Review

Tumor necrosis factor in myocardial hypertrophy and ischaemia — an anti-apoptotic perspective

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1. Introduction

Tumour necrosis factor-alpha (TNF\textalpha) is a multifunctional cytokine that has been implicated as mediator of diverse physiologic and pathophysiologic events. These processes include inflammation, cellular survival, growth, differentiation and apoptosis. The local homeostatic cellular effects of cytokines may be considered ‘autocoid’ in nature. This word is derived from the Greek words autos (‘self’) and akos (‘remedy’), and refers to locally acting, biologically active agents, both peptides and non-peptides, that are distinct from neurotransmitters and hormones [1].

Recent studies have highlighted the pathogenic role of TNF\textalpha in the development of myocardial disease, such as the direct correlation between serum TNF\textalpha levels and the severity and progression of heart failure [2–4] and the development of myocarditis in mice that overexpress TNF\textalpha specifically in the heart [5]. Taken together, these studies suggest that TNF\textalpha is a mediator of cardiac pathology, acting at least in part via inflammatory pathways and via the activation of programmed cell death i.e. apoptosis [6,7].

However, in addition to mediating inflammation and apoptosis, cytokines play a critical role in the control and maintenance of signalling pathways that regulate mammalian physiology in multiple organ systems. Specifically in the heart, biomechanical stress promotes endogenous TNF\textalpha production by myocytes and non-myocytes. Therefore, as in other organ systems, the question arising is whether TNF\textalpha plays an adaptive role in mediating cardiac cell growth and in remodelling the cardiac extracellular matrix in response to biomechanical stress. The hypothesis that TNF\textalpha could have a short term adaptive and long term maladaptive role was proposed and reviewed by Mann [8] in 1996. Recent studies support the view that TNF\textalpha can have beneficial effects in cardiac homeostasis.

In this mini-review we will focus on the role of TNF\textalpha in mediating myocardial cell survival, growth and differentiation as adaptation responses to acute/subacute biomechanical stress, rather than emphasizing its role in apoptosis. We believe that these perspectives need to be reviewed due to the recent therapeutic advances in the ability to suppress TNF\textalpha production and release in the clinical arena [9,10].

2. TNF\textalpha — a prototypic pleiotropic cytokine

TNF belongs to a family of signaling molecules that exist as type II membrane proteins characterized by the C-terminus being extra-cytoplasmic. A conserved 150-amino acid region within the C-terminus characterizes this family and is the region used by the members of the TNF family to recognize their cognate receptors [11]. Currently two isoforms of TNF have been identified and share similar inflammatory activities. TNF\textalpha, the smaller and more abundant of the two peptides and is thought to be the peptide that mediates cardiac homeostasis. TNF\textbeta, first described as lymphotoxin [12], is less abundant, thought to be produced mainly by T cells and will not be discussed further in this review.

TNF receptors signal as homotrimeres and can exist either as membrane-bound or as truncated soluble forms...
3. TNFα signalling pathways

The binding of TNFα to its cognate homotrimeric receptor results in the formation of multimolecular signal transduction complexes which can rapidly activate several divergent downstream signalling pathways (Fig. 1). In brief, the coupling of the adaptor Fas-associated death domain protein (FADD) to TNFR1 is associated with the recruitment and activation of apoptotic proteases with subsequent progression to programmed cell death. However, TNFR1 activation is not usually associated with apoptosis, due to the induction of a complex cytoprotective response that requires TNFR associated factor 2 (TRAF2), a signal transducer which couples to TNFR1. This complex activates both NFκB-dependent and -independent transcriptional events implicated in the induction of cytoprotective genes involved in cellular growth, survival and proliferation [17]. Cytoprotective pathways downstream of the TNF receptors that may be relevant to the maintenance of cardiac homeostasis in response to pressure and volume overload were recently reviewed [18]. These include the hypertrophic growth programmes downstream of: protein kinase C (PKC); stress activated protein kinases (SAPK) and downstream of c-Jun N-terminal kinase (JNK). The putative TNF mediated cardioprotective programmes against ischaemia–reperfusion injury may be downstream of PKC [19], NFκB [20] and SAPK [21]. The regulatory balance between these alternate pathways with consequential propagation of divergent cellular fates (cell survival vs. apoptosis) are currently poorly understood and under active investigation. Further details of these pathways have been extensively reviewed [17,22,23] and are beyond the scope of this mini-review.

4. TNFα production in the adult heart

TNFα biosynthesis is largely controlled at the translational level [24]. Moreover, as TNF is efficiently secreted from macrophages, immunocytochemical detection of these proteins within cells is difficult to assess. To overcome these limitations, Girior et al. [25] generated transgenic mice harbouring the TNFα promoter upstream of a CAT reporter gene. The CAT enzyme activities were evident in heart lysates after the mice were exposed to intraperitoneal lipopolysaccharides (LPS). These data suggested that TNFα may be secreted by heart cells, while not excluding the contribution of macrophages in the heart lysate assays. To confirm the production of TNFα by intrinsic cardiac cells, primary cardiocytes were extracted from mice and exposed to LPS in the cell culture environment. TNFα levels, measured in the culture media, again demonstrated the increased production of TNFα in the presence of LPS [25]. Moreover, using immunohistoch- emical analysis, TNFα peptide was shown to be produced in myocardial cardiocytes, smooth muscle cells and endothelial cells in response to endotoxin in vitro [26]. Following ischaemia, TNFα expression is upregulated in isolated cardiac myocytes and fibroblasts [27–29]. Following the onset of experimental myocardial infarction [30], there is a diffuse increase in TNFα mRNA expression persisting for over 1 month. Taken together, these data demonstrate that the heart has an endogenous capacity to produce TNFα in response to diverse pathophysiological stimuli.

5. TNFα and cardiac growth

Genetic ablations of TNFα [31], its cognate receptors (TNFR1 and TNFR2) and of the combined receptors have been generated in mice. Although not rigorously studied, no obvious defects in cardiac development have been described in any of these genetic manipulations. This suggests that either the TNF signalling cascade is not required for normal cardiogenesis or that this pathway is redundant and compensated for by alternate signalling pathways. However, in response to pressure-overload and to isolated stretch, TNFα expression and peptide production are upregulated in the adult heart [32], suggesting that in postnatal life this cytokine is directly induced in response to haemodynamic pressure overload. Such a load elicits cardiac myocyte hypertrophy and extracellular matrix remodelling. These phenotypic alterations are thought to be adaptive responses in order to maintain normal cardiac contractility and homeostasis in response to the increased workload. In keeping with a possible role of TNFα signalling in postnatal cardiac growth, Kapadia et al. [32] showed that simple passive stretch of cat papillary muscle induced the expression of TNFα mRNA. Physiologically relevant concentrations of TNFα provoke a hypertrophic response by increasing the synthesis of both
Fig. 1. Signaling pathways elicited by tumour necrosis factor receptors (TNFRs) after activation by TNF. This figure shows the recruitment of various docking proteins to TNFR1 and TNFR2. These include: tumour receptor associated death domain protein (TRADD), receptor interacting protein (RIP), TNF receptor associated factor 2 (TRAF2) and Fas-associated death domain protein (FADD, alternate name, Mort1, mediator of receptor induced toxicity). Recruitment of these docking proteins are coupled to the activation of numerous signalling pathways culminating in inflammation, apoptosis or multiple gene activation as illustrated. Abbreviations, are described from proximal to distal in each pathway. The phospholipase A2 pathway (PLA2) include: cyclooxygenase (COX); prostaglandins (PGs) and thromboxanes (TXs). The PLC pathway (phospholipase C) includes: diacylglycerol (DAG), sphingomyelinase (SMase) and protein kinase C (PKC). The mitogen activated kinase (MAPK) pathway include: NFκB inducing kinase (NIK), the NFκB transcription factor subtypes-p50 and p65 and the NFκB cytosolic anchoring protein complex (IκB). An alternate TRAF2 or PKC activated pathway is represented by Janus N-terminal kinase/stress activated protein kinase (JNK/SAPK) with downstream activation of genes via activation protein-1 (AP-1).
structural and contractile protein in adult feline cardiocytes [33]. This response appears to depend on a preserved interaction between cell integrin and the extracellular matrix [33]. Moreover, Nakamura et al. [34] have suggested that an additional pathway whereby TNFα induces hypertrophy in neonatal rat cardiac myocytes is via activation of reactive oxygen intermediates.

In a preliminary report, there is further in vivo evidence to support the role of TNFα in the induction of cardiac hypertrophy [35]. Transgenic mice overexpressing TNFα specifically in the heart, develop cardiac hypertrophy [36], these same mice develop myocarditis in response to the inflammatory response mediated by TNFα. Interestingly, the inflammatory response but not the hypertrophic response can be attenuated by genetic attenuation of the TNFRI signalling pathway [35]. In preliminary data we have shown that with the genetic ablation of TNFα, mice have a significantly reduced right ventricular hypertrophic response to three weeks of hypoxia compared to wild type littermate controls (unpublished results).

Taken together, these preliminary data in genetically modified mice, together with the in vitro data reviewed, strongly suggest that TNFα binding to its cognate receptor, and the subsequent activation of the downstream signalling cascade play a role in the postnatal adaptive myocardial growth response to multiple biomechanical stresses.

6. TNFα modulation of cardiac contractile function and its role in myocardial ischaemia

In septic shock, experimental evidence links TNFα to reduced cardiac contractility [37]. The putative mechanisms for this include: a NO-independent reduction in peak systolic [Ca2+]I [38]; or a NO-mediated decrease in the sensitivity of the myofilaments to Ca2+ concentrations [39]. Alternatively, a neutral sphingomyelinase pathway may be involved [40]. Acute reduction in cardiac contractile function does not, however, necessarily result in long term functional impairment [41]. Theoretically, however, chronic TNFα production in the heart may lead to permanent functional impairment via NO-dependent apoptosis [42,43]. Interestingly, myocardial TNFα production has been well documented during acute ischaemia with or without reperfusion [30,44]. Such myocardial TNFα release may theoretically reduce cardiac contractility, with subsequent cardioprotection by the attenuation of myocardial energy demand. Thus, hypothetically, the acute TNFα induced reduction in contractility during acute ischaemic syndromes may be an additional adaptive action of this pleiotropic peptide.

Further preliminary evidence for a cardioprotective action of TNFα in acute ischaemia is found in mice with combined genetic ablation of the TNFR1 and TNFR2 receptors. These mice developed larger myocardial infarcts, compared to wild-type littermate controls when subjected to an acute infarction in vivo [45]. These data suggest that TNF signalling, through an unknown mechanism, is required for endogenous myocardial resistance to ischaemic cell death.

6.1. Preconditioning

TNFα may also function as a preconditioning peptide, thereby providing an additional and alternate putative mechanism that could confer cardioprotection against myocardial ischaemia–reperfusion injury. Preconditioning agents are by definition extrinsic stimuli which when introduced to the heart prior to an ischaemic insult and subsequent reperfusion period, help the heart to be more resistant to the latter insult. Nelson et al. [46] demonstrated that pretreatment of rabbits with intravenous TNFα 24 h before ex vivo simulated ischaemia and reperfusion resulted in improved cardiac contractile functional recovery and reduced lactate dehydrogenase release. These investigators noted that TNFα pretreatment, with the resultant reduction in ischaemia–reperfusion injury, correlated with an increase in myocardial manganese superoxide dismutase activity, suggesting a possible mechanism involved in this cardioprotection. In preliminary data from our laboratory, we have pretreated isolated rat hearts with TNFα (0.5 ng/ml) for 7 min followed by a washout of 10 min prior to a simulated ischaemic–reperfusion protocol. Consistent with the data of Nelson et al. [46], left ventricular developed pressure and cardiac contractile functional recovery markedly increased in the TNFα group compared to vehicle pretreated controls (unpublished results). These data suggest that the known production of TNFα by the myocardium in response to an ischaemic and reperfusion insult [29], may in fact be an endogenous pathway activated by the heart to induce short-term intrinsic cardioprotection against subsequent ischaemia–reperfusion injury. Contrary data were published by Meldrum et al. [47], where they demonstrate that ischaemic preconditioning or adenosine pretreatment reduces TNFα peptide in the myocardium following a subsequent ischaemic–reperfusion episode. TNFα peptide production by the myocardium immediately following the preconditioning stimuli was, however, not measured and it is hypothetically possible that the preconditioning stimuli activates TNFα production with a resultant autoregulatory negative feedback signal on subsequent and/or excessive TNFα production. We are currently investigating TNFα production following classic ischaemic preconditioning stimuli.

7. Bifunctional role for TNFα

7.1. Arguments from signalling pathways

Signalling from the type I TNFα receptor, thought to be the more important of the two receptor types in the heart,
may lead either to cell survival or to apoptosis [23]. One proposal is that apoptosis is not the physiological end point for this path, but only results when protein synthesis is blocked [48]. TNFα mediated apoptosis in the heart has been described [49] and the regulatory pathways are being characterised [50]. The anti-apoptotic pathway is not yet fully clarified, but may converge on the activation of the transcription factor NFκB. The latter promotes the expression of cytoprotective genes (described in Section 3 and illustrated in Fig. 1). Activation of this pathway is thought to involve the protein factor TRAF-2, that binds directly to TNF α, and indirectly via another protein factor called TRADD, which binds to the type 1 receptor [48].

The alternate pathway, linked to apoptosis, results in activation of caspases, followed by apoptosis. One of the activators of caspase-8 is FADD (Fas-associated death domain protein, also called MORT1 (mediator of receptor-induced toxicity). As expected, in FADD-deficient embryonic fibroblasts, stimulation of the TNFR1 did not induce apoptosis [51]. Thus it would be anticipated that FADD-deficient mutant mice would show increased cell growth (apoptosis absent or lessened). Unexpectedly, FADD was required for the development of the ventricular myocardium [51]. Thus, at least in embryogenesis, an apparently pro-apoptotic path has a role in organogenesis and in embryonic survival. Therefore, whether the TNFα-mediated signal results in apoptosis or not may depend on a complex interaction between the various post-receptor signalling pathways, and the exact physiological milieu involved.

7.2. Concentration-dependent effects

The concentration of TNFα interacting with myocardial cells may also determine its homeostatic effect. In non-cardiac cultured cells, TNFα-induced apoptosis is concentration-dependent, with levels in excess of 0.1 ng/ml required to produce apoptosis [13]. In neonatal rat ventricular cardiomyocytes, TNFα also has a concentration dependent effect, partially to inhibit the phosphorylation of phospholamban and of troponin I [52]. Moreover, Franco et al. [53] have demonstrated, using magnetic resonance imaging and invasive techniques, that the rate and severity of TNFα induced left ventricular dysfunction is dependent on the degree of TNFα overexpression in transgenic mice. These data elegantly confirm the earlier observation by Kubota and colleagues [5,36].

Our preliminary data on the isolated rat heart (unpublished results) show that post-ischaemic recovery of function is improved by low concentrations of TNFα (0.5 ng/ml). Conversely, Nutt et al. [54] have demonstrated in the ex vivo heart that cardiac contractile function is markedly attenuated at higher concentrations of TNFα infusion (i.e. 20 ng/ml). In apparent conflict with our results, Cain et al. [55] found that severe simulated ischaemia for 45 min in the human heart resulted in increased production of TNFα during simulated reperfusion with adverse effects. Inhibition of the synthesis of TNFα by an inhibitor of p38 MAP kinase decreased production of TNF and improved post-ischaemic function. Differences from our data include the different means of production of ischaemia, the different duration of ischaemia and the species used. Hypothetically, we induced milder ischaemia with less production of TNFα, allowing a positive response to added low concentration of TNFα. Moreover, the question which needs to be answered is, whether TNFα’s role in inducing an acute reduction of cardiac contractility is adaptive and confers cardioprotection or whether it is maladaptive leading to long term dysfunctional sequelae.

8. Suppression of TNFα release — therapeutic potentials/pitfalls

The modulation of inflammatory and apoptotic pathways in the heart are thought to be potential therapeutic approaches for the treatment of myocarditis, cardiac ischaemia and heart failure [10]. TNFα modulation may be an integral component of both these inflammatory and apoptotic programmes, although other therapeutic targets include antagonists to reactive oxygen species, to nitric oxide synthase and to angiotensin II production. Treatments directly targeted at the attenuation of TNFα production include: the inhibition of p38/MAPK signalling pathway in an ex vivo ischaemia–reperfusion model, using SB203580 [55], and the use of pentoxyfylline in subjects with dilated cardiomyopathy [9]. In the latter pilot randomized study, Sliwa et al. [9] demonstrated that subjects with idiopathic dilated cardiomyopathy had reduced symptoms, increased left-ventricular systolic function and reduced plasma TNFα concentrations compared to matching placebo treated control patients. Additional therapeutic options for blocking TNF activity have recently been made clinically available and include monoclonal antibodies to TNFα, and the recombinant TNF soluble receptor [56]. The soluble TNFR–fusion protein has been used with reasonable efficacy as an adjuvant agent in the treatment of rheumatoid arthritis [57]. These therapeutic strategies are currently being evaluated in the treatment of heart failure and as a potential adjunctive therapy to control TNFα mediated cardiac cachexia. In a pilot study, Deswal et al. have demonstrated that the intravenous administration of a soluble p75 TNF receptor is well tolerated in humans with advanced heart failure [58]. In addition, this therapy seems to: reduce biologically active levels of TNF; enhance the ability of patients to exercise and improves the heart failure patients perception of quality of life [58].

However, these therapies should be viewed in the context of the contrary view initially proposed by Mann [8] and expanded on in this review. Our hypothesis states...
that the release of TNFα from the myocardium following acute/subacute haemodynamic and/or ischaemia–reperfusion biomechanical stress may activate signalling pathways that promote cardiac adaption and/or protection. If these adaptive actions of TNFα are proven correct, then there would have to be careful patient selection to ensure that ablation or antagonism of the function of TNFα would give beneficial rather than adverse effects. The hypothesis would also postulate that maladaptive excess TNF signalling for prolonged periods, as in severe inflammatory conditions (myocarditis) or during chronic heart failure, could be reduced with clinical benefit.

9. Proposed hypothesis for future work

In conclusion, we have reviewed data that suggest that the production of the locally acting cytokine TNFα by the heart may play a role in protective cardiac adaptation to acute or subacute haemodynamic and/or ischaemia/reperfusion induced biomechanical stress. Our working model for the role of TNFα in adaptive and maladaptive myocardial homeostasis is described in Fig. 2. The molecular mechanisms conferring these putative adaptive events are unknown and need to be investigated. Different local myocardial concentrations of the peptide may be important. Moreover, this hypothesis needs to be tested further with specific reference to the temporal activity, the response of the TNFα signalling path in different pathophysiologic events and the putative divergent pathways leading either to cardiac protection or to apoptosis.

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References


