Review

NO-cGMP and TNF-α counter regulatory system in blood: Understanding the mechanisms leading to myocardial dysfunction and failure

Maqsood Elahi, Sanjay Asopa, Bashir Matata

* Wessex Cardiothoracic Centre, Chalybeate Close, Southampton, SO16 6UY, UK
b The Cardiothoracic Centre, Liverpool NHS Trust, Thomas Drive, Liverpool L14 3PE, UK

Received 6 July 2006; received in revised form 1 September 2006; accepted 5 September 2006

Abstract

One of the major conceptual advances in the understanding of the pathogenesis of heart failure has been the insight that myocardial dysfunction and heart failure may progress as the result of the sustained over-expression of nitric oxide (NO) metabolites locally and in blood modulated by inducible nitric oxide synthase (iNOS). This by virtue of their deleterious effects is sufficient to contribute to disease progression by provoking left ventricular (LV) remodeling, hypertrophy and progressive LV dysfunction. Recently, tumor necrosis factor-alpha (TNF-α) has also been identified in this setting of heart failure. Analogous to the situation with NO, the over-expression of TNF-α is sufficient to contribute to disease progression in heart failure phenotype. Although important interactions between TNF-α and the NO have been recognized in the cardiovascular system for over a decade, the nature and importance of the interactions between these biologically active molecules in cardiac hypertrophy has become apparent only in the recent times. Therefore, we focused on the prevailing updated evidence which suggests that there is a functionally significant cross-regulation between NO and TNF-α signaling in blood thus playing a part in cardiac hypertrophy and failure. The discussions presented here will have a bearing on the therapeutic potential via inhibitors of these pathways in reducing cardiomyocyte hypertrophy and the LV dysfunction.

© 2006 Elsevier B.V. All rights reserved.

Keywords: TNF-α; Proinflammatory cytokines; Nitric oxide; Cardiomyopathy; Left ventricular function

1. Introduction

In light of the data painstakingly gathered over the last 10 to 12 years, the relevance of the well-established experimental models of myocardial dysfunction and failure overload to human cardiovascular disease remains perplexing [1–4]. Typically heart failure is the final culmination of protracted disease states (Fig. 1) precipitated by underlying hypertension, ischemic changes and atherosclerosis, valvular insufficiency, viral myocarditis or mutations in genes encoding sarcomeric proteins [5]. However, with the advent of the unifying understanding of the redox-inflammation interactions via gain- and loss-of-function approaches, a reductionist investigative approach for defining the pathways and processes of reactive myocardial adaptation became possible [discussed in ref. [6]. In summarizing the literature there is almost a consensus that augmentation, inhibition, or ablation of specific signaling events through targeted gene deletion or over-expression as being responsible for the various degrees of myocardial failure [7–9].

Our understanding of the pathogenesis of the myocardial dysfunction has evolved from viewing cardiac failure as an illness resulting from the “defective pump” to the new concept that one of the major determinants of the progression of heart failure as the persistent over activation of various compensatory systems involving reactive oxygen species (ROS) [10,11]. In addition, other emerging evidence indicates that under conditions of oxidative stress, the activation of the inflammatory system occurs, resulting in the production and release of proinflamma-
Cytokines, activation of the complement system, production of autoantibodies and over-expression of class II major histocompatibility complex molecules as well as adhesion molecules that may perpetuate the inflammatory state [12–14].

Tumor necrosis factor-alpha (TNF-α) though traditionally considered cardio-depressant mediator, yet its contribution in myocyte contractile responses is complex and bimodal, with an early response of variable direction, (stimulatory or depressant), depending on the ambient physiologic milieu and the relative contributions of other underlying signaling pathways especially nitric oxide (NO) [15,16]. This is subsequently followed by a profoundly late cardio-depressant response lasting hours to days depending on the production of secondary mediators and the combined influence of NO generated from inducible nitric oxide (iNOS), ROS and alteration in β-adrenergic receptor signaling. Supportive evidence comes from the fact that partial blockade of the rennin-angiotensin and β-adrenergic systems leads to improved cardiomyocyte survival [17–19]. Hence, the interrelationships between these pathways and the time dependence of their activation are important considerations in the evaluation of cytokine-dependent dysfunction during both acute cardiac injury and chronic pathologies (Fig. 1). Certainly, the stimuli are entirely different, as are the cellular responses and it is not yet known what the important relevant pathophysiological events are but accumulating data suggest that they differ substantially [20–22]. Additionally, considering that many of the data, by necessity, collected using genetically altered mice, the basic differences between mice and humans with respect to heart rate, cardiac reserve, and strain variations mitigate against easy comparisons [discussed in ref [23].

Hence, the major focus of this review will be the regulation and effects of TNF-α and NO in the modulation of myocardial function and their potential involvement in the strain induced mechanotransduction mechanism summarized in Fig. 2. The discussion will provide rationale for the sometimes-conflicting results in the literature regarding the underlying mechanisms and patterns of myocardial dysfunction.

1.1. TNF-α induced myocardial dysfunction

Accrued evidence indicates that cytokines are important mediators of cardiovascular disease. A working understanding of inflammatory cytokines and their relationship to myocardial disease is of growing importance to basic and clinical cardiovascular scientists, immunologists and clinicians. In this regard, TNF-α is a proinflammatory cytokine that has been
Implicated in the pathogenesis of cardiovascular diseases including acute myocardial infarction, chronic heart failure, atherosclerosis, viral myocarditis, cardiac allograft rejection and septic cardiac dysfunction [24–27]. Initially, TNF-α was described as predominantly the product of lipopolysaccharides-stimulated macrophages where NF-κB activation is necessary for the induction of iNOS and expression of TNF-α. Evidence now indicates that cardiac myocytes themselves produce substantial amounts of TNF-α in response to ischemia [28].

The intracellular signal pathways that provoke TNF-α production are being elucidated with increasing clarity. Evidence from a transgenic mouse model indicated that TNF-α over-expression causes shock due to a decrease in peripheral vascular resistance and direct cardiac effects [29]. Mann and his colleagues have argued that the net effect of TNF-α on cardiac function will depend on the amount and duration of TNF-α expression [29]. Short-term expression of TNF-α within the heart may be an adaptive response to ‘stress’, whereas long-term expression may be maladaptive resulting in cardiac decompensation. Several investigators have also demonstrated that infusions of micromolar concentrations (40–200 μg/kg) of TNF-α can produce left ventricular dysfunction [30,31], cardiomyopathy, and the clinical manifestation of heart failure. In addition, TNF-α can affect heart failure, in part, by stimulating myocyte hypertrophy, through the generation of ROS intermediates in cardiac myocytes, and also by inducing ventricular remodeling, through stimulating extracellular matrix protein production and increased turnover of the matrix metalloproteinases [32,33]. TNF-α can also cause cardiomyocytes loss, through necrosis, or apoptosis, as demonstrated by in vitro models. It can induce apoptosis directly, via the TNF receptor, or indirectly, through stimulation of NO production [15,34].
It is suggested that TNF-α can depress myocardial function through NO-dependent pathways [35]. Activation of the NO-dependent pathway can induce negative inotropic effects on isolated cardiac myocytes, causing immediate cell contraction, through stimulation of iNOS production [36]. The resultant increase in NO will act as an important intracellular signaling molecule that mediates the negative inotropic effects. Reports have recommended that TNF-α, infused at low non-toxic concentrations (~2.5 μg/kg), depresses myocardial contractile performance independently of NO, through blocking α- and β-adrenoceptor-stimulated contractility [discussed in ref [37]. Several other studies have analyzed the effects of TNF-α on myocardial calcium handling as one mechanism of TNF-α-induced contractile dysfunction [38,39], TNF-α-induced disruption of calcium handling may lead to dysfunctional excitation–contraction coupling causing systolic and/or diastolic dysfunction. The actions of TNF-α on left ventricular dysfunction were shown to be reversible in animal models [40–43].

1.2. Effects of NO on inducing left ventricular dysfunction

NO imparts cardiac effects via two general signaling modalities: (1) the activation of soluble guanylate cyclase and the subsequent formation of cGMP, which in turn activates protein kinase G (PKG) and PKG-dependent phosphorylation events; and (2) the direct oxidation of thiol residues on critical regulatory proteins (S-nitrosylation) [44,45]. At nanomolar concentrations of NO, levels that are lower than those required to promote S-nitrosylation, this blocks mitochondrial respiration through direct and sensitive inhibition of cytochrome-c oxidase (complex IV of mitochondrial respiration) [46]. Since myocytes are cells packed with mitochondria, bioavailability of NO at nanomolar concentrations could have significant implications on myocardial function [46]. These events induce complex myocardial functional responses that include bimodal, concentration-dependent effects on contractility and the Ca\(^{2+}\) transient, desensitization of the myofilaments to Ca\(^{2+}\), and suppression of mitochondrial respiration. A large number of studies, performed in a variety of experimental models, have examined whether NO, contributes to early cytokine-induced contractile dysfunction. These investigations have yielded variable (and sometimes conflicting) results. In a study of isolated, superfused hamster papillary muscles, Finkel et al. [15] demonstrated that relatively high concentrations of TNF-α, profoundly depressed contractile function within 5 min, and that these effects were fully reversible on washout. The nonspecific NOS inhibitor \(N^{\omega}\)-monomethyl-L-arginine blocked these effects, a response that was overcome by the addition of excess L-arginine. The rapid time course and reversibility of these effects implicated the activation of myocardial NOS. Since this initial report, several additional studies have supported a role for NO in the early negative inotropic effects of the TNF-α and/or IL-1β (alone or in combination) in adult and neonatal mammalian myocytes, guinea pig, and rat muscle strips, isolated crystalloid-perfused rat hearts, and human atrial trabeculae [47–52].

In addition to acute contractile dysfunction, NO has been linked to a variety of functional abnormalities in the depression of the Ca\(^{2+}\) transient [53,54], TNF-α-mediated reductions in myofilament Ca\(^{2+}\) sensitivity [47], and the deterioration of mechanical efficiency induced by a combination of IL-1β, TNF-α and IFN-γ [52]. Several authors have further shown that these NO-mediated responses were cGMP dependent, requiring activation of soluble guanylate cyclase [49,53]. Importantly, Kumar et al. [49] demonstrated that although TNF-α and IL-1β increased the myocyte generation of NO gas, there was no difference in Ca\(^{2+}\)-dependent (or independent) NOS activity measured in vitro. This suggested that cytokines impact NOS activity by regulating cofactor and substrate conditions rather than via increases in abundance or fixed post-translational enzyme modifications. The above-mentioned studies have evaluated a wide range of cytokine concentrations (picomolar to nanomolar) and have uniformly used pharmacological NOS blockade and/or NO scavengers to probe the role of NO [55,56]. To date, no studies have been performed in mice with genetic ablation of the NOS isoforms, an investigation that would provide the most conclusive delineation of the role of NOS in early cytokine-mediated contractile dysfunction.

Although these studies persuasively argue that NO has an important role in the pathogenesis of acute cytokine-mediated contractile dysfunction, they stand in direct contrast to several reports that dispute this concept [54,57–59]. In isolated adult feline left ventricles and cardiomyocytes, Yokoyama et al. [59] demonstrated that TNF-α-dependent immediate reductions in contractility and Ca\(^{2+}\)-transients were not accompanied by increased NO oxidation products or cGMP production. Furthermore, the contractile dysfunction was not prevented by two different pharmacological NOS inhibitors. Other studies examining TNF-α and/or IL-1β in isolated rat hearts and guinea pig myocytes have similarly failed to show an acute increase in NO oxidation products or cGMP and have also reported no effect of NOS inhibition on early cytokine-mediated cardio-depression [57,58]. The basis for the discrepancies between these studies is not entirely clear but may be related to species differences and differences in experimental models and conditions. Importantly, the magnitude of NOS activation may be cytokine specific. In this regard, Sugushita et al. [54] have demonstrated that in guinea pig myocytes, whereas both IL-6 and TNF-α reduced cell shortening and peak systolic Ca\(^{2+}\), NOS blockade prevented IL-6-mediated effects but had no effect on the response to TNF-α.

1.3. TNF-α depress cardiac efficiency via nitrosative mediated mechanism

Some studies on isolated cardiomyocytes have described a delayed cytokine-mediated depression of basal unstimulated contractility, an observation that is in agreement with results from other models such as in the left ventricles (LV) of isolated hearts or in whole animals, multicellular preparations such as papillary muscles and atrial trabeculae, and in transgenic mice with cardiac-specific over-expression of TNF-α [65–67]. Most studies have implicated cytokine-mediated induction of iNOS.
gene expression, de novo iNOS protein synthesis, and increased NO generation in the depression of basal mechanical function [68,69].

In this context, modulation of basal function by NO may be more dependent on S-nitrosylation of thiol residues of regulatory proteins [60]. Consistent with this notion, studies in isolated myocytes have demonstrated that the late contractile dysfunction (24 to 48 h of exposure) induced by TNF-α is linked to NO-dependent depression of the Ca\(^{2+}\) transient. In addition, within a similar time frame interleukin (IL)-1 beta (IL-1β) causes an NO-dependent (but cGMP-independent) inhibition of oxidant-sensitive mitochondrial enzymes and the inward Ca\(^{2+}\) current resulting in the inhibition of mitochondrial respiration, energy depletion, and depressed contraction [61–64]. As S-nitrosylation is a redox-driven event, ambient levels of ROS significantly modulate the ensuing covalent protein–thiol modifications and the ultimate balance between nitrosative and oxidative stress. If TNF-α stimulation induces high-output iNOS, the high levels of NO generated would be expected to result in secondary oxidative modifications in the presence of superoxide. Studies have demonstrated an inducible NO production originating from within the heart, and that TNF-induced NO mediates alterations in cardiac contractility, although the cytotoxic potential of NO with respect to the heart has yet to be defined. Evidence from studies [63] suggest that increased activity of an inducible nitric oxide synthase (iNOS; type 2 NO synthase) in primary isolates of adult rat ventricular myocytes after exposure to soluble mediators in medium conditioned by lipopolysaccharide-activated macrophages is associated with a decrease in their contractile responsiveness to beta-adrenergic agonists. However, the studies failed to clarify specifically which inflammatory cytokines in the medium contributed to the induction of iNOS activity in myocytes and whether the induction of iNOS would result in an obligatory decline in contractile function. In this context, IL-1 beta and TNF-α were both reported to be present in the lipopolysaccharide-activated macrophage-conditioned medium and therefore it is not clear whether both cytokines were equally as effective in induction of iNOS.

Indeed, as in above, several studies have established that cytokine-mediated contractile dysfunction results not only from increased iNOS-derived NO but also from increased ROS generation particularly from peroxynitrite formation. Using isolated working rat hearts perfused with a Krebs–Henseleit solution containing a combination of cytokines (5 ng/mL human IL-1β, 9 ng/mL rat IFN-γ, 20 ng/mL human TNF-α), Ferdinandy et al. [65] demonstrated a marked decline in myocardial contractility over 20 min. This was preceded by an increased activity of superoxide sources such as xanthine oxidoreductase and NAD (P) H oxidase pathways together with an enhanced activity of iNOS. In addition, there was an increased myocardial contents of NO, superoxide, and the peroxynitrite markers nitrotyrosine and dityrosine. Furthermore, cardiac dysfunction and nitrotyrosine levels were inhibited by concomitant administration (individually) of a NOS inhibitor, superoxide scavenger, and peroxynitrite decomposition catalyst.

Studies using an in vivo canine model of IL-1β-mediated chronic cardiac dysfunction have similarly demonstrated the importance of superoxide and peroxynitrite [60]. Coronary injection of IL-1β coated microspheres (but not control microspheres) resulted in increased myocardial superoxide, nitrotyrosine, and persistent LV dysfunction at 7 days, effects that were prevented by concomitant treatment with dexamethasone, iNOS inhibition with aminoguanidine, or pharmacological inhibition of superoxide production [65]. These authors also suggested that inflammatory cells infiltrating in response to cytokines may contribute to superoxide generation as inhibition of the adhesion molecule P-selectin ameliorated the LV dysfunction [66]. These findings are consistent with the demonstration that cell-to-cell contact between inflammatory cells (macrophages) and myocytes enhances the delayed contractile dysfunction induced by the TNF-α because of the production of ROS and NO [49]. As oxidative modifications attributable to peroxynitrite would tend to be irreversible, post hoc inhibition of NO generation would not be expected to reverse the mechanical dysfunction. Indeed, whereas NOS inhibitors given concurrently with cytokines improve the delayed contractile dysfunction that is manifested in vivo NOS inhibition after the establishment of cytokine-mediated dysfunction does not acutely reverse the loss of contractile function [57,67].

1.4. INOS-TNF-α intracellular signaling in heart failure

Mann et al. have argued that as TNF-α is produced following all forms of cardiac injury, it may act as a stress response gene in the heart [68]. Using mammalian hearts, TNF-α-mRNA was induced within 30 min of a stressful stimulus with endotoxin challenge and TNF-α-mRNA levels returned to baseline levels soon after the removal of the stimulus [69,70]. TNF-α stimulation of cultured cardiac myocytes resulted in increased heat shock protein (HSP 72) expression that was completely abolished by a neutralizing antibody against TNF-α [71]. Very high concentrations of TNF-α (>100 U/mL) in physiological condition resulted in lower levels of HSP expression via the modulation of the iNOS activity [71]. Similarly, immunoreactivity for NO has been demonstrated in myocardial dysfunction and that the increased levels of iNOS in heart failure coincided with elevated TNF-α level (>50 U/mL) not present in ischemic heart disease or normal myocardium [31]. However, the stimulus for this mechanism in patients with chronic heart failure remains unclear, but may be related to the local ischemia associated with reduced blood flow. In vitro experiments on perfused hearts suggest that the resultant oxidation products (H₂O₂, ONOO⁻, O₂⁻, etc.), CREB/activating transcription factor (ATF) family, Ser133 phosphorylation products may induce TNF-α production. Ser133 phosphorylation products are mediated by a variety of protein kinase pathways such as (1) protein kinase A (PKA), (2) Ca\(^{2+}\)/calmodulin-dependent protein kinase (CaMK) II, (3) extracellular signal-regulated kinase (ERK), (4) p38 mitogen-activated protein kinase (p38MAPK), and (5) phosphatidylinositol 3-kinase (PI3K) may activate a P38 mitogen activated protein kinase (MAPK) [72].
Hitherto, multiple intracellular pathways that participate in the signaling cascade during heart failure may contribute to the production and release of macrophage-derived proinflammatory mediators. Under normal circumstances, lipopolysaccharides (LPS) interaction with CD14 is an obligate trigger of the intracellular signals that transmit LPS-induced TNF production. LPS–CD14 interaction provokes rapid activation of protein tyrosine kinase (PTK) causing tyrosine phosphorylation of several intracellular protein kinases [73,74]. PTK activates a pathway involving Ras/Raf-1/mitogen-activated protein kinase kinase (MEK)/MAPK/NF-κB [75]. Several studies have demonstrated that LPS activates PTK and that PTK inhibition abolishes downstream activation of MAPK, TNF and IL-1 production, and macrophage-mediated tumoricidal activity. An early target of activated PTK is Ras that is able to interact directly with Raf-1 [76], which is an important intermediate of MAPK activation. Studies by Reimann and colleagues [76] demonstrated that LPS resulted in PTK-dependent rapid phosphorylation and activation of Raf-1. These findings were supported by Geppert and associates [77], who observed that transfection of the dominant-negative repressors of Ras and Raf-1 into macrophages, resulted in a suppression of LPS-induced activation of the TNF promoter. However, transfection with a constitutively active form of Raf-1 did not reproduce LPS-induced macrophage activation, suggesting that Raf-1 activation is necessary but not sufficient to induce macrophage TNF production. Raf-1/MEK appears to activate members of the MAPK family of protein kinases; of these the P38 MAPK appears to be a pivotal MAPK in the cascade leading to TNF gene induction [78]. Thus LPS interaction with CD14 may lead to rapid intracellular tyrosine phosphorylation of Ras, a process that initiates the protein kinase cascade leading to NF-κB activation and TNF production during heart failure.

1.5. Evidence from in vivo studies

The view that proinflammatory cytokines induce LV dysfunction was first gleaned from animal and human studies of sepsis. These established circulating myocardial depressant substances, later identified, in large parts, caused that sepsis-associated cardiac dysfunction to be inflammatory cytokines (including TNF-α, IL-1β, IL-6, IL-2, and interferon-γ [IFN-γ]) [26,27]. In intact animals, intravenous administration of TNF-α [79–81] recapitulated the hemodynamic changes of endotoxemia and endotoxic shock, and anti-TNF-α antibodies [25] ameliorated the cardiovascular abnormalities and mortality. In addition to these insights from studies of sepsis, it was noted that immunomodulatory therapy for cancer using TNF-α was limited by dose-dependent cardiovascular depression and negative inotropy (1 to 200 μg/m² body surface area by alternating intramuscular and intravascular bolus injections) [82,83]. These observations were extended by studies in rats demonstrating that sustained infusion of TNF-α caused time-dependent contractile dysfunction and LV dilatation that was partially reversed by stopping the infusion or on concomitant treatment with a TNF-α antagonist [31]. This concept was subsequently confirmed by the observation that cardiac-specific TNF-α over-expression in mice induced LV dilatation, reduced ejection fraction, depressed catecholamine responsiveness, and induced premature mortality [32].

Several studies have specifically evaluated the in vivo effects of cytokines on cardiac function, often using TNF-α. Early studies in conscious dogs indicated that a single infusion of recombinant human (rh) TNF-α resulted in the appearance of LV systolic dysfunction 2 h to 2 days post-infusion as assessed by the load-sensitive index LV ejection fraction that could persist for up to 10 days, depending on the dose administered [81]. The decline in left ventricular ejection fraction was not normalized by volume resuscitation, suggesting intrinsic cardiac dysfunction rather than a preload-dependent response. Subsequent investigations using LV pressure-volume (or pressure-dimension) indexes confirmed the delayed onset of LV dysfunction following TNF-α infusion (from 2 to 24 h after initiation of the infusion, depending on the contractile parameter examined), as assessed by the relatively load-independent parameters end-systolic elastance (Ees), preload-recruitable stroke work (PRSW), or peak dP/dt normalized for end-diastolic volume (EDV) [40–42]. Reduced LV performance has also been associated with several diastolic abnormalities including slowing of relaxation, LV dilatation without changes in end-diastolic pressure (myocardial creep), and a leftward shift of the end-diastolic pressure–strain relation, indicating increased diastolic stiffness. Generally, cytokine-mediated contractile dysfunction is reversible over an extended time period of several days following exposure [31,81].

In contrast, the few studies that have examined the immediate contractile effects of cytokines in vivo have generally reported a distinct early stimulatory effect of TNF-α on inotropy and lusitropy [40,41]. Specifically, within the first 1 to 3 h after TNF-α administration, there is an augmentation of several contractility indexes including Ees, the slope of the dP/dmax–EDV relation, and dP/dmax normalized for EDV, together with a leftward shift of the PRSW relation and a reduction in the time constant of relaxation [41]. Importantly, the increase in contractility and lusitropy was not related to variations in heart rate or to cardiovascular reflex responses, as they persisted in the presence of β-AR and vagal blockade, and occurred before the maximal surge in circulating catecholamines [41].

Thus, taken together, the aforementioned in vivo studies indicate that TNF-α induce a biphasic effect on intrinsic cardiac function comprised of an early positive inotropic and lusitropic effect of relatively short duration, followed by a delayed and prolonged phase of profound systolic and diastolic dysfunction. The rapid onset of the early stimulatory response as early as 5 to 15 min in one study [41] suggests a direct myocardial effect via mechanisms not requiring gene expression, whereas the delayed onset and prolonged duration of the late cardiodepressant response suggest an indirect effect requiring the induction of de novo gene expression and protein synthesis and the activation of secondary mediators. Generally, proinflammatory cytokines can be considered to impart negative inotropic and cardiodepressant effects in pathophysiological states with sustained, chronic augmentation of cytokine expression.
1.6. Evidence from in vitro and ex vivo studies

Analogous to investigations in vivo, a biphasic contractile response has also been observed in single myocyte, muscle strip, and isolated heart preparations, with early effects generally within 30 min and delayed effects occurring thereafter up to 48 to 72 h. These studies have uniformly reported delayed cytokine-mediated depression of either basal [57,67] or stimulated [63,64] myocardial function, but have reported conflicting results regarding the immediate effects, with the preponderance of in vitro and ex vivo studies also indicating early cytokine-induced cardio-depression. Pressure-volume studies of the isolated, blood-perfused canine heart have shown that TNF-α acutely impacts LV mechanoenergetics by increasing the oxygen cost of contractility, an effect ascribed to alterations in energy utilization for excitation–contraction (E–C) coupling [84]. Coronary vasconstriction also contributes indirectly to TNF-α-induced early [63,64] and late [67] contractile dysfunction in isolated, crystalloid-perfused rat hearts.

The basis for the sometimes discrepant results in the aforementioned studies regarding the immediate inotropic effects of cytokines is not fully clear but may be related to several factors like single myocyte, papillary muscle, and isolated heart models which can inherently be less physiological than the intact state, which is subject not only to direct myocardial effects but also to complex and integrated vascular, neural, hematologic/immunologic, and endocrinologic changes effected by the TNF-α. From an alternative physiological context, TNF-α primarily acts locally to induce effects that are determined not only by the target cell type but also by the prevailing cellular and biological milieu [85]. Furthermore, TNF-α are generally not elaborated in isolation but rather as part of a complex network involving multiple other cytokines and biologically active molecules [20,21,26]. As TNF-α can have additive, synergistic, or antagonistic effects [26,80], this will complicate the responses seen in vitro or in blood-perfused ex vivo models, such that the end-effects may not be determined solely by the index cytokine. Also the ambient cellular redox and/or metabolic state and impacted NO bioavailability should be considered [86] under the circumstances. Therefore, careful attention needs to be focused on the experimental methodology and cytokine concentrations used. *In vitro* studies perspective suggest that an immediate positive inotropic effect (when reported) generally occurs very early (within 5 to 15 min) after exposure and at lower cytokine concentrations [67,86] whereas negative inotropic effects become pronounced with longer periods of exposure and/or higher concentrations. Finally, Stamm and co-workers have reported significant species-specificity with regard to the inotropic effects of recombinant cytokines; this factor may also account for some of the variability among studies that have used cross-species sources of cytokines [87].

1.7. TNF-α induced modulation of cardiac function: specific target for intervention

Normal myocardium does not contain TNF-α, but expresses both its receptors-TNF receptor types 1 and 2. However, in failing myocardium, there is increased expression of TNF-α and the receptors for TNF-α are downregulated as discussed earlier. Furthermore, in the patient with heart failure, circulating levels of TNF-α are elevated and increased levels are found as the patient’s heart failure (HF) worsens [discussed in ref [88]. These observations in humans as well as evidence in experimental animals that TNF-α was capable of inducing reversible myocardial dysfunction resulted in the evaluation of the new neurohormonal pathways as a target for therapeutic intervention. Indeed, pilot studies have shown that pharmacologic blockade of the biologic effects of TNF-α in human beings was associated with improved functional status as well as with improved myocardial infarction [89,90]. With these positive results, clinical (RENAISSANCE and RECOVER) trials depicted findings in patients with moderate to severe heart failure treated with anti-TNF-α blocking agents (Etanercept and Infliximab). Hitherto, the clinical results of these trials were disappointing. Two important notions could possibly explain this conflicting paradigm; Firstly, studies on isolated myocytes have shown that TNF-α acts as a growth stimulus and protects myocardial cells from hypoxia and helps to retain systolic function. Thus it is likely that systolic protection in heart failure is not the direct result of cardiac TNF-α expression. Hence in patients with established HF, anti-TNF-α therapy may no longer have the ability to change outcomes because injury at that stage is not dependent on TNF-α expression only. Once persistent activation of TNF-α results in hypertrophy and dilation, therapy would only put patients at risk of potential side effects of the therapy, with no potential clinical benefit. Secondly, the immune system is redundant after HF is established and other proinflammatory cytokines (IL-1, IL-6) known to be elevated can participate and or substitute for the absence of TNF-α in the anti-TNF-α treated patients.

1.8. Unresolved issues and perspectives

Although the NO-cGMP-TNF-α axis has been shown to attenuate cardiac hypertrophy, full therapeutic exploitation of this system faces at least two challenges, the first being concomitant untoward events. For NO, these would include reaction with superoxide, negative inotropic effects, and depressed mitochondrial respiration. Addressing these issues requires consideration of features of cardiac NOS such as isoform identity, spatial confinement, and substrate and cofactor availability. A better understanding of myocardial factors that protect against nitrosative stress is also needed. For anti-TNF-α, which are used in treating heart failure, receptor downregulation or uncoupling may limit their effectiveness in restricting cardiac hypertrophy.

The second challenge arises from the observation that although maladaptive signaling leading to LV hypertrophy may be inexorably linked to apoptosis and heart failure, survival pathways are likely to be activated as well. Thus, there is a need to identify the “good” from the “bad” signaling events occurring during maladaptive cardiac hypertrophy. For instance, although the calcineurin–nuclear factor of activated T-cells (NFAT) pathway is activated in pressure overload and over-expressing calcineurin results in cardiac hypertrophy that progresses to
heart failure, calcineurin–NFAT has been linked to anti-apoptotic events [91]. Also, it is important to look at the effects of NO-cGMP-TNF-α related signaling on all aspects of cardiac remodeling, including apoptosis, matrix deposition, gene expression, metabolism, and contractile function. After all, the pathogenesis of heart failure is complex, involving a host of events that include alterations in sarcomeric proteins, interstitial fibrosis, endothelial dysfunction, myocardial apoptosis, and abnormalities in cardiomyocyte cell-to-cell connection, excitation–contraction coupling, and mitochondrial morphology.

These unresolved issues highlight the need to move as far “downstream” as possible in developing therapeutic strategies based on exploiting any of the recently identified endogenous anti-hypertrophic systems, not just those involving NO-cGMP-TNF-α regulatory system. In this regard, deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop, a recently identified growth-suppressing transcriptional repression mediated by the atypical homeodomain Hop, a recently identified growth-suppressing counter-regulatory effects of several cytokines acting in concert. These unresolved issues highlight the need to move as far “downstream” as possible in developing therapeutic strategies based on exploiting any of the recently identified endogenous anti-hypertrophic systems, not just those involving NO-cGMP-TNF-α regulatory system. In this regard, deacetylase-dependent transcriptional repression mediated by the atypical homeodomain protein Hop, a recently identified growth-suppressing transcriptional repression mediated by the atypical homeodomain Hop, a recently identified growth-suppressing counter-regulatory effects of several cytokines acting in concert.

2. Conclusions

TNF-α impact on cardiac mechanical function may be either cardiostimulatory or cardiodepressant, depending on the prevailing cellular and biological milieu, specifically with regard to the redox and metabolic state, the magnitude of extra-cardiac adaptive and reflex responses and the synergistic and/ or counter-regulatory effects of several cytokines acting in concert. All of these variables will thus influence TNF-α mediated myocardial dysfunction and cardiac failure triggered by the nitrosative stress together with persistent activation of NO-dependent signaling pathways as depicted schematically in Fig. 2. However, we are just beginning to recognize the limitations of these analyses, as well as the identification of the primary genetic etiology; both have provided only limited insight into understanding the resultant heart failure pathways or pathology. To fully understand cardiovascular failure, we now realize that the overall intracellular and intercellular regulatory networks in terms of the localization of TNF-α will need to be described. Wrapping the profiling data within the biological phenomenology, a plausible effort should be put forth in delineating the relative importance of complex interrelationships between the various cellular mechanisms underlying TNF-α-NO-induced functional effects on the heart, so that this understanding can be successfully translated into more effective therapy.

References

[26] A. Kumar, V. Thota, L. Dee, J. Olson, E. Uretz, J.E. Parrillo, Tumor
Nitrosylation. The prototypic redox-sensitive protein is nitrotyrosine, which is formed by the reaction of peroxynitrite with tyrosine residues. This modification plays a crucial role in the regulation of various signaling pathways, including the activation of tyrosine kinases and the inactivation of serine/threonine kinases.


