Anti-cytokine therapy in chronic heart failure: new approaches and unmet promises

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Introduction

Until some decades ago chronic heart failure (CHF) has been considered a haemodynamic disorder with neurohormonal activation. Thus, the therapeutic strategies were oriented to correct cardiovascular disarrangement and counteract neurohormones. Although these therapeutic interventions improved CHF prognosis, mortality and morbidity CHF related remained high. In the recent years experimental and clinical researches increased our knowledge on CHF pathophysiology. Specifically, the involvement of inflammatory cytokines in the progression of CHF become more and more evident.

Therefore, correction of cytokine network may represent a new therapeutic strategy approach in the management of CHF. However, results obtained from the first clinical trials with engineered anti-cytokine molecules, such as Infliximab and Etanercept, are discouraging.

More knowledge on the complex interplay among inflammatory molecules in heart failure will allow its transfer to the design of more effective therapeutic candidates. The present review addresses current information on this controversial issue.

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TNF and receptors

TNF, which was originally identified as a factor able to induce necrosis of tumor tissue, is one of the most prominent inflammatory mediators, being able to trigger other
cytokines production, adhesion molecules expression, cytotoxicity and proliferation. TNF is expressed by many different cell types of the immune system such as macrophages, monocytes, T and B cells, granulocytes and mast cells.\(^1\)

The TNF molecule exists either as a membrane bound pre-protein or as mature soluble TNF, which is cleaved off from the pre-protein by metalloproteinases.\(^2,3\) Both mature and membrane-bound TNF form homotrimers. Trimerisation is essential to activate TNF receptors.\(^4\)

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Recently, a new TNF-R2 isoform originated from alternative splicing of TNF-R2 has been described.\(^12\) Since this isoform is expressed within the cell and not found on the cell membrane it was termed intracellular TNF-R2 (icTNF-R2 or icp 75TNF-R). Over expression of icTNF-R2 results in binding TNF and in intracellular signal transduction in a TRAF-2 dependent manner, with subsequent enhanced NF-kB activation which ultimately protects the cell from TNF-mediated cytotoxicity.\(^12\)

### IL-6 family and receptors

Originally identified as T cell-derived cytokine, IL-6 has been actually recognized as multifunctional cytokine produced by several cell types of non-immunological origin, as well.

Head of a larger family of structurally related cytokines with overlapping biological effect,\(^13\) the IL-6 family is composed by at least seven known members listed in Table 1.

The redundancy of the biological effect exerted by these molecules is mainly due to the assembly of the cytokines to a specific set of receptors. There are two different varieties: 1 — a *private ligand-binding receptor* (called membrane alpha receptor[\(\alpha\)] and 2 — a *membrane beta receptor* (called transmembrane glcoprotein [gp] 130).

The association of the cytokine to its proper a receptor is the necessary step for the gp130 recruitment and its own activation. All the IL-6 members work through membrane receptors with the exception of OSM and LIF that bind directly the gp130 receptor. Unfortunately, alpha receptor show a restricted tissue distribution, thus cells or tissues lacking this receptor can be functionally activated by the use of circulating soluble receptors.

The mechanism that evokes the signal transduction at intracellular level is the gp130 dimerization with (heterodimerization) or without (homodimerization) association to the LIF receptor. Both these receptors are expressed in every cell and tissue explaining the redundancy of the biological response. This latter is controlled by distinct regions of gp130 through phosphorylation events that principally involve protein tyrosine kinase (Janus kinase = JAK family),\(^14,15\) tyrosine phosphates SHP2/signal transducer and activator factors (STATs),\(^13–17\) mitogen-activated protein kinase (MAPK)\(^18\) and phosphatidylinositol 3-kinase (PI3K).\(^19\)

#### Table 1 IL-6 family and its receptor component for signalling transduction

<table>
<thead>
<tr>
<th>IL-6 members</th>
<th>Short name</th>
<th>Proper receptor</th>
<th>Signal transduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukin-6</td>
<td>IL-6</td>
<td>IL-6R, sIL-6</td>
<td>gp130/gp130</td>
</tr>
<tr>
<td>Interleukin-11</td>
<td>IL-11</td>
<td>IL-11R, sIL-11</td>
<td>gp130/gp130</td>
</tr>
<tr>
<td>Oncostatin M</td>
<td>OSM</td>
<td>LIF-R, OSM-R</td>
<td>LIF-R/gp130</td>
</tr>
<tr>
<td>Cardiotrophin-1</td>
<td>CT-1</td>
<td>CT-1R</td>
<td>LIF-R/gp130</td>
</tr>
<tr>
<td>Leukemia inhibitor factor</td>
<td>LIF</td>
<td>LIF-R</td>
<td>LIF-R/gp130</td>
</tr>
<tr>
<td>Ciliary neurotrophic factor</td>
<td>CNTF</td>
<td>CNTF-R</td>
<td>LIF-R/gp130</td>
</tr>
<tr>
<td>Neurotrophin1/B cell-stimulatory factor 3</td>
<td>NNT-1/BSF3</td>
<td>?</td>
<td>LIF-R/gp130</td>
</tr>
</tbody>
</table>
Transient over expression of STAT3 has been shown to induce a hypertrophic response both in vivo and in vitro suggesting a role for IL-6 related cytokines in the pathophysiology of the failing heart.

Cytokines activation during CHF

Circulating and myocardial levels of TNF and IL-6, found to be elevated in CHF patients with left ventricular dysfunction, became strong independent prognostic marker of aethiology (ischemic vs. dilated cardiomyopathy) and severity of prognosis. Relatively little is known regarding other IL-6 family cytokines. However, myocardial LIF mRNA levels and CT-1 serum levels have shown to be elevated in CHF patients. Circulating levels of soluble TNF-receptors and soluble gp130 were found to be increased in CHF patients in close relation to functional class. On the contrary plasma levels of sIL6R in CHF patients were not different from healthy subjects.

Considering that in vitro TNF can regulate IL-6 in vitro, we might speculate that increased levels of TNF during CHF could be involved in the regulation of the IL-6 expression thus evoking apoptosis or hypertrophic damage at cardiac level.

Cytokines and endothelial function

The cytokine TNF negatively affects endothelium function in terms of reduced nitric oxide (NO) production, induction of cell death and oxidative stress. In vitro studies by Rosenkranz-Weiss and our group have shown that TNF is able to down regulate eNOS time-dependently in cultured endothelial cells. The reduced availability of endothelial nitric oxide synthase (eNOS) results in reduced activity of the eNOS enzyme and reduced accumulation of nitrite/nitrate in the conditioned medium of cultured cells, likely suggesting reduced NO production after cytokine treatment.

Both reduced NO-mediated endothelial-dependent vasodilatation, after acetylcholine infusion and reactive hyperemia, and elevated circulating levels of TNF are hallmarks of CHF. In this disease, the functional role of TNF in modulating endothelial function was first suggested by our group, showing that the serum of patients with CHF induces apoptosis and down regulates eNOS in human umbilical vein endothelial cells. The importance of TNF was revealed by adding an anti-TNF antibody that partially antagonised the effects of CHF sera on endothelial cell apoptosis and eNOS. Reduced eNOS protein and NO production from the coronary circulation of dogs with cardiac decompensation has an impact on cardiac metabolism, increasing cardiac oxygen consumption and inducing a shift in cardiac substrate use from fatty acid to glucose. The role of TNF-induced endothelial dysfunction is further supported by studies by Anker et al. who have shown that circulating TNF levels are inversely correlated with peak blood flow in CHF patients, independently of age, ejection fraction, peak oxygen consumption, and New York Heart Association (NYHA) class, thus suggesting that TNF might contribute to peripheral skeletal muscle weakness/fatigue in patients with CHF.

Finally, TNF is able to cause endothelial dysfunction also by inducing oxidative stress. Indeed we showed that an anti-TNF antibody was able to reduce the depletion of thiol groups in endothelial cells that were incubated with the sera from CHF patients, suggesting a possible link between TNF, oxidative stress and apoptosis. Carvedilol, added on a group of endothelial cells of the same experiment, reduced the rate of apoptosis and maintained the physiological levels of thiol groups, suggesting that reduction of oxidative stress likewise reduces also the rate of CHF-induced apoptosis in endothelial cells. Another study confirmed such speculation as vitamin C suppressed the induction by TNF of apoptosis in endothelial cells both when added in vitro and when given to CHF patients as a therapy.

Therefore, cytokines may well be considered as contributors to endothelial dysfunction in CHF.

Experimental and clinical application of the anticytokines therapy

Several experimental studies in animal models show the beneficial effects of anti-cytokines therapy. The first work which shows that TNF-inhibition during CHF can provide suggestions for the efficacy of the anti-TNF therapy was published by Bozkurt and colleagues in 1998. These Authors demonstrate that continuous infusion of TNF caused the development of dilated cardiomyopathy in rats. However, discontinuation of TNF infusion resulted in a recovery of normal or near to normal left ventricular dimension and functions. These effects were evident after weeks of treatment.

Other Authors approached the use of soluble TNF binding proteins to antagonize the toxic effect of TNF during remodeling and inflammation, obtaining very promising results in mice. The efficacy of anti-TNF monoclonal antibodies approach in modulating the genesis and developing of CHF was confirmed by other studies in transgenic mice over-expressing TNF. However, despite these promising evidences, Kadokami’s paper suggests that the role of TNF in activating relating cytokines is not fully understood. In this work, the Authors induced in mice TNF over-expression by lipopolysaccharide injection. These mice were subsequently treated with adenovirus-mediated TNF receptor fusion protein (AdTNFR1) in order to antagonize the cytokine protein expression. The results obtained show that the treatment decreases IL1beta blood levels and TNF bioactivity and did not modify other cytokines such as MCP-1 and IL-6 suggesting a limitation in the single anti-cytokine approach.

Moreover, the cytokine strategies can be affected by the time of intervention as shown in experimental CHF-
induced by pacing in swine, a species closer to humans, in which a role for TNF in preventing early cardiac remodeling was demonstrated.45

Whilst anti-TNF-strategies have been approached and developed in the clinical setting, as far as IL-6 is concerned the efforts are actually concentrated on the comprehension of the mechanisms that regulate gp130-mediated signalling. The development of engineered animal models over expressing IL-6 and sIL6R46 and gp13047 or IL6R/soluble gp130 fused receptors48 confirms that a lot of work has to be done before approaching therapies in humans. In this respect, a small trial using a mouse monoclonal antibody that blocks IL-6 action therapies in humans. In this respect, a small trial using a mouse monoclonal antibody that blocks IL-6 action has been developed in non-cardiac disease.49–51 However its application in humans elicits an immune response that limits its efficacy.52

In light of these results, clinicians decided to test anti-cytokine therapy (basically as anti-TNF) in human affected by CHF. The approach was mainly developed on the use of soluble cytokine receptors (SCRs) as immunotherapeutic agents (Fig. 1), but anti-cytokine monoclonal antibody against TNF has been developed as well. Moreover, recent development of "cytokine traps" opens the study of a new class of high affinity therapeutic candidates designed to specifically inhibit cytokines, like those of the IL-6 family, using complex multi-component receptor systems (Fig. 1).53

SCRs have been generated by molecular biology techniques such as cloning of cDNAs encoding soluble receptors and engineered truncated versions of the membrane cytokine receptors. SCRs, being commonly devoid of intracellular signalling activity, by binding their ligand inhibit cytokine biological activity. Therefore they are considered as "cytokine inhibitors", since they compete for the ligand with membrane receptors.

A number of considerations must be taken into account in relation to the use of SCRs as therapeutic agents.54 First, the antagonistic effect of SCRs is cytokine-specific. Second, the antagonistic effect of SCRs is directly proportional to their concentration and inversely proportional to the concentration of the cytokine to be buffered. Third, their antagonistic effect is influenced by the binding affinities of the SCR and the functional membrane receptors, the latter generally having a higher affinity for their natural ligand. However, one should also consider that SCRs may provide the cytokine with increased molecular stability and half-life, thus playing a potential role as "cytokine carriers" in the circulation, and ultimately enhancing the activity of the cytokine.55–57

The overall effect of SCRs in vivo is likely to be dictated by the balance between their antagonistic and agonistic effects, which in turn is the resultant of the specific local concentrations of the cytokine, SCRs and membrane cytokine receptors on target cells.

As therapeutic agents SCRs have several advantages over the use of other immunosuppressive drugs. Among these, specificity, high affinity and low immunogenicity, the two latter being particularly true with respect to anti-cytokine monoclonal antibodies.

SCRs disadvantages are: their short half-life; their carrier effect, possibly leading to undesired effects; their high molecular weight, which implies low stability and increased sensitivity to proteolytic degradation; and last but not least their high cost.

The preliminary results of a phase I study in which was tested the effect of a construct that contains the extracellular domain of human P-75 TNFR, which binds and inactivates circulating TNF, named Etanercept was published as an Abstract in the 1998.58 The results obtained show that Etanercept is well tolerated and it reduces the blood TNF.

A subsequent phase II randomised study which investigated the role of Etanercept administered at two doses in 47 patients with CHF (Class III—IV NYHA) confirms the salutary effects of the experimental drug evaluated as improvement of cardiac size and functions.59

Based on these preliminary results two large scale randomised trials named RENAISSANCE and RECOVER were organised in parallel but in two different areas of the globe (RENAISSANCE was organised in North America, RECOVER in Europe and Australia). These studies used...
Etfaximab. Infliximab.60 globulin G monoclonal antibody against TNF named TACH) which used a chimeric (mouse–human) immuno-death.

Similar results were obtained in a similar study (ATTACH) which used a chimeric (mouse–human) immunoglobulin G monoclonal antibody against TNF named Infliximab.60

In the ATTACH study were randomly enrolled patients with stable CHF NYHA Class III or IV and left ventricular EF of less than 35%. β-blockers and ACE inhibitors were allowed.

Active treatment did not influence the variable measured. In fact, the composite end-point after 14 and 28 weeks of treatment was not different between the groups studied. Moreover there was an increased number of death and hospitalisation in patients treated with Infliximab. The conclusion was that TNF antagonism did not produce any advantage for the CHF patients.60

A question raises spontaneously: Why was anti-cytokine therapy unsuccessful in CHF patients in spite of encouraging data obtained in vitro and in animal experimental models?

Some possible explanations to this question are recently provided by Feldman and Colleague:61

(1) a failure to select the appropriate patient enrolled in the studies,
(2) a failure to understand the pathophysiology of TNF overexpression,
(3) an absence of information regarding TNF antagonists pharmacodynamics,
(4) an absence of information regarding the role of TNF genetic polymorphisms,
(5) a limited knowledge regarding the role of gender in TNF turnover,
(6) a limited knowledge regarding the possible pharmacodynamic interactions between cytokines agents and routine therapy used in CHF patients.

We can add to this list that complexity of the cytokines network and intracellular signalling pathways complicate this scenario. The current knowledge suggests that the block of a single cytokine not necessarily results in blocking downstream cytokines, therefore making unpredictable the ultimate efficacy of this therapy. It should be considered that TNF plays a pivotal role in the immune defence and patients with CHF are at high risk for inflammation: thus, it may be that hiding positive results is actually the side effect of blocking or reducing the natural defence against inflammation.

In conclusion cytokines are a complex network of molecules, receptors and intracellular signal which can cross-talk among themselves. The final clinical results depend on the balance among different reactions including individual response and/or interactions with external modifications.

Further basic and clinical work is necessary to clarify the role of cytokines system in the genesis and evolution of CHF in order to identify optimal therapeutic strategies.

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