Tumor necrosis factor in CHF: a double facet cytokine

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1. Introduction

Tumor necrosis factor (TNF) gained its popularity in the world of biology and medicine in a weird way. In 1985, Old in New York City discovered the presence of a protein in blood after lipopolysaccharide (LPS) injection, which is able to induce necrosis of some mouse tumors [1]. He called it ‘tumor necrosis factor’, a term actually coined in the 1970s. At the same time and, virtually, on the other side of the street, Beutler and Cerami, working at what may appear as an unsurmountable biological distance, discovered that the intriguing state of cachexia is associated with the presence of a protein called ‘cachectin’ in the serum [2]. Its purification made them realize that ‘cachectin’ is an essential mediator of the state of LPS-induced shock and that it is the same molecule of ‘tumor necrosis factor’ [2]. It took some years for these three parents to recognize that their child, whatever called, was unusually gifted. They could not foresee that TNF turned out to be a prodigy molecule eliciting the publication of many papers per day [3].

Two names are not enough to describe TNF, as we now distinguish TNF-α from its twin, TNF-β or ‘lymphotoxin’ (LT), a lymphokine which has only a 30% homology with TNF-α but which binds to the same receptors [4]. Despite similarities, these two proteins do not always transduce identical signals; they have distinct cellular sources and regulation mechanisms. Strangely enough, and without any particular reason, there are many more studies on TNF-α than TNF-β.

Herein the current knowledge on the involvement of TNF-α in congestive heart failure (CHF) is reviewed. For the sake of simplicity, we will refer to TNF-α as TNF.

2. What is it and what does it?

TNF is a trimeric 17-KDa polypeptide mainly produced by monocytes and macrophages, but also by other cell types [4]. It is expressed as a 26-KDa integral membrane precursor protein from which the 17-KDa subunit is released after proteolytic cleavage [5]. This is prevented by metalloprotease inhibitors which hold promise as suppressors of TNF-mediated diseases. It exists both as secreted (type I) and transmembrane (type II) form, [3]. TNF exerts several effects, either beneficial or detrimental.

Even its classical trilogy of action is now considered from a different perspective. TNF is more than a tumor necrosis factor, (as it can promote growth), more than a cachectin as it can induce obesity, more than a mediator of shock as it can protect the host. In contrast to original and popular thinking, TNF acts as a growth factor, playing a major role in embryogenesis and in a wide variety of tumors [3]. This, together with its dose-limiting toxicity, explains the disappointing results when used as therapeutic agent against cancer. It regulates hematopoiesis. It affects several endocrine organs, causing downregulation of progesterone and testosterone secretion and upregulation of ACTH and GH release [3]. Paradoxically, it has a role in cachexia and anorexia as well as in obesity [4]. In obese human adipose tissue its expression increases and TNF causes the insulin resistance related to obesity [4]. It is a mediator of inflammation and of septic shock, its effects being considered either harmful or beneficial to the host [3]. It triggers the release of practically all known mediators of inflammation such as cytokines and all metabolites of arachidonic acid, the final results ranging from hemorrhagic necrosis to extensive local fibrosis. It damages the host by inducing and enhancing, often in association with IFN-γ, the production of reactive oxygen derivatives, such
as nitric oxide (NO). However, even under these pathological conditions, TNF has a dual effect, allowing the host self-protection against this type of injury by the induction of oxygen free radicals-scavenging enzymes, such as mitochondrial superoxide dismutase and of protective substances such as heat shock proteins [3]. It is able to act very rapidly, so much that it can kill, but also slowly, so much that it can create new, well-organized, highly differentiated and specialized structures such as immune granulomas or lymphocyte infiltration. Thus, probably depending upon timing, extent of release and stability in the circulation, it may either be the killer or the protector of the host. It has a remarkable tendency to initiate its own circulation, it may either be the killer or the protector of this region in the functional neutralization and physical clearance of TNF from the circulation.

3. How does it act?

The wide range of TNF activities is explained by the presence of TNF-receptors (Rs) on almost all nucleated cell types. Both in man and in mouse, two distinct types of TNF-Rs have been identified and molecularly cloned: TNF-R55 (also referred as TNF-RI) and TNF-R75 (TNF-RII), with a molecular mass of 55 and 75-KDa, respectively [6]. The extracellular portion (182 residues) of TNF-R55 contains four cysteine domains sharing a significant sequence homology. On the other hand, homology does not occur at the cytoplasmic domains, suggesting that the two receptors activate different intracellular pathways. The precise role of each receptor in different cell types is far from being solved. Antagonistic antibodies to both TNF-Rs inhibit TNF-induced cytotoxicity of tumor cell and TNF-enhanced expression of inducible nitric oxide synthase (iNOS) [6]. This suggests that both receptor types contribute to these bioactivities. Paradoxically, however, the use of agonistic antibodies or of high concentrations of TNF suggests that, in most of the cases, only triggering with the TNF-RI is responsible for cytotoxic activity or gene induction, making it difficult to explain the effects of antagonistic antibodies to TNF-RII [6,7].

The ligand-passing model reconciles these paradoxical findings [6]. TNF-RII has a higher affinity and dissociation rate than TNF-RI. Therefore, it binds preferentially circulating TNF at low ligand concentration. The ligand is then passed to neighbouring TNF-RI which monopolizes all TNF-mediated signalling. Thus TNF-RII enhances and synergizes the effects mediated by TNF-RI.

How TNF induces such diverse and often opposing signals is not clear. Truncation and deletion mutagenesis of the cytoplasmic intracellular region of TNF-RII has identified a domain of 80 amino acids near to the C-terminus responsible for cytotoxicity [8]. An homologous region in the Fas is also sufficient to produce a signal that leads to cytotoxicity. These regions are called the ‘death domain or death region’ [6].

Other TNF functions that require the TNF-RI ‘death region’ are antiviral activity and induction of iNOS [6]. Over-expression of the ‘death region’ leads to apoptosis and NF-kB activation. Surprisingly, TNF-RI lacks any inherent kinases activity and their role is fulfilled by associated proteins [6].

Another peculiar aspect of TNF-RI is that it is capable of self-associating. This in turn, causes cell death [9]. Normally, cells expressing TNF-RI do not display TNF activities unless the receptor is triggered and self-associated. It follows that there are mechanisms preventing spontaneous self-association, probably by maintaining extremely low levels of the gene encoding TNF-RI. On the contrary, TNF-RII does not self-associate and this is the reason why it is more abundantly present. Like TNF-RI, TNF-RII has no intrinsic kinase activity, suggesting that associated kinases are responsible for intracellular crosstalk between the two receptors.

On the whole, the TNF-Rs system is quite unique in that one ligand, TNF, is able to interact with two receptors containing different intracellular domains. TNF-RI mediates almost all TNF activity. TNF-RII is signal transducing only in few cell types, such as T cells.

Activation and over expression of TNF-RII permit fine tuning of TNF-RI-mediated processes and the rapid induction of soluble (s) TNF-RII molecules which are involved in the functional neutralization and physical clearance of TNF from the circulation.

4. Is TNF involved in CHF?

For more than 2500 years physicians recognized that patients with end stage CHF share clinical features with those having chronic inflammatory or neoplastic disorders [10]. Cardiologists marvelled at the clinical wasting of patients with advanced CHF and thought that the body was consuming itself, worsening the cardiac condition. The pathogenesis of ‘cardiac cachexia’ remained obscure for many years until 1990, when Levine et al. [11] recognized that these patients had circulating levels of TNF as high as those with neoplastic and inflammatory disorders.

Subsequent studies confirm that circulating levels of the cytokine are increased in patients with most advanced symptoms and that there is a relation between circulating levels of TNF and clinical features of the disease, successful cardiac transplantation being associated by a decrease in circulating TNF [12–16]. Besides CHF, abnormal levels of circulating TNF are found in a variety of other cardiac diseases, including acute viral myocarditis [17], dilated cardiomyopathy [7], cardiac allograft rejection [18], myocardial infarction [8] and after cardiopulmonary bypass.
surgery [19]. In these reports, the assay of TNF is performed either by the cytotoxicity or the immunologic method, and it is often assumed that measurement with both assays would be highly correlated. This, however, is not the case and there is no relation between circulating levels of the cytokine measured by one assay and those measured by the other [20]. There are several explanations for this discrepancy, including the presence of high levels of circulating TNF antagonists which block the cytotoxicity but not the immunogenity of TNF.

Such circulating antagonists are soluble forms of the TNF receptors (sTNF-Rs), shed from target cells upon interaction with the cytokine and circulating in the blood stream. Both sTNF-Rs are increased in patients with severe heart failure and after myocardial infarction [20–22]. This finding raises questions far beyond the interpretation of differences between assays [23].

5. Increased sTNF-Rs in CHF: is it good or bad?

sTNF-Rs are naturally occurring inhibitors of TNF activity and exert a counteraction that could be either advantageous or injurious for the organism, reasserting the concept that TNF is a molecule with a ‘double facet’. The shedding of TNF receptors in patients with high circulating levels of the cytokine (like in those with CHF) could be an adaptive response that effectively neutralizes its biological action [20,23]. In addition, the shedding reduces the number of active receptors [24]. Thus, the formation of sTNF-Rs complexes could lessen TNF toxicity [25]. Accordingly, some investigators challenge the concept that TNF exerts any important biological action in cardiovascular diseases [23].

Others contrast with the formers saying that sTNF-Rs enhance, rather than attenuate, the biological action of TNF. By binding to trimeric TNF, sTNF-Rs prevent its monomerization and subsequent inactivation, increasing its half-life and biological function. According to this way of thinking sTNF-Rs act as a circulating ‘slow release reservoir’ and this may be quite relevant as the effects of TNF are more related to the persistence of the cytokine rather than to its peak levels [23].

Another easier interpretation is that increased sTNF-Rs are neither good nor bad, but simply reflect activation of the cytokine at local level produced. We found that high levels of sTNF-RII in patients with advanced CHF (NYHA class IV) represent the most important single independent variable in predicting short term mortality [20]. This could imply that TNF and sTNF-Rs have pathological effect in CHF.

6. Which are the biological effects of TNF in CHF?

It is tempting to speculate that, besides causing the clinical feature of cardiac cachexia, TNF contributes directly to the syndrome of CHF. Many of the hallmarks of the syndrome, including ventricular dysfunction and exercise intolerance, could be explained by the known biological effects of TNF. Before discussing these aspects in detail, a word of caution is necessary. At the present time the concept that TNF contributes to CHF is no more than a hypothesis. There is experimental evidence supporting a role of TNF in CHF which is indeed challenging. Such evidence, however, is not always unanimously proven and not necessarily applicable to patients.

7. How could TNF contribute to ventricular dysfunction in CHF?

The possibility that the heart itself is a target for TNF is supported by the evidence that circulating levels of TNF are high in CHF patients and the myocardial TNF-Rs are low [21]. Although several different stimuli other than TNF can affect the level of its receptor it is possible that this finding is the result of the shedding of TNF-Rs as the cytokine attaches itself to the cardiac cells.

Such an interaction in many species will induce expression of a ‘cytokine-induced high output’ isoform of NOS (iNOS or NOS2, together with cationic amino acid transporters necessary for the uptake of the NO-preursors L-arginine and the enzymes necessary for the production of tetrahydrobiopterin, a cofactor essential for iNOS activation [20–23,26,27]. TNF mediated induction of iNOS is part of the innate immune response, a phylogenetically primitive and rapidly activated form of host defence [28].

Whether TNF is able to induce human iNOS either in endothelial cells, myocytes or monocytes is not clear. There is evidence, although not unanimous, of iNOS expression in the myocardium and in circulating monocytes of patients with advanced CHF [18,29,30]. Whether this is caused by high circulating levels of TNF is not certain and several other cytokines are likely involved. The resulting increase of NO production from cardiac myocyte and other cell type is expected to further impair ventricular function by increasing intracellular cGMP or by altering specific sarcoplemmal ion channels [31–35].

The hypothesis that TNF in CHF decreases cardiac contraction by increasing NO production needs further testing as systemic infusion of recombinant TNF to intact animal preparation yields different and contrasting results [36–38].

The decline in contractile function observed in experimental preparations occurs at a very high concentration, unlikely to be of clinical relevance [38]. A recent report indicates that NO induction in failing myocytes does not alter baseline sarcomere mechanics but only attenuates the inotropic response to isoproterenol [39].

It is also emerging that programmed death of myocytes (apoptosis) occurs in decompensated human heart, despite the enhanced expression of Bcl-2, the proto-oncogenes which protect cells from apoptosis [40–42]. In vitro TNF is
Fig. 1. Proposed model on tumor necrosis factor (TNF) worsening ventricular dysfunction during congestive heart failure. NO: nitric oxide; iNOS: inducible nitric oxide synthase.

8. How could TNF contribute to exercise intolerance in CHF?

In CHF impairment in functional capacity is related not only to a reduced performance of the heart, but also to a defect in the periphery [43]. This, in turn, involves a reduced peripheral vasodilating capacity due to endothelial dysfunction and a decrease in strength and endurance of skeletal muscle, due to the atrophy of muscle fibers [44]. TNF can be responsible for both these abnormalities. It may cause endothelial dysfunction by inducing oxidative stress, which, in turn, destroys NO and causes apoptosis [45–47]. In vitro TNF is a powerful trigger of apoptosis in endothelial cell and this together with induction of pro-coagulant activity and fibrin deposition, is one of the mechanisms involved in the TNF-induced haemorrhagic necrosis of tumor neovasculature [47]. We have shown that incubation of human endothelium with serum from CHF patients with enhanced TNF increases the rate of endothelial apoptosis [48]. However, even the concept that TNF contributes to endothelial dysfunction in CHF should be considered with reservation. The TNF mediated induction of iNOS (if it occurs in man) may be viewed as a beneficial effect, counteracting the vasoconstriction mediated by the activation of sympathetic and renin–angiotensin–aldosterone, systems. NO-mediated peripheral vasodilation in CHF is either positively or inversely related to TNF levels [49]. In addition, elevated levels of TNF deactivate the release of superoxide anions from neutrophils [50]. This is considered an example of self-protection against the deleterious effect of an increased production of oxygen free radicals, possibly involved in endothelial dysfunction. Here emerges, once again, the ‘double face’ of this molecule, mediating effects which can be either beneficial or detrimental.

Besides altering the endothelium, TNF can contribute to peripheral muscle wasting by impairing the synthesis and increasing protein catabolism, although cachexia is not a prerequisite for TNF activation in CHF [23] (Fig. 2).

9. What is the stimulus to the increased TNF production in patients with CHF?

There is no definitive answer to this fundamental question. The major source of TNF in many disease states is activated macrophages. It has been suggested that the increase in prostaglandin E₂ seen in CHF patients stimulates macrophages to produce TNF [51]. There is also evidence that in CHF, the cytokine is activated independently from inflammation and from the cause of the dis-
ease, suggesting that the TNF increase is more related to the presence of CHF than to its cause [23].

Other possibilities are that the failing human heart directly produces TNF or that blood flow reduction causes local ischaemia and hypoxia with consequent macrophage stimulation. Interestingly, it has been observed that in CHF increased mesenteric venous pressure causes intestinal edema. The consequent mesenteric venous congestion leads to increased bowel permeability with consequent bacterial translocation and endotoxin release. The increased endotoxin challenge causes immune activation with increased TNF production. In support of this hypothesis, CD14 levels (indicative of endotoxin–cell interaction) are increased in patients with CHF and especially in those with elevated TNF and cachexia [52].

10. Conclusions

There is substantial evidence that TNF is increased in severe CHF. Due to the different methodologies and wide variations, the reported plasma levels of TNF are quite different, but within the range of those found in other terminal diseases. Activation of TNF is not proportional to the severity of the syndrome as the cytokine increases only in advanced CHF, although independently from the presence of cachexia. Measurements of soluble receptors of TNF are likely to be a better marker for local TNF activation and have prognostic implication.

TNF could either have a beneficial or a deleterious action in CHF. In several species it increases endothelial and monocytic expression of iNOS, thus mediates vasodilation and improves vascular and peripheral muscle function. On the other hand it may induce endothelial dysfunction by facilitating apoptosis and by inducing oxidative stress. It could also further impair peripheral muscle dysfunction by causing muscular wasting. At myocardial level it could increase the expression of iNOS and induce myocyte apoptosis, further reducing myocardial dysfunction.

The possibility that the syndrome of CHF leads to excessive production of endogenous substances with opposite effects is not new. Neuroendocrine activation in CHF, for instance, may be beneficial in a short term but it is certainly dangerous in the long term. Whether this is also true for TNF is not known.

Patients with end stage CHF are not so different from those with end stage cancer or AIDS. TNF increases in all of them. Another interpretation is that TNF activation reflects the severity of the condition independently from whether or not it contributes to worsen the inherent pathological syndrome. It could be just a part of the mosaic leading to the end of the line.

The whole issue is complicated as TNF is part of a network that includes positive, such as stimulating factors and interleukins, as well as negative regulators, such as transforming growth factors and cytokines. The network requires an appropriate balance between positive and negative regulators and allows considerable flexibility. We are just at the beginning of what will be an important exploration of the effects of CHF on such a complex network and TNF is just a small part of it. The fact that it is the most valuable arm of the immune system but that it often attacks the body’s own tissue is indeed very challenging, but it makes interpretation of the results very difficult.

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