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Review

Tumour necrosis factor alpha and the cardiovascular system: its role in cardiac allograft rejection and heart disease

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1. Introduction

Tumour necrosis factor alpha (TNFα) is a pleiotropic cytokine that has many proinflammatory actions with negative inotropic effects. It has been implicated in the pathogenesis of many non-infectious disorders, from rheumatoid disease [1], to multiple sclerosis [2]. This cytokine also affects the heart [3] where it is produced by immune cells and the myocardium in some diseases. Raised serum TNFα is seen in patients with cardiomyopathy, myocardial infarction, and chronic heart failure [4], thus implicating TNFα in disease pathogenesis. Raised serum levels of this cytokine have also been identified in transplant patients following episodes of acute cellular rejection. There is a correlation between TNFα expression and rejection grade [5] suggesting that the cytokine is a candidate marker of rejection.

In this review, we consider the basic biology of TNFα in relation to certain cardiac diseases. There are interrelationships between some of the conditions described, but these have been arbitrarily divided into three main groups: allograft rejection, coronary artery disease, and heart failure.

2. Basic biology and function of TNFα: a summary

Tumor necrosis factor alpha, cachectin (TNFα), was originally identified for its potent toxicity against tumour cells, hence its name [6]. Today, TNFα is recognised as a pleiotropic cytokine functioning within a complex and tightly regulated cytokine network. It activates multiple transduction pathways, inducing or suppressing a wide variety of genes, including those encoding the production of cytokines, adhesion molecules, and inducible nitric oxide synthase (iNOS). TNFα has many proinflammatory actions: orchestrating the inflammatory response through activation of proinflammatory cytokine genes, such as IL-1 and IL-6, as well as its own production [7]. This section will summarise our current understanding of the biology and function of TNFα with particular emphasis on the heart, and review recent work on the regulation of its production.

2.1. TNFα molecule

The TNFα molecule (a 157 amino acid polypeptide; M_r 17 356) exists as both a membrane bound and a secreted molecule, both bioactive [8]. TNFα acts at the cellular level via both type I (p55) and type II (p75) receptors. Both receptors have been localized in the human myocardium [9]. Activation by TNFα of different transduction mechanisms may operate through separate TNFα receptors. Both receptors can be up or downregulated under different pathophysiological conditions [9].

The activated macrophage is the main source of TNFα, containing both cell associated and membrane bound TNFα [10]. Analysis of the kinetics of TNFα secretion, demonstrated that bioactivity appeared after 2 h of macrophage culture stimulation, reaching a maximum by 4–8 h and disappearing by 12 h. The cytokine is rapidly synthesised and released on demand, and not stored in the cytoplasm. Other cells releasing TNFα include lymphocytes, fibroblasts, neutrophils, smooth muscle and mast cells. The ability of adult mammalian myocardial cells to release TNFα after endotoxin stimulation has been shown [11].

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2.2. Molecular regulation of TNFα production

The TNFα gene is one of the earliest genes to be transcribed after T lymphocyte activation. Transcription of mRNA does not require de novo protein synthesis, as do other cytokines, such as IL-2 [12]. There is a high baseline transcription of TNFα mRNA by comparison to other cytokines such as TNFβ [13].

Both positive and negative regulatory gene elements affect TNFα gene transcription [14], as well as other cytokines, such as GM-CSF and IL-4. These can differentially regulate transcription, through acting on upstream promoter elements [15].

Production of the TNFα gene product is regulated at the post-transcriptional level. The TNFα mRNA has a very short half-life (30 min) by comparison to the mRNA half-life of TNFβ (5.5 h) [13]. A conserved consensus sequence (UUAUUUAU) in the 3′ untranslated region of the mRNA destabilizes the mRNA and shortens its half-life [16,17]. Various degradative pathways can degrade mRNA [18], for example, via poly A tail removal [19]. These regulatory mechanisms limit the use of in situ hybridisation techniques in detecting mRNA transcripts. Messenger RNA's sensitivity to tissue fixation, can further result in false negative results. TNFα production is also subjected to translational control. Translational derepression, occurs following lipopolysaccharide (LPS) induction and the translational rates of TNFα mRNA are accelerated [18,20]. TNFα mRNA expression is not constitutive in adult myocardium, but induced [10,21]. However, constitutive expression has been documented in other normal tissues, such as the spleen, liver, and kidney [21,22].

2.3. Genetic variation in TNFα levels

Within the normal population, there is considerable variation in the amount of TNFα produced. Such variation is also seen when peripheral blood lymphocytes [23] and mononuclear cell populations [24] are stimulated with endotoxin [25,26]. Polymorphisms within microsatellite regions flanking the TNF locus have been identified [27,28]. Five microsatellite regions are described: TNF a, b, c, d and TNF-e. High levels of in vitro TNFα production from mononuclear cell populations are associated with the TNFa2 allele and low levels with TNFa6 allele, respectively. In addition, there are polymorphisms within the TNFα promoter region as follows: a point mutation of G to A in position -308 (termed TNF1 and TNF2, respectively) and in position -238 predisposes to higher TNF production. Studies using reporter gene assays have demonstrated an increase in the level of TNF gene transcription when the TNF2 allele is inserted [29–30]. Two additional G/A mutations at position -376 and -163 are described [31]. An insertional 'C' polymorphism is also described at position +70 [32] resulting in an 8'C' rather than 7'C' repeated sequence.

2.4. Effects of TNFα on cardiac function

Administration of large amounts of TNFα to the circulation causes shock due to a decrease in peripheral vascular resistance and direct cardiac effects [33]. Mann has argued that the net effect of TNFα on cardiac function will depend on the amount and duration of TNFα expression. Short term expression of TNFα within the heart may be an adaptive response to 'stress', whereas long term expression may be maladaptive by producing cardiac decompensation [34]. Excessive TNFα levels can produce left ventricular dysfunction [35], cardiomyopathy, and the clinical manifestation of heart failure [36,37]. TNFα can affect heart failure, in part, by stimulating myocyte hypertrophy, through the generation of reactive oxygen intermediates in cardiac myocytes [38], and also by inducing ventricular remodeling, through stimulating extracellular matrix protein production and increased turnover of matrix [35,39]. TNFα will cause cardiomyocyte loss, through necrosis, or apoptosis, as demonstrated in in vitro models. It can induce apoptosis directly, via the TNF receptor, or indirectly, through stimulation of nitric oxide (NO) production [40]. Evidence now suggests that cardiotropin-1, produced by cardiomyocytes, is able to inhibit cytokine-induced cardiomyocyte apoptosis in vitro [40]. The effects of TNFα on myocardial contractility and left ventricular dysfunction, were first demonstrated in animal models [41,42].

TNFα can depress myocardial function through two major pathways. The NO-dependent pathway [35,36,43], and the sphingomyelinase (sphingosine-dependent) pathway [44]. Activation of the NO-dependent pathway can induce negative inotropic effects on isolated cardiac myocytes, causing immediate cell contraction, through stimulation of iNOS production [45]. The resultant increase in NO will act as an important intracellular signalling molecule, that mediates the negative inotropic effects. Even though TNF has negative inotropic effects on isolated myocytes, its net effect on the whole heart is an increase in end diastolic pressure, resulting in increased ventricular volume. The induction of iNOS and its effects on cardiac function in allograft rejection, have been reviewed in detail [46].

Activation of the sphingomyelinase pathway will result in breakdown of the phospholipid, sphingomyelin, to its metabolites ceramide and sphingosine [47]. Both can act as second messengers in the signalling pathways. Edmunds and Woodward showed that the early increase in coronary perfusion pressure following TNFα treatment of rat perfused hearts, was due to early coronary vasoconstriction, mediated by sphingosine and thromboxane A2 [44].

TNFα, at low non-toxic concentrations, depresses cultured myocyte contractile performance independently of NO, through blocking α- and β-adrenoceptor-stimulated increase in contractility [35,48]. Several other studies have analysed the effects of TNFα on myocardial calcium handling as one mechanism of TNFα-induced contractile

Table 1
Summary of the effects of TNFα on the cardiovascular system

Process			Effect	Manifestation of condition
↑	Adhesion molecule expression MHC molecule expression Vascular permeability	≫	Increased immunogenicity, rejection response	Allograft rejection
	Activation of inflammatory cells And cytokine release	≫	Orchestration of the inflammatory response	Allograft rejection, CAD, CHF
↑	Turnover of matrix, Extracellular matrix proteins	≫	Ventricular remodeling	CHF
↑	Reactive oxygen intermediates	≫	Myocyte hypertrophy	CHF
↓	Disruption of calcium handling Uncoupling of β-adrenergic receptors Reduced inotropic response Left ventricular ejection fraction	≫	Contractile dysfunction Ventricular dysfunction	CHF, Allograft rejection
	Apoptosis	≫	Cardiomyocyte loss	Allograft rejection, CHF
↑ ↑	Reduce lipoprotein lipase activity Triglyceride levels, hepatic fatty acid synthesis Procoagulant activity	≫	Contribute to atheroma formation	CAD

CAD: coronary artery disease; CHF: congestive heart failure.

dysfunction [49]. TNFα-induced disruption of calcium handling may lead to dysfunctional excitation–contraction coupling causing systolic and/or diastolic dysfunction [37]. The observations of Schreiner suggest that the effects of TNFα on cardiac contractile cell function, are reversible, both in animal models, and in clinical manifestations of heart failure, including allograft rejection [35]. The actions of TNFα on left ventricular dysfunction were shown to be partially reversible in animal models following treatment with TNFα anatgonists [50]. The effects of TNFα are listed in Table 1.

3. Role of TNFα in allograft rejection

3.1. Allograft rejection: the need for a predictor

Acute cardiac allograft rejection is an immune-mediated response, hallmarked by cellular infiltration and myocyte damage in the transplanted heart. The infiltrate consists largely of T lymphocytes and macrophages. In more severe forms, polymorphonuclear cells and eosinophils are also recruited [51]. Both the CD4+ T lymphocytes and macrophages play a major role in directing the rejection response, through elaboration of initiator cytokines, such as TNFα, and the induction of effector molecules such as NO [52]. Moderate to severe rejection episodes may initially be asymptomatic in some cases, but are often followed by breathlessness, pyrexia, and raised intracardiac pressures (end-diastolic). Lesser grades of rejection have no clinical signs or symptoms. In the early 1970s, endomyocardial biopsy (EMB) was introduced, and is now the 'gold' standard for monitoring cardiac transplants. Rejection is graded histologically from mild to severe, depending on the extent and type of cellular infiltrate and degree of myocyte damage, according to the Working Formulation of the International Heart and Lung Transplant Study Group (ISHLT) [51]. The immunosuppressive regimen following transplantation is altered according to the grade of rejection and clinical symptoms. However, due to limitations of the biopsy procedure (such as sampling error), the risk of over-immunosuppression or under-immunosuppression remains a problem, particularly for the management of mild to focal moderate rejection (ISHLT grades 1 and 2, respectively) [53–55]. Hence there is a pressing need to identify a marker that can predict rejection. An alternative non-invasive method of diagnosis, such as serum analysis, would be of great benefit.

3.2. Involvement of TNFα in rejection

TNFα has many proinflammatory functions and hence has been implicated in the initiation and orchestration of the rejection response. The rejection response is initiated by activation of CD4+ T helper cells by alloantigen, either through direct stimulation by donor antigen presenting cells, or indirectly by recipient antigen presenting cells. Activated T helper cells will release initiator cytokines such as IL-1β, IL-2, and interferon γ, which in turn activate macrophages to release TNFα [56]. TNFα participates in initiating the response through upregulation of MHC molecule expression required for specific T cell activation and increased cellular infiltration through endothelial cell activation and adhesion molecule expression

[57]. TNFα will further maintain the inflammatory response within the rejection infiltrate through upregulation of adhesion molecules, increased vascular permeability, and activation of inflammatory cells [57] (Table 1).

Early suggestions that TNFα may play a role in the rejection response, came from two sources. Firstly, observations were made that cardiac [58], renal [59,60], and liver [61], transplant recipients, had raised serum levels of TNFα following rejection episodes, although no causal relationship was established. The second source of evidence came from animal models of acute cardiac allograft rejection. Anti-TNF antibody therapy prolonged cardiac allograft survival in the rat [62,63], although this was not demonstrated for allogenic skin in Rhesus monkeys [64]. In addition, localization of the protein and mRNA transcripts within the rejection infiltrate of both cardiac [5,65], and renal [66,67] allografts, provided supporting evidence for the role of TNFα in rejection. In cardiac allografts, the presence of protein TNFα correlated with higher grades of rejection [5]. When EMB were examined for both TNF mRNA transcript and protein product expression, using combined in situ hybridisation and immunohistochemistry [65], approximately half of the cases positive for TNF mRNA transcript were also positive for protein product. Although there was no correlation between expression and grade of rejection, there was a trend to an increased number of positive cells in samples taken early after transplantation.

Analysis of serum TNFα levels have shown that in most cases with elevated levels, the amount of TNFα in samples taken within the first 30 days post-transplantation did not relate to grade of rejection [65]. In another study, serum levels were significantly increased in patients with moderate to severe rejection ($P=0.001$) for samples taken in the first week post-transplantation [68]. Jordan et al. showed that for three patients with humoral allograft rejection, elevated levels coincided with rejection episodes [69]. However, in other studies [69–72], there was no association between serum TNFα levels and grade of cellular rejection. Grant et al. [71] found that when samples were taken after the initial 30 days of transplantation, (in order to overcome any stimulating effects of surgery, cardiopulmonary bypass, or RATG therapy on TNFα levels [73–76]), there was no demonstrable relationship between elevated levels of TNFα and grade of rejection. To assess whether the in situ expression of TNFα is reflected in the serum, the expression of TNFα in situ (for both mRNA and protein) was compared with serum TNFα levels [65]. Serum TNFα levels did not correspond with the number of TNFα positive cells/mm^2 within the biopsy ($r=0.126$; $P=$NS). Since TNFα is a locally acting potent cytokine, serum analysis will not accurately reflect in situ levels within a graft. Also, the methodology used in studies to detect intragraft TNFα mRNA and protein, suffer from a number of limitations and caution is necessary during interpretation. Since rejection is patchy, sampling error is the major disadvantage of the EMB technique [77,78], calculated at 2% if four samples are taken, and 5% if only three samples are taken [79].

Subject variation in serum TNFα levels have led to studies on the genetic control of TNFα production [80–81]. Turner et al. (1995), studied the relationship between TNFα gene polymorphisms and TNFα production in immunosuppressed heart transplant recipients. Endotoxin stimulation of whole blood samples demonstrated a significant association between the microsatellite allele TNFd3 with TNF production [80]. When TNFα gene polymorphisms are studied in relation to those of interleukin-10 (IL-10), (an immunosuppressive cytokine that down-regulates the expression of TNFα [81]), certain combinations of TNF-A and IL-10 promoter gene polymorphisms are associated with acute cardiac rejection. In patients with high levels of rejection, significantly more patients were typed as high TNFα/low IL-10 producers compared with patients of low rejection levels [81]. In addition, we have identified a subgroup of heart transplant recipients who suffered acute cellular rejection that did not resolve with immunosuppressive therapy and ultimately led to their death. TNFα genotyping of these cases has identified a significant increase in the frequency of the TNF2 alleles by comparison to the whole population of heart transplant recipients. More than 82% of acute rejectors had the TNF2 allele, by comparison to only 35% in the whole transplant population ($P<0.01$) [Azzawi, unpublished observations]. Although these genetic studies represent investigations into a few out of many different alleles for TNFα and its promoters, they provide early, but promising, findings.

3.3. Can TNFα be used as a predictor for rejection?

We showed a period of higher expression of TNFα early after transplantation, and suggest that its release may not be specific to rejection. Expression of TNFα following ATG (anti-thymocyte globulin) therapy [73,74] and ischaemia [75,76], have been well documented. ATG stimulates cytokine release, including TNFα [73,74]. Thus detection of cytokines during this stage may not be a reflection of rejection per se. Conversely, induction of TNFα mRNA transcripts can be blocked by immunosuppressive drugs, such as FK506 and cyclosporin [14]. Long term immunosuppression may therefore dampen down cytokine release. Our demonstration of the early, but not subsequent release of TNFα following transplantation, may reflect this process. Variations in immunosuppression protocols between centres may contribute to discrepancies in the results of cytokine studies.

The studies described in this review [5,60,61,68,69], have not analysed the relationship between intragraft TNFα expression and grade of rejection at subsequent biopsy occasions, when all contributing factors are excluded, including time post-transplant [5,68,72], ATG therapy [60], OKT3 therapy [61], and infection [68,69].

The detection of TNF protein in cardiac allografts in the absence of histological or clinical evidence of rejection [72] needs to be assessed in isolation from these contributing factors. When such factors are excluded, our own studies have shown that TNFα expression can be predictive of the next biopsy grade. Some cases with biopsies positive for the in situ protein expression of TNFα (over 70% of biopsies) will often have a higher grade of rejection on the subsequent biopsy occasion. This is true only for mild grades of rejection (ISHLT grades 1A and 1B). Larger numbers of samples need to be analysed to substantiate these findings. Our studies further concluded that intragraft expression of TNFα is not reflected in the serum, rendering the analysis of such levels unacceptable, as an alternative method of TNFα detection. Although the studies described in this review, do not establish whether increased TNFα expression is the 'cause' or 'effect' of rejection, the genetic studies indicate that stimulation of the inflammatory response (by alloantigen, ATG, or ischaemia) will result in large amounts of TNFα being secreted by individuals with a genetic predisposition to produce higher levels of TNFα.

4. Role of TNFα in coronary artery disease (CAD)

A large inflammatory component may exist within the atherosclerotic lesion of CAD. The inflammatory response is initiated by the presence of memory T cells specific to oxidised low density lipoprotein. The elaboration of inflammatory cytokines, such as TNFα, is orchestrated by monocytes transmigrating into the lesions, and differentiating into macrophages. TNFα affects lipid metabolism and hyper-triglyceridaemia by decreasing lipoprotein lipase activity in cultured adipocytes. [82] It increases hepatic fatty acid synthesis [83] and is associated with increased levels of triglycerides [84]. TNFα probably plays a pivotal role in obesity-related insulin resistance. This is by attenuation of insulin receptor signalling by decreasing both insulin-stimulated autophosphorylation and the tyrosine kinase activity of the insulin receptor in cultured adipocytes [85]. TNFα could contribute to the development of atheroma through its direct action on endothelial function, stimulation of growth factors and chemoattractants, as well as synthesis and stimulation of adhesion molecules. Finally, TNFα increases the risk of CAD by interfering with the thrombotic process by enhancing procoagulant activity (PAI-1, von Willebrand factor) and suppressing the antithrombotic protein C pathway in endothelial cells [86] (Table 1).

TNFα has been demonstrated in 88% of atherosclerotic lesions but is absent from normal tissues [87]. TNFα expression increases with the severity of the lesion, suggesting it may play a role in disease evolution [88]. Arbustini et al. [89] showed by Western blotting and immunohistochemistry that TNFα was present in lipid-rich plaques, with or without thrombosis. It was absent in normal control coronary arteries and present in minimum concentrations in a few fibrous plaques. Smooth muscle cells, macrophages and intimal cells were immunoreactive for this cytokine. Medial cells do not express TNFα mRNA, whereas 96% of intimal smooth muscle cells express this cytokine.

That TNFα expression in smooth muscle could be triggered by lipid intake was confirmed by electron microscopy [89]. Cultured human smooth muscle cells produce TNFα when incubated with low density lipoproteins [88]. TNFα mRNA is synthesised by smooth muscle cells and by macrophages [90]. The cytokine was present as a margin of lesions in reactive areas, where there was little lipid and fibrosis. Smooth muscle cells from plaques respond to both IL-1 and TNFα by manufacturing GM-CSF [91]. Cytokines may act in an autocrine fashion within a plaque, for example TNFα may induce expression of TNFα mRNA during monocyte differentiation [92].

Herrman et al. (1998) studied TNFα gene polymorphisms in relation to coronary artery disease and obesity [93]. The genotypic frequencies were similar in cases and controls in the high-risk populations in Belfast and France. However, the TNFα -308 A allele was more frequent in Belfast than in France and carriers of this allele were more frequently obese than non-carriers. Thus polymorphisms are unlikely to contribute to CAD risk but TNFα -308 polymorphism may be related to obesity. The TNFα -308 allele was associated with a parental history of myocardial infarction in both countries, but the lack of a case–control difference suggested this association might be spurious.

4.1. Coronary angioplasty

Circulating TNFα is not released during transient ischaemia in patients undergoing coronary angioplasty [94]. The liberation of TNFα during myocardial infarction probably reflects an acute phase response to myocardial necrosis rather than ischaemia. A study of cardioatherectomy specimens taken at the time of percutaneous transluminal coronary angioplasty (PTCA) and subsequent restenosis were examined, The lesions were clinically and angiographically similar with equivalent lumina diameter before and after atherectomy. Restenotic lesions had increased expression of TNFα and fibronectin compared with the primary lesions ($P = < 0.05$). There was a trend for a greater number of T cells and increased expression of IL-1 in restenoic areas. An immune-inflammatory reaction probably contributed to neointimal formation and may represent a form of wound healing, possibly secondary to mechanical injury [95]. In this study there were no significant differences in the relative amounts of fibrosis or cellularity when comparing primary atherectomy tissue with restenotic tissues.

Recent studies on coronary graft arteriopathy suggest there is a reciprocal co-induction of TNFα and IL-1β. Both

cytokines regulate endothelial and smooth muscle fibronectin synthesis [96]. Neutralisation of TNFα activity in rabbits after cardiac transplantation reduces both the severity and number of coronary artery lesions. This was associated with less inflammation and a reduced accumulation of fibronectin in the vessel wall [97].

4.2. Cardiopulmonary bypass and coronary artery grafts

A series of papers have studied cytokine levels following cardiopulmonary bypass. A study of two groups of patients, one with an ejection fraction of less than 0.45 (study group) and those of an ejection fraction of greater than 0.55 (control group) having coronary artery bypass grafting were investigated. The study and control groups did not differ with regard to age, sex, vessel involvement, number of grafts, cross-clamp time, extracorporeal circulation time, or duration of ventilation [98]. There were no complications in the control group. In the study group there were higher levels of IL-2, TNFα, and a higher maximum cytokine response to extracorporeal circulation for IL-2, soluble IL-2 receptor, IL-6 and TNFα. Preoperative left ventricular dysfunction is associated with a higher degree of proinflammatory cytokine release during elective coronary artery bypass grafting. Such a response is associated with impaired haemodynamics and a higher incidence of perioperative complications. This study examined arterial and mixed venous samples taken at ten points in time (24 h before, until 48 h after extracorporeal circulation). TNFα release proceeded IL-6 liberation. This investigation was carried under normothermic conditions, which could have contributed to the higher level of cytokine release [99], compared with hypothermia.

Other published data concerning TNFα release during extracorporeal circulation are conflicting. Other studies have given varying results [100–104]. Wan et al. [105] studied pro-inflammatory cytokines such as TNFα, IL-6, IL-8 and anti-inflammatory cytokines, such as IL-10, in patients undergoing cardiopulmonary bypass. The cytokines were measured by arterial, coronary sinus and pulmonary artery catheters. Levels of TNFα and IL-6 were significantly higher in coronary sinus than arterial blood after aortic declamping. TNFα and IL-6 levels were higher in mixed venous than arterial blood within 1 h of declamping. They confirmed the myocardium was a major source of TNFα and IL-6 in patients undergoing cardiopulmonary bypass. Removal of TNFα and IL-6 by haemofiltration has beneficial effects on children undergoing cardiopulmonary bypass [106] but the data do not exclude significant cytokine release from other organs. After cardiopulmonary bypass other organs may have an inadequate blood supply and could be an important source of such mediators. The release of endotoxin and complement activation, seen during bypass, could trigger cytokine release. The lungs release TNFα during ischaemia and it is known that during cardiopulmonary bypass the lung does suffer damage [107]. TNFα and IL-6 levels were significantly higher in mixed venous than arterial blood shortly after reperfusion [107]. The lungs were thought to consume rather than release pro-inflammatory cytokines in the early phase of reperfusion.

Meldrum et al. (1998) studied the levels of myocardial TNFα before and after cardiopulmonary bypass, and showed that it induced an increase in myocardial TNFα. They used immunolocalisation techniques to demonstrate that myocardial TNFα was locally produced, subsequent to bypass, by the myocytes themselves [76].

5. Role of TNFα in heart failure

Elevated circulating levels of TNFα and its soluble receptors have been demonstrated in advanced congestive heart failure (CHF) [4,42,108–110]. TNFα has various effects on cardiac function, leading to heart failure (discussed in Section 2.4). TNFα may thus have a causative role. Mann [34] has argued that as TNFα is produced following all forms of cardiac injury, it may act as a ‘stress response’ gene in the heart. Using whole mammalian hearts, TNFα mRNA can be induced within 30 min of a stressful stimulus with endotoxin challenge. TNFα mRNA levels returned to baseline levels soon after removal of the stimulus. TNFα stimulation of cultured cardiac myocytes resulted in increased heat shock protein (HSP 72) expression, which was completely abolished by a neutralising antibody against TNFα [111]. Very high concentrations of TNFα, within the pathophysiological range, resulted in lower levels of HSP expression [34].

Immunoreactivity for NO has been demonstrated in dilated cardiomyopathy [112]. Tissue was obtained from patients with end stage CHF undergoing primary orthotopic cardiac transplantation or endomyocardial biopsy. Inducible NOS was strongly expressed in myocytes in dilated cardiomyopathy, especially in subendocardial areas, and may contribute to the low contractility and the thromboembolic tendency in these patients. Dilated cardiomyopathy may have an inflammatory component and this will contribute to the pathogenesis and consequent cytokine production and iNOS induction [113]. The increased levels of iNOS in cardiomyopathy coincided with an abundant source of TNFα, not present in ischaemic heart disease or normal myocardium. There is no increase in concentrations in IL-1α, soluble IL-2 receptor or TNFα concentrations in mild to moderate heart failure versus controls. In myocarditis, dilated cardiomyopathy and hypertrophic cardiomyopathy, TNFα was increased in up to 46% of patients in some groups [114]. TNFα may be raised in less than 25% of patients with acute myocardial infarction with large infarcts complicated by hypotension and pulmonary oedema [106].

Familial hypertrophic cardiomyopathy has been associated with HLA-DR4 and with other major histocom-

patibility complex haplotypes [115,116]. Dilated cardiomyopathy is associated with HLA-DR4 and HLA-DQw4 [117]. Thus raised concentrations of plasma TNFα in hypertrophic and dilated cardiomyopathy are probably associated within a major histocompatibility index. Kubota et al. (1998), however, were unable to identify any association between TNFA nor TNFB polymorphisms, and the elevation of circulating TNFα levels associated with congestive heart failure [118].

The stimulus for increased TNFα production in patients with CHF remains unclear [109], but maybe related to the local ischaemia associated with the reduced blood flow. In vitro experiments on perfused rat hearts suggest that the resultant oxidation products (H_2O_2) may activate a P38 mitogen-activated protein kinase (MAPK) which in turn induces TNFα production [119]. The suppression of this production by adenosine suggests a possible intrinsic mechanism for cardiac protection [120].

6. Conclusion

TNFα is a potent cytokine which acts not just on coronary arteries but also on myocardial fibres. Although TNFα cannot be used as a marker of allograft rejection, per se, it might be useful as a general indicator of 'stress' or damage. TNFα may act in concert with other cytokines during the rejection response, and may contribute to the development of atheroma in CAD. The association of TNFα with heart failure is apparent, although further study is necessary to establish any causal relationship.

Immunomodulation by antibodies to cytokines have not lived up to their promise. This may be because they are not given at the correct time clinically. Also, expecting a single cytokine to have an effect when it is part of an 'orchestra' is overoptimistic. The recognition of individuals with a genetic predisposition to high TNFα production, is necessary, and may require its assessment in relation to the overall cytokine genotype, in order to enable closer surveillance of high risk individuals.

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