). Moderate stimulation of the survivor activating factor enhancement pathway leads to cell survival but intense stimulation is detrimental.
the JAK/STAT3 pathway also occurs with many other cardioprotective agents, such as erythropoietin, cannabinoid agonists, insulin, prostaglandins, and high density lipoproteins (HDL).80,81 Downstream effectors of the SAFE pathway following cytokine and STAT3 activation still need to be defined. Several targets of STAT3 have been identified including proteins that are involved in cell survival and proliferation, such as Bcl-2, Bcl-xL, McI-1, Fas, cyclin D1, E1, p21, some growth factors (VEGF), and other transcription factors (see review).89 In the preconditioning setting, STAT3 plays a critical role by increasing the anti-apoptotic gene Bcl-2 and reducing the pro-apoptotic gene bax.82 Also, STAT3 mediates cardioprotection via the phosphorylation and inactivation of the pro-apoptotic factor Bad.83 In late preconditioning, STAT3 activates superoxide dismutase,83 inducible nitric oxide synthase,82 and cyclooxygenase 2 expression. Similarly, the JAK/STAT pathway protects in opioid-induced cardioprotection via phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3 β).84 Mitochondrial connexion 43, a key element of the signal transduction cascade of the protection by ischaemic preconditioning,85 may also be a downstream target of STAT3.86 By definition, STAT3 is a transcription factor but its effects observed in a setting of ischaemia/reperfusion or classic preconditioning do not occur at a transcriptional level, as the timeframe between the activation of STAT3 and its action is too short to allow an effect at the gene level. This observation suggests that STAT3 has additional effects, such as direct phosphorylation of target molecules. With the recent discovery that STAT3 translocates to the mitochondria, the protective effect of STAT3 in pre- and post-conditioning is likely to be mediated by modulating the opening of the mitochondrial permeability transition pore.75

Tumour necrosis factor can also promote cardioprotection via TRAF2, nuclear factor kappaB, reactive oxygen species, and protein kinase C.4,72,73 With regards to sphingolipids, TNF-induced cardioprotection is mediated via ceramide, but sphingosine-1 phosphate protects via TNF and STAT3.3,87 Whether these intermediates form part of the SAFE pathway or constitute an alternative protective signalling path activated by TNF merits further investigation.

How to activate the survival activating factor enhancement pathway?
The SAFE pathway being recently discovered, few upstream targets have been delineated so far. Initially discovered as a downstream target of ischaemic preconditioning and ischaemic postconditioning54,67,88 (see Figure 2), it is now recognized that various cardioprotective agents can activate both TNF and STAT3, such as bradykinin, adrenoreceptors, leptin, opioids, and cannabinoids.89 Similarly, HDL (whose low levels are associated with poor prognosis in patients with HF)90 or one of its components sphingosine-1 phosphate, confers protection via the activation of TNF and/or STAT3.80,87 Moderate consumption of red wine may also confer cardioprotection via the activation of the SAFE pathway. Hence, resveratrol and two biogenic amines present in red wine (ethanola- mine and melatonin) protect the ischaemic isolated mouse heart against MI but this effect was lost in TNF knockout mice or STAT3 knockout mice.91,92 Surprisingly, adenosine, a well-known preconditioning mimetic does not seem to activate the SAFE pathway, therefore confirming the existence of alternative cardioprotective signalling paths, such as the well-described reperfusion injury salvage kinase pathway or protein kinase G-dependent pathway.63,77,93

**Activation of the survival activating factor enhancement pathway in heart failure: a safe therapy to consider?**

**Evidence for a survival activating factor enhancement therapy in heart failure**

As demonstrated in the previous section, experimental data provide evidence that activation of the SAFE pathway is protective in MI, but what about HF?

In humans, the concept that activation of the SAFE pathway may protect against HF is supported by the alteration in left ventricular STAT3 and gp130 proteins and phosphorylation of JAK observed in patients with dilated cardiomyopathy.14,94 The expression of SOCS is also attenuated in terminally failing human hearts.94 Similarly, women with postpartum cardiomyopathies have a decrease in STAT3 levels compared with healthy women.95 In experimental studies, although cardiomyocyte STAT3 deficient mice do not develop any cardiac abnormalities of function or morphology at a young age,96 abnormalities appear with aging and severe cardiac fibrosis is observed at 6 months (see review).75 Female cardiomyocyte STAT3 deficient mice develop postpartum cardiomyopathy suggesting that activation of STAT3 is essential to protect the maternal heart from postpartum oxidative stress.95 Mice with cardiomyocyte overexpression of STAT3 activate a hypertrophic signal but also a protective signal against doxorubicin-induced cardiomyopathy by inhibiting reduction in cardiac contractile gene expression and inducing cardiac protective factors.97 Similarly, mice with cardiac restriction of gp130 display rapid onset of dilated cardiomyopathy and massive

**Ways to activate the SAFE pathway**

![Figure 2](http://eurheartj.oxfordjournals.org/content/2/1/56.F2)

**Figure 2** Multiple ways of activating the SAFE pathway to protect against myocardial infarction. SAFE, survivor activating factor enhancement; HDL, high density lipoprotein cholesterol.
induction of myocyte apoptosis during aortic pressure overload vs. the control mice that exhibit compensatory hypertrophy. Also, activation of STAT3 by granulocyte colony stimulating factor in dilated cardiomyopathy improves survival and cardiac function. Furthermore, activation of gp130/STAT3 with IL11 attenuates cardiac fibrosis following MI.

Evidence against a survival activating factor enhancement therapy in heart failure

These promising data in favour of a protective effect of the SAFE activation in HF should however be considered with precaution for several reasons. First, as mentioned earlier, TNF can activate two types of receptors, TNFRI and TNFRII. Activation of the SAFE pathway involves the activation of TNFRII only. In experimental ischaemic HF, TNFRI and TNFRII have disparate and opposing effects on remodelling, hypertrophy, inflammation and apoptosis with TNFRII exacerbating, and TNFRII ameliorating these effects. Potential drugs targeting the activation of the SAFE pathway should therefore be specific to TNFRII. Second, continuous gp130-mediated JAK/STAT3 activation obtained by blockage of the tyrosine 757 on the gp130 site (and therefore inef- ficient gp130-mediated JAK/STAT3 activation obtained by the SAFE pathway should therefore be specific to TNFRII. Second, continuous gp130-mediated JAK/STAT3 activation obtained by blockage of the tyrosine 757 on the gp130 site (and therefore inef- ficient gp130-mediated JAK/STAT3 activation obtained by the SAFE pathway should therefore be specific to TNFRII. Second, continuous gp130-mediated JAK/STAT3 activation obtained by blockage of the tyrosine 757 on the gp130 site (and therefore inef- ficient gp130-mediated JAK/STAT3 activation obtained by the SAFE pathway should therefore be specific to TNFRII. Second, continuous gp130-mediated JAK/STAT3 activation obtained by blockage of the tyrosine 757 on the gp130 site (and therefore inef- ficient gp130-mediated JAK/STAT3 activation obtained by the SAFE pathway should therefore be specific to TNFRII. Second, continuous gp130-mediated JAK/STAT3 activation obtained by blockage of the tyrosine 757 on the gp130 site (and therefore inef- ficient gp130-mediated JAK/STAT3 activation obtained by the SAFE pathway should therefore be specific to TNFRII. Second, continuous gp130-mediated JAK/STAT3 activation obtained by blockage of the tyrosine 757 on the gp130 site (and therefore inef- ficient gp130-mediated JAK/STAT3 activation obtained bylerendising these effects. These data suggest that an overstimulation of the SAFE pathway would lead to an adverse outcome. Third, the putative beneficial role of cytokines/STAT3 in ischaemic HF may not translate in non-ischaemic HF. The kinetics of production of cytokines varies as a function of the aetiology of HF with, for instance, gp130 increased to a greater extent in patients with dilated cardiomyopathy than ishae- mic cardiomyopathy. Thus defining beneficial/deleterious roles of the SAFE pathway in HF will require intensive investigation taking into account the aetiology of the disease.

Conclusion

In HF, there is an undoubted imbalance between pro-inflammatory and anti-inflammatory cytokines. This imbalance correlates with the severity of the disease and the aetiology of HF. Animal studies conducted essentially on ischaemic HF models with over-stimulation or repression of the pro-inflammatory cytokines are in favour of a causal role of these cytokines in the pathogenesis of HF. In contrast, while human studies suggest that measuring cytokines can be used as a biomarker for HF, they presently do not support a contribution of pro-inflammatory cytokines in the development of the disease. Over the last decade, new experimental data and human studies have demonstrated the Janus nature of cytokines, being either cytoprotective or detrimental. It underlines the dynamic aspect of cytokine action, where questions such as concentration, the time when produced and the type of receptors that they activate need to be addressed. The protective effect of the cytokines has been clearly demonstrated when there is a moderate activation of the SAFE pathway. Conversely, an uncontrolled activation of the SAFE pathway may be detrimental. Targeting a controlled activation of the SAFE pathway in HF is undoubtedly a valid therapeutic objective but the precise kinetic and bioactivity characteristics of pro-inflammatory cytokines in either chronic or acute HF should be carefully delineated before this can be considered.

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