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REVIEW ARTICLE

Targeting the Vicious Inflammation–Oxidative Stress Cycle for the Management of Heart Failure

Neelam Khaper,[1] Sean Bryan,[1] Sanjiv Dhingra,[2] Rohit Singal,[3] Anju Bajaj,[2] Chander M. Pathak,[4] and Pawan K. Singal[2]

Abstract

Oxidative stress and inflammation are each implicated independently in the development and progression of heart failure. Their interaction, however, is also evident throughout the process from initial injury to cardiac remodeling and failure. In the failing heart, the linkage between excessive reactive oxygen species (ROS) and the cytokine elaboration is manifested in shared elements and cross-promotion within downstream signaling pathways. In spite of this, the failure of anticytokine immunotherapy and antioxidant therapy, which had previously shown promise, suggests that a more complete perspective of ROS–cytokine interaction is required. The present review focuses on two of the major cytokines that are demonstrably connected to oxidative stress—the pro-inflammatory tumor necrosis factor-alpha (TNF-α) and the anti-inflammatory interleukin-10 (IL-10)—and their interactions in cardiac remodeling and failure. It is proposed that an optimal balance between TNF-α and IL-10 may be of crucial importance in mitigating both inflammation and oxidative stress processes leading to heart failure. *Antioxid. Redox Signal.* 13, 1033–1049.

Introduction

Although our understanding of the pathophysiology of heart failure has evolved significantly, it remains a major health problem, causing unacceptable levels of morbidity and mortality. A wide variety of stimuli and mechanisms, many of which operate simultaneously, contribute to the cardiac remodeling that can ultimately lead to heart failure. Pronounced oxidative stress, immune activation and inflammation are increasingly recognized as key features within many of these processes. Indeed, elevated oxidative stress (74, 106, 119, 125, 135) and inflammation (7, 14, 22, 108, 109, 116, 147, 153, 154, 167) that correlate with the severity of disease have been reported in a variety of pathophysiological conditions including hypertension, atherosclerosis, coronary artery disease, cardiomyopathy, and heart failure. Most of the components of neurohumoral and inflammatory activation such as catecholamines, angiotensin II, aldosterone, and pro-inflammatory cytokines observed in heart failure induce oxidative stress through diverse mechanisms (105, 148, 157). In this regard, there has been special interest of late in the pathological relevance of the interrelation and interaction of oxidative stress and inflammatory cytokines in cardiovascular disease.

This review focuses on the interplay of oxidative stress with the key inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10), in the pathophysiology of heart failure, as well as on the potential significance of the balance between these inflammatory mediators. The extensive literature on the topic has necessitated some selectivity in what reasonably can be covered. For more details on the discrete roles of oxidative stress and inflammation in cardiovascular disease, readers are directed to the following excellent reviews (19, 41, 44, 92, 96, 98, 108, 114, 130, 136, 167).

Oxidative Stress and Inflammation Are Key Features of the Failing Heart

In order to better understand the linkages between oxidative stress and inflammatory cytokine expression, a brief discussion of the two processes and their contributions to the development and progression of heart failure is required.

Oxidative stress refers to the total burden of potentially harmful reactive oxygen species (ROS) that form during cellular metabolism. Potential endogenous sources of free radicals include the mitochondrial electron transport chain, nicotinamide adenine dinucleotide/phosphate (NADH/NADPH)

[1]Medical Sciences Division, Northern Ontario School of Medicine, Lakehead University, Thunder Bay, Ontario, Canada.
[2]Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Center and Department of Physiology, Winnipeg, Manitoba, Canada.
[3]Cardiac Sciences, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada.
[4]Department of Biophysics, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

oxidase, the xanthine–xanthine oxidase system, and many other redox reactions. Examples of ROS include superoxide anion, hydrogen peroxide, hydroxyl radical, and hypochlorous acid. Reactive nitrogen species (RNS) include free radicals such as nitric oxide (NO) as well as nonradicals such as peroxynitrite, which is generated via the ready reaction of NO and superoxide. ROS and RNS trigger a cascade of harmful events including DNA damage, protein nitration, lipid peroxidation, and activation of matrix metalloproteinases (MMPs), contributing to cardiac remodeling (44, 96). As a defense mechanism, cells have evolved various enzymatic (e.g., superoxide dismutase, catalase, and glutathione peroxidase) and nonenzymatic antioxidants (e.g., glutathione, antioxidant vitamins) that detoxify free radicals. When an abundance of ROS, from endogenous or exogenous influences, exceeds the capacity of the antioxidant defenses to detoxify them, oxidative stress results (44, 125). However, despite these detrimental effects, it is essential to note that ROS generated under physiological conditions play important beneficial roles, including their acting as signaling molecules in a variety of cell signaling pathways and often as second messengers. Indeed, ROS act to modulate the activity of specific transcription factors including NF-κB and AP-1, and are also integral to defense mechanisms such as oxidative burst in phagocytosis, neutrophil function, and shear stress-induced vasorelaxation (44, 114, 125).

Oxidative stress is involved in a variety of pathophysiological conditions, such as ischemia-reperfusion (I-R) injury, hypertrophy and heart failure, hypertension, catecholamine-induced cardiomyopathy, diabetic cardiomyopathy, and adriamycin-induced cardiomyopathy (64, 74, 114, 128, 134–136). For example, in patients with dilated cardiomyopathy and congestive heart failure, plasma malondialdehyde (MDA)—a marker of oxidative stress—is increased, correlating with the severity of symptoms and inversely correlating with ejection fraction and exercise capacity (106, 157). Similarly, an increase in oxidative stress and a deficit in antioxidant level have been reported in various animal studies related to cardiovascular complications (46, 74, 125, 128, 135). Indeed, we have observed clear changes in oxidative stress level and antioxidant capacity in a broad variety of experimental *in vivo* and *ex vivo* models of heart failure (Table 1) (29–31, 36, 59, 61–64, 70–75, 84, 113, 117, 134, 137, 140). Importantly, we have also documented significant cardioprotective effects of various antioxidants in some of these models (Table 2) (30, 60, 70, 72, 74, 79, 113, 126, 138).

Substantial evidence has implicated ROS signaling in cardiac hypertrophy. ROS stimulates myocardial growth and matrix remodeling in cardiac hypertrophy through a variety of signaling kinases and transcription factors such as tyrosine kinase, Src, GTP binding proteins, Ras, protein kinase C, ERK, and JNK (33, 96, 122, 125, 136), leading to altered signal transduction, calcium regulation, and apoptosis (3, 20, 96, 128). This modifies the collagen and overall matrix arrangement (139), resulting in further apoptosis, fibrosis, or necrosis (referred to as ventricular remodeling), leading to cardiac dysfunction. ROS also play an important role in cardiac remodeling by angiotensin II and activation of AP-1 (164). Moreover, it is increasingly recognized that ROS are both a contributor to and product of many cardiovascular disease processes, such that a positive feedback relationship can exist. It is for this reason that ROS level is generally observed to scale with the severity of disease.

Like oxidative stress, inflammation represents an integral aspect of homeostatic regulation that can exert both beneficial effects and contribute to disease pathogenesis. Inflammation is a tightly regulated, complex tissue response to harmful stimuli that attempts to attenuate stressors and is also involved in the healing process (22, 41, 108, 167). The inflammatory response is mediated by a variety of signaling molecules, including prostaglandins, C-reactive protein, soluble CD40 ligand, adiponectin, and inflammatory cytokines, such as TNF-α. Many of these are increasingly used as biomarkers for the systemic inflammation associated with cardiac remodeling and heart failure (19, 22, 41). Cytokine signaling is essential for the function of the innate and adaptive immune system, playing numerous roles in the host's inflammatory response including chemoattraction of neutrophils, monocytes, and dendritic cells by chemokines, and

Table 1. Antioxidants and Oxidative Stress Changes in Acute and Chronic Experimental Heart Failure

Experimental model	*Δ Antioxidants*	*Δ Oxidative stress*	*References*
• Catecholamine-induced cardiomyopathy (rats)		↑ MDA	134
• Adriamycin-induced cardiomyopathy (rats)	↓ GSHPx	↑ H_2O_2 ↓ GSH	84, 137, 61
• Pressure overload hypertrophy & heart failure (guinea pigs)	↓ SOD ↓ GSHPx	↓ GSH/GSSG ↑ Lipid peroxides	31, 30
• Myocardial infarction and chronic heart failure (rats)	↓ GSHPx ↓ CAT ↓ SOD ↓ Vit E	↓ GSH/GSSG ↑ Lipid peroxides	71, 70, 73, 59
• Monocrotaline-induced right heart failure (rats)	↓ Vit E	↑ Lipid peroxides	117, 36
• Ischemia-reperfusion (isolated rat hearts)	↓ Vit E ↓ Retinol ↓ GSH ↓ Ascorbic acid	↑ Lipid peroxides ↑ H_2O_2 ↓ GSH/GSSG	113, 140
• Hypoxia-reoxygenation (isolated rat hearts)	↓ SOD ↓ GSHPx		29, 75
• Diabetic cardiomyopathy (rats)	↓ SOD ↓ GSHPx ↑ CAT	↑ Lipid peroxides	64

Table 2. Cardioprotection by Antioxidants in Acute and Chronic Experimental Heart Failure

Antioxidant	*Experimental model*	*Δ Oxidative stress*	*References*
• Vitamin E	Pressure overload hypertrophy & heart failure (guinea pigs)	↑ GSH/GSSG ↓ Lipid peroxides ↑ Vit E	31
	Myocardial infarction and chronic heart failure (rats)	↑ Vit E	113
• Probucol	Adriamycin-induced cardiomyopathy (rats)	↑ SOD ↑ GSHPx ↓ TBARS ↑ GSH/GSSG	138, 60
• Captopril	Myocardial infarction and chronic heart failure (rats)	↑ SOD ↑ GSHPx ↑ CAT ↓ TBARS ↑ GSH/GSSG	73, 70
• Propanolol	Ischemia-reperfusion (isolated rat hearts)	↑ SOD ↑ CAT ↑ GSHPx ↓ Lipid peroxides	79, 72
• Methionine	Subchronic methionine treatment (rats)	↑ GSHPx ↓ TBARS	126
• Losartan	Myocardial infarction and chronic heart failure (rats)	↑ GSHPx ↑ CAT ↑ GSH/GSSG	74

triggering of fever (22, 108). In short, inflammatory cytokines are ubiquitous, critical mediators of the protective inflammatory response to a wide variety of harmful or potentially harmful stimuli throughout the body. However, in pathophysiological conditions the dysregulation of various processes can lead to chronic inflammation, where the unchecked activity of inflammatory mediators and processes can be massively damaging (41, 92, 108).

Pro-inflammatory cytokines have been shown to contribute to cardiac dysfunction under various pathophysiological conditions associated with heart failure, including I-R injury, myocardial infarction (MI), atherosclerosis, hypertrophy, and acute viral myocarditis (7, 26, 90, 95, 110, 116, 123, 146, 147, 153, 157, 167). Inflammatory cytokines may modulate cardiovascular function by various mechanisms including altered adrenergic signaling, increases in NO, or alteration of calcium homeostasis and redox balance (37, 46, 151). Several studies have shown that the inflammatory response resulting from MI serves to further exacerbate myocardial injury, leading to deleterious remodeling of the heart and of the extracellular matrix. This can include MMP activation and excess collagen formation, increased apoptosis and hypertrophy, and, importantly, self-amplification of the inflammatory signal transduction pathways leading to depressed contractility (1, 16, 54, 85, 92, 102, 108).

Cytokines and chemokines implicated in the progression of heart failure include the pro-inflammatory TNF-α, IL-6, IL-1, interferon-gamma (IFN-γ), cardiotrophin-1 (CT-1, an IL-6 family cytokine), IL-18, IL-13, IL-8, monocyte chemoattractant peptide-1 (MCP-1), and macrophage inflammatory protein-1 alpha (MIP-1α), and the primarily anti-inflammatory transforming growth factor-beta (TGF-β) family and IL-10 (7, 20, 26, 40, 90, 103, 110, 144, 153). Of these, there has been special interest in the prognostic potential of TNF-α, IL-1β, IL-6, and IFN-γ, since the circulating levels of these cytokines at both the protein and mRNA levels are elevated in relation to the clinical severity of heart failure (8, 26, 35, 90, 98, 129, 153, 154, 167).

Inflammatory cytokines and oxidative stress, in conjunction with neurohumoral activation, contribute to physiological and pathological cardiovascular events preceding and including ventricular remodeling and failure in response to a given cardiac stress. Both ROS and pro-inflammatory cytokines impact cardiac function over time in a biphasic manner. The early phase is characterized by an activation of the cellular signaling mechanisms that are adaptive and may be beneficial depending on the existing cellular milieu, leading to pro-survival cellular processes including an increase in antioxidant reserve and anti-inflammatory responses leading to physiological hypertrophy (125, 136). This early phase is followed by a more prolonged, often maladaptive phase, which is prominent in pathological states such as heart failure, and leads to pathlogical hypertrophy, fibrosis, apoptosis, necrosis, autophagy and pro-oxidant and pro-inflammatory responses (Figs. 1 and 2). Within these pathways, the sum of the balance between pro-oxidant and pro-inflammatory mediators with antioxidant and anti-inflammatory mediators determines the overall response of either protection (physiological) or damage (pathological). However, there is significant evidence for an additional level of balance *between* the oxidative stress and inflammatory pathways that may be important in regulating their respective roles in the diseased heart, as will be highlighted here.

The clinical trials on antioxidants have provided mixed results with no concrete evidence of their protective role in cardiovascular disease. Several review articles have been published, highlighting the main findings of the major trials. An analysis of the trials suggests that patient population, primary and secondary endpoints, choice and dosages of antioxidants, and lack of inclusion of reliable markers of oxidative stress are some of the contributors to the failure of these trials (10, 53, 63, 159). Moreover, adjuvant therapies such as statins, ACE-1, beta-blockers and others may also impact

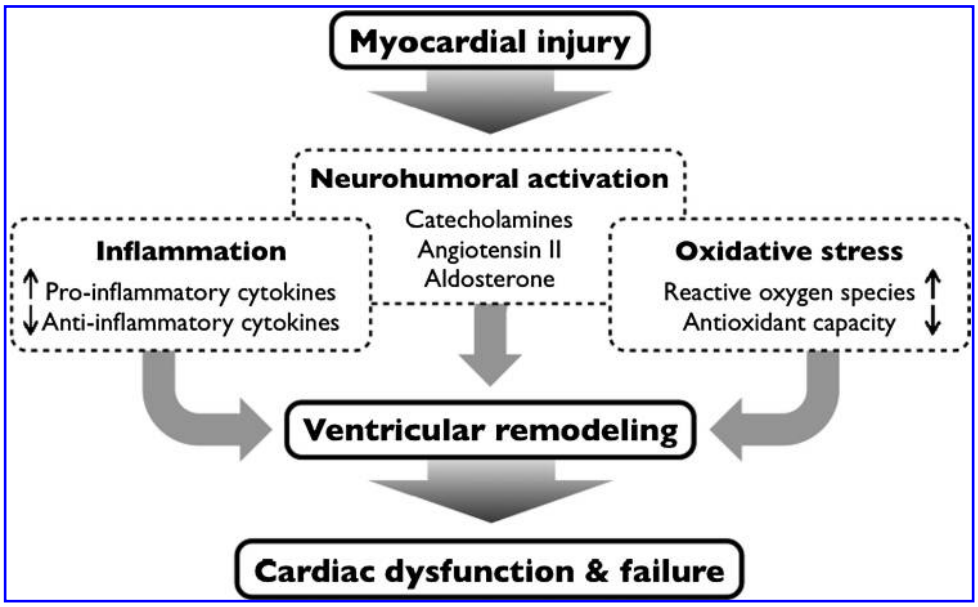

FIG. 1. Local as well as central responses to myocardial injury/stress. Neurohumoral mediators initiated by myocardial stress further involve processes resulting in the development and progression of heart failure. The resulting increase in oxidative stress as well as inflammatory cytokines can contribute not only to the initial myocardial injury, but also stimulates a myriad of secondary pathways that induce or exacerbate ventricular remodeling processes. The latter, at the organ level, includes fibrosis, apoptosis, autophagy, and necrosis. Thus, oxidative stress and inflammation are integral aspects of the pathogenesis of cardiac dysfunction and heart failure.

the efficacy of antioxidants. However, the lack of unequivocal clinical evidence on the beneficial effects of antioxidants in heart disease should not deter us from more basic and clinical research on this topic. To this end, it is suggested that substantial effort should be made in delineating the complex interrelationships between the oxidative stress and inflammatory mediators, wherein the promise of antioxidant and anti-inflammatory therapies may be realized.

The Hard Lessons of Anti-Cytokine Therapy

Due to the apparently significant role of cytokines in the pathogenesis of heart failure, anti-cytokine targeted therapeutic approaches have received a great deal of attention. Here, we have summarized all currently available information on anti-cytokine therapy in heart disease in human studies (Table 3). At the outset, a series of preclinical studies and phase 1 trials suggested that anti-cytokine therapy could be useful in combating heart failure (15, 28, 39). A continuous infusion of TNF-α caused the development of dilated cardiomyopathy in rats, whereas discontinuation of TNF-α resulted in normalization of cardiac function (14), supporting the potential value of anti-TNF-α therapies. Other anti-TNF-α studies followed, supporting the indication that the modality conferred meaningful cardioprotection and attenuation of pro-inflammatory cytokine expression, including that of IL-1β and TNF-α, and reduced oxidative stress in the myocardium. A later study showed that ACE inhibition with enalapril caused a marked reduction in IL-6 bioactivity, which was associated with a reduction in left ventricular thickness (48).

Another randomized, placebo-controlled clinical trial of pentoxifylline, which reduces TNF-α production, showed significant improvements in cardiac function associated with a reduction in TNF-α levels in patients with dilated cardiomyopathy (141). In order to obtain a more global inhibition of cytokines, the immunomodulatory (IVIg) strategy was also employed. This double-blind trial used patients with moderate heart failure who were receiving conventional therapy, including ACE-inhibitors and beta-blockers. Results obtained from this trial suggested that in comparison to placebo, IVIg induced a marked increase in plasma levels of anti-inflammatory cytokines, including IL-10, and these changes were accompanied by a significant improvement in cardiac function, emphasizing that the IL-10/TNF-α ratio may be an important determinant of desirable outcome (47).

Two large-scale, double-blind, randomized trials; RENAISSANCE ("Randomized Etanercept North American Strategy to Study Antagonism of Cytokines") and RECOVER ("Research into Etanercept: Cytokine Antagonism in Ventricular Dysfunction") were organized to study the effects of etanercept, an anti-TNF-α agent in the form of a TNFR-2 fusion protein that binds to and inactivates TNF-α (91). The trials differed in the doses of etanercept administered, and the primary end-point was a change in the clinical status from baseline to 24 weeks. The results obtained showed that etanercept did not influence the variables measured (mortality, hospitalization, and clinical scores); as a result, the study was interrupted (80). The combined analysis of the two trials, RENIASSANCE and RECOVER, was termed RENEWAL ("Randomized Etanercept Worldwide Evaluation") and it, too, reported no clinical improvement with etanercept (91). Here, the patients involved had varying degrees of heart failure severity and received other concomitant therapies for their conditions. Furthermore, many of these patients had co-morbid conditions such as diabetes, hypertension, and coronary artery disease (91). In another randomized, double

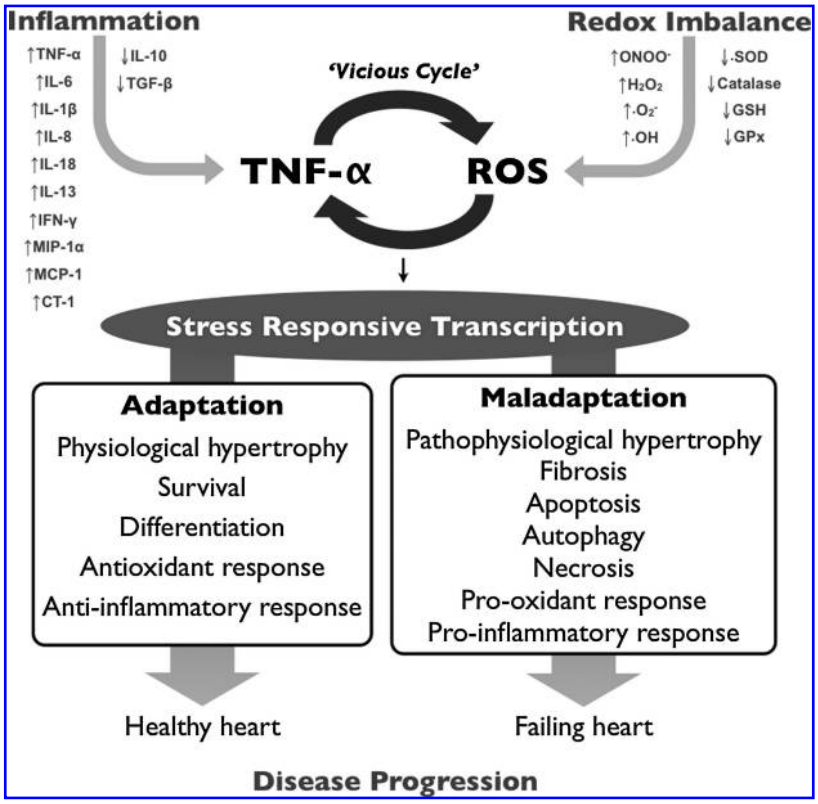

FIG. 2. The 'vicious cycle' of inflammatory cytokine expression and oxidative stress and the stress response spectrum leading to heart failure. Linkages between oxidative stress and inflammatory cytokine/chemokine expression have been documented in the pathophysiology of heart failure. Both pathways exist in delicate balance between their respective pro-oxidant/pro-inflammatory and antioxidant/anti-inflammatory elements, and each has been shown to correlate with the severity of disease. These pathways also interact at a more global level, with TNF-α and other pro-inflammatory mediators contributing to the production of reactive oxygen species (ROS), which in turn stimulates the expression of more such mediators, leading to a perpetuating, 'vicious cycle'. Pro-inflammatory cytokines TNF-α as well as ROS activate transcription factors, which regulate the expression of a broad variety of stress response pathways. The extent of the response depends on the intensity and duration of the stimuli. Thus it can range from acute to chronic in nature, producing phenotypic changes ranging from adaptive (hypertrophy, survival, differentiation, antioxidant response, and anti-inflammatory response) to maladaptive (fibrosis, apoptosis, autophagy, necrosis, pro-oxidant response, and pro-inflammatory response). This continuum is evident in the cardiac remodeling changes from normal to compensatory hypertrophy to heart failure. Importantly, inflammatory cytokines and ROS both stimulate the stress response and are regenerated by it, creating yet another common, positive feedback cycle that scales with the disease progression.

blind, placebo-controlled trial entitled ATTACH ("Anti-TNF Therapy Against Congestive Heart Failure"), which used infliximab, an immunoglobulin monoclonal mouse–human chimeric antibody against TNF-α, there was a significant increase in death and hospitalization (24, 80). Another unpublished study by Gullestad and Aukrust showed that most of the CHF patients in the group with immunoglobulin therapy showed a decrease in left ventricular ejection fraction one year after termination of the study, suggesting that maintenance is necessary for an extended period of time to sustain the effect.

Several possible explanations have been provided for the lack of positive results in these trials including insufficient dosage of etanercept to block not only TNF-α but also other inflammatory mediators, inclusion of a small number of patients, as well as duration of treatment (5, 8, 91). It has even been reported that bioavailability of TNF-α as drug/cytokine complex in the circulation is extended (2) which may also perpetuate its toxic effects. Given the complicated nature of cytokine network interaction as well as the added complexity of disease-related perturbations within it, it is perhaps not surprising that the removal of a single component is insufficient to produce therapeutic benefit (91). Indeed, recent additional evidence indicates that the suppression of TNF-α in rat models of chronic heart failure is an ineffective strategy because it leads to the upregulation of IL-6 and overall pathophysiological aggravation (55). Importantly, another factor that was overlooked in the analysis of these failed trials was the inclusion of reliable markers of oxidative stress. Given its ubiquitous role in pathologies leading to heart failure, and, as will be discussed, its significant cross-promotion and interrelationship with inflammatory cytokines, consideration of redox status may have provided key insight into the underlying reasons for the trials' failure.

That said, there remains cautious interest regarding the future application of anti-cytokine therapies in heart failure. This sentiment is bolstered by the recent successful application of anti-TNF-α therapy in the randomized, controlled, double-blind EFECT study ("Effect of Etanercept on Cardiac Transplantation") wherein it was found that etanercept appeared to decrease post-transplant left ventricular

Table 3. Pertinent Literature on Anti-Cytokine Therapy in Patients with Heart Disease

Supportive findings	*Population & duration*	*Primary endpoints*	*References*
• Treatment with the TNF-α antagonist, pentoxifylline, decreased TNF-α levels ($p = 0.0001$), improved EF ($p = 0.04$), and helped to preserve NYHA functional class ($p = 0.01$) in patients with idiopathic dilated cardiomyopathy.	- 28 patients - 6 months	- NYHA functional class - LV function	141
• A single intravenous infusion of etanercept decreased levels of biologically active TNF-α ($p = 0.05$) and led to improvements in ejection fraction ($p = 0.04$) and quality of life ($p = 0.003$).	- 18 patients - 14 days	- Quality of life assessment - Inflammatory markers	28
• Treatment with intravenous immunoglobulin resulted in markedly increased plasma levels of the anti-inflammatory mediators IL-10, IL-1 receptor antagonist, and soluble TNF receptors that correlated with significantly improved function in patients with chronic CHF.	- 60 patients - 26 weeks		47
• Treatment with etanercept led to a significant dose-dependent improvement in LVEF ($p = 0.05$) and LV remodeling, as well as a trend toward an improvement in patient functional status in patients with NYHA class III to IV HF.	- 47 patients - 3 months	- Safety/efficacy of etanercept - Patient functional and clinical status	15
• In the double-blind (EFECT) Effect of Etanercept on Cardiac Transplantation clinical trial, treatment with etanercept appeared to decrease allograft-associated LV hypertrophy (p = ns) by decreasing extracellular matrix deposition ($p = 0.08$).	- 49 patients - 6 months	- LV mass - Degree of collagen deposition	155
• In the double-blind, placebo-controlled (ACCLAIM) Advanced Chronic Heart Failure Clinical Assessment of Immunomodulation study of a device-based non-specific immunomodulation therapy in patients with NYHA functional class II–IV chronic HF, significant reductions in risk of primary endpoint events of 26% ($p = 0.02$) and 39% ($p = 0.0003$) were observed in patients with no history of myocardial infarction and NYHA functional class II status, respectively.	- 2408 patients	- Composite of time to death or first hospitalization for CV compliance	152
Nonsupportive findings	*Population & duration*	*Primary endpoints*	*References*
• In the large-scale, randomized, double-blind (ATTACH) Anti-TNF Therapy Against Congestive Heart Failure clinical trial, treatment with infliximab did not improve and high doses adversely affected the clinical condition of patients with moderate-to-severe chronic HF.	- 150 patients	- Clinical status - Inflammatory markers - Quality of life assessment	24
• The results of the (RENEWAL) Randomized Etanercept Worldwide Evaluation analysis concluded from the two large-scale, double-blind, prematurely terminated randomized clinical trials: RENAISSANCE (Randomized Etanercept North American Strategy to Study Antagonism of Cytokines) and RECOVER (Research into Etanercept: Cytokine Antagonism in Ventricular Dysfunction), that etanercept had no effect on clinical status or on the rate of death or hospitalization due to chronic HF.	RECOVER = 1123 patients RENAISSANCE = 925 patients	- Clinical status at 24 weeks - Death or hospitalization	91
• RENEWAL and ATTACH trials unsuccessful.			80

CHF, congestive heart failure; EF, ejection fraction; HF, heart failure; LV, left ventricle; New York Health Association (NYHA); sIL-2R, soluble IL-2 receptor; sTNF-RII, soluble TNF-a receptor type II.

hypertrophy by reducing extracellular matrix deposition (155). Furthermore, the Federal Department of Food and Drug Administration of the United States approved three anti-TNF-α agents, adalimumab (Humira), infliximab (Remicade), and etanercept (Enbrel). Each of these has been widely used in the treatment of autoimmune diseases such as rheumatoid arthritis, psoriasis, Crohn's disease, and ankylosing spondylitis, with as yet unsubstantiated concerns for treatment-associated cardiovascular complications (86). Finally, the encouraging recent ACCLAIM study ("Advanced Chronic Heart Failure Clinical Assessment of Immunomodulation"), wherein a broad-spectrum, nonspecific immunomodulatory approach improved the outcome in a select group of patients (152). These findings suggest the need for continued research in this area, and certainly, in pursuance of these next-generation anti-cytokine treatment modalities, co-investigation of oxidative

stress should be of foremost concern, as it will not only reveal further the nature of redox-cytokine interplay but also aid us in refining what remains a promising approach to managing heart failure.

The Link Between Oxidative Stress and Inflammatory Mediators

Besides its central role in challenging and aggravating myocardial injury directly, the oxidative stress pathway is demonstrably connected with key aspects of the inflammatory response in the progression of heart failure (Fig. 2). Recent findings suggest that oxidants promote inflammatory processes via the activation of downstream, redox-sensitive factors such as NF-κB, AP-1, and p38 MAP kinase, leading to the induction of various inflammatory cytokines (3, 20, 92, 96, 97, 103, 145, 160). In this way, oxidative stress contributes to the development of cardiovascular disease both directly and indirectly. Conversely, pro-inflammatory cytokines and chemokines have been implicated in the cascade of events leading to increased oxidative stress, which, in turn, contributes to disease progression via intensification of the inflammatory response (6, 33, 46, 105, 151). Moreover, components of neurohormonal activation (Fig. 1), such as catecholamines, angiotensin II, aldosterone, endothelin-1 are potential contributors to the pro-inflammatory phenotype of heart failure and have been shown to enhance oxidative stress both directly and indirectly (122, 148, 162, 164).

However, despite a considerable amount of direct and indirect evidence for an important redox-cytokine relationship, only a limited number of studies have co-investigated markers of oxidative stress and inflammation in cardiac pathologies. One recent study demonstrated that cardiac and systemic glutathione deficiency as well as elevated levels of blood soluble TNFR1 correlated with NYHA functional class in patients with coronary artery disease, aortic stenosis, and cardiomyopathy (27). Additionally, several studies have demonstrated that cytokine-mediated cardiac dysfunction results from increased ROS generation and peroxynitrite formation (23, 37, 42). Indeed, Ferdinandy *et al.* demonstrated that a combination of IL-1β, TNF-α, and IFN-γ augmented the enzymatic activity of xanthine oxidoreductase, NADH oxidase, and iNOS, concomitant with an increase in NO, superoxide, and peroxynitrite levels, which correlated with depressed cardiac function (37). These effects were normalized by treatment with each of a superoxide scavenger, NOS inhibitor, and peroxynitrite decomposition catalyst (37).

Thus, current literature indicates that increased pro-inflammatory cytokine expression and ROS production are linked in a 'vicious' perpetuating cycle that may be of major significance in the development and progression of heart failure (Fig. 2). In the following sections, we discuss in greater detail the pro-inflammatory cytokine, TNF-α, and the anti-inflammatory cytokine, IL-10, as these mediators in particular have received the greatest attention in the literature with regard to their effects on oxidative stress.

Tumor Necrosis Factor-Alpha and Oxidative Stress

Although cross-promotion of inflammatory cytokines and oxidative stress may be manifested in many ways, the vast majority of research has specifically focused on the link between oxidative stress and TNF-α (Figs. 3 and 4). TNF-α is expressed in numerous cell types, including macrophages, monocytes, T and B cells, mast cells, and cardiomyocytes. TNF-α exerts its effects via specific receptors (TNFR1 and TNFR2), and both the soluble and membrane-bound TNF-α forms are capable of activating these receptors. Both TNFR1 and TNFR2 possess sequences called TNFR-associated factors (TRAFs) that are capable of binding intracellular adapter proteins that link TNFR stimulation to the activation of many signaling processes (54, 93, 98). Interestingly, Higuchi *et al.* (58) discovered that ablation of the TNFR1 gene blunted heart failure and improved survival, while the ablation of TNFR2 exacerbated heart failure and reduced survival, suggesting differential roles for TNFR1 and TNFR2. Moreover, in a recent murine model of MI, despite having no effect on TNF-α levels, TNFR1 was found to be responsible for increased transcript levels of IL-6, IL1β, TGF-β, and MCP-1, while TNFR2 acted to oppose these effects (100).

TNF-α acts as a master mediator of cytokine and other downstream pathways. Consistent with its normal physiological role in the innate and adaptive immunity, it is a key factor in the host response to injury. However, this function can also be detrimental within a disease state such as that within the infarcted myocardium, where exaggerated TNF-α signaling via activation of other pro-inflammatory mediators

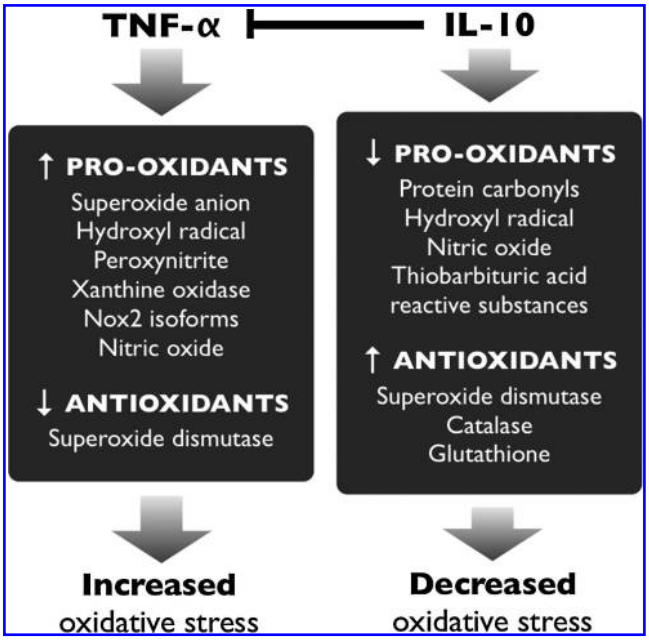

FIG. 3. The interplay of pro-inflammatory (TNF-α) and anti-inflammatory (IL-10) cytokines. Both TNF-α and IL-10 exert a significant influence over numerous pro-oxidant and anti-oxidant mediators. TNF-α is known to suppress antioxidants, while stimulating the production/expression of pro-oxidants. By contrast, IL-10 has been shown to enhance/preserve expression of antioxidants as well as to suppress pro-oxidants. Thus, TNF-α and IL-10 have pro- and antioxidant effects, respectively. Moreover, IL-10 is also known to inhibit the synthesis of TNF-α, and thus of its numerous downstream redox targets. These cogent and opposing roles in regulating oxidative stress are integral to the cytokine–redox interaction as well as the potential significance of IL-10/TNF-α imbalance in heart failure.

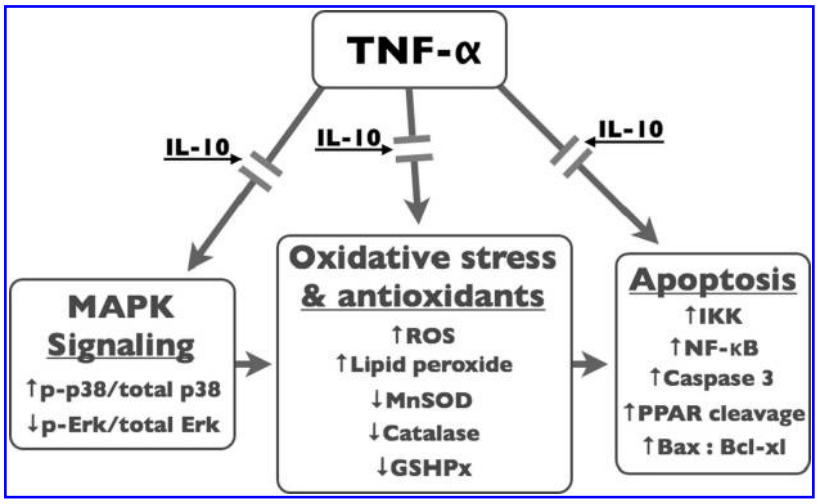

FIG. 4. IL-10-mediated inhibition of TNF-α and its cellular effects on oxidative stress and apoptosis. We have observed that TNF-α influences oxidative stress and apoptosis via MAPK activation in cardiomyocytes. IL-10 prevents these pro-apoptotic and pro-oxidant cellular effects of TNF-α.

such as IL-1β, IL-18, and the IL-6 family of cytokines can exacerbate an already highly stressed environment (14, 20, 41, 98). This altered level of activity can serve to advance disease; studies using transgenic mice have confirmed this role of TNF-α in the acute and chronic remodeling process (81, 88, 108, 146, 147). In response to MI or pressure overload, increases in TNF-α were associated with an elevated local inflammatory response, MMP-2 and MMP-9 expression, collagen turnover, and apoptosis, which correlated with marked cardiac dysfunction (81, 85, 146, 147). Moreover, anti-TNF-α treatment was shown to attenuate MMPs and prevent cardiac fibrosis, further supporting a critical role for TNF-α in the matrix remodeling and regulation of cardiac function (14, 85). Likewise, in human studies, protein and mRNA expression of TNF-α and other pro-inflammatory markers increases during remodeling such that a direct correlation with New York Heart Association (NYHA) functional class has been demonstrated (154). In a model of cardiac-restricted TNF-α overexpression in mice, TGF-β receptor antagonism was found to attenuate the associated myocardial fibrosis, indicating that TGF-β was in fact the specific pro-remodeling factor (121). Thus, it is evident that there is a significant need for further characterization and attribution of TNF-α's actions to other, discrete mediators.

Numerous studies have demonstrated a link between TNF-α and ROS (Figs. 2 and 3). For example, increases in oxidative stress, cellular injury, and apoptosis have been shown upon exposure to TNF-α (3, 33, 105). TNF-α has been reported to contribute to progressive cardiac dysfunction in pacing-induced heart failure that is mediated by an increase in oxidative stress and apoptosis (99). This is accompanied by impaired mitochondrial function, and *in vivo* TNF-α inhibition ameliorates cardiac mitochondrial dysfunction, oxidative stress, and apoptosis in this model (99). Several potential signaling pathways are active during TNF-α-induced apoptosis, including ceramide signaling, JNK pathway, and caspase activation that regulate mitochondrial function. All these pathways can lead to increased oxidative stress and depletion of antioxidants (51, 131). TNF-α enhanced the formation of NO, superoxide, and peroxynitrite via the stimulation of iNOS, xanthine oxidase, and NADPH oxidase activities, contributing to depressed cardiac function in isolated perfused hearts of both rats and mice (25, 37, 151). Increased formation of peroxynitrite by cytokines other than TNF-α, such as IL-1β and IFN-γ, have also been reported in cardiomyocytes (68). Cardiac function was preserved by the inclusion of a peroxynitrite inhibitor, suggesting that the mechanism by which cytokines depress cardiac function was NO-dependent and perhaps mediated by ceramide signaling (37). Ceramide-dependent signaling was also found to mediate the ability of TNF-α to directly induce mitochondrial ROS generation within cardiomyocytes and to cause DNA damage (145). In another study, TNF-α-mediated stimulation of iNOS and ROS generation were also linked to the modulation of potassium ion channels, indicating the broad range of redox-sensitive targets of TNF-α (38). Consistent with these results, increased production of the hydroxyl radical and impaired antioxidant capacity for myocardial SOD were found in a transgenic mouse model of TNF-α over-expression (88). Furthermore, in a rat coronary artery ligation model, TNF-α blockade was associated with significant decreases in ROS, iNOS, catecholamines, and renal sympathetic nerve activity preventing further decrease in cardiac function (46).

Recent studies indicate that the pathway for TNF-α-induced oxidative stress is mediated by the activation of NADPH oxidase and production of superoxide, which was associated with mitochondrial dysfunction by depleting mitochondrial and cytosolic antioxidants. Treatment with the SOD mimetic, Tempol, mitigated oxidative stress and improved membrane permeability transition pore, thereby improving cardiac function (94). Other cellular mechanisms that underlie the various effects of pro-inflammatory cytokines include sphingolipid mediators (4, 45) and β-adrenergic signaling, resulting in altered intracellular Ca^{2+} concentration and leading to impaired contractility (92, 93). Several studies have demonstrated that sphingolipid-based signaling contributes to the negative inotropic effects of TNF-α (112). The intracellular redox state plays an important role in activation of sphingomyelinase (SMase), thereby affecting the negative inotropic role of TNF-α (87). In this regard, Callieret *et al.* demonstrated that N-acetyl cysteine (GSH precursor) increased GSH content, prevented TNF-α-induced ROS generation and SMase activation, thereby improving cardiac function (17).

The potential for antioxidant-based regulation of TNF-α levels has been suggested in rodent models of MI and

hypertension (1, 13). In these studies, treatment with N-acetylcysteine was found to normalize serum and/or cardiac TNF-α, TNFR1, and glutathione levels, attenuate MMP expression and collagen deposition, and improve cardiac function. In other rodent studies of I-R injury, the antioxidant probucol decreased levels of cardiac MDA and expression of IL-1β and IL-6, and augmented levels of GSHPx and CAT, contributing to cardioprotection and increased survival (133). Importantly, there is evidence of common TNF-α and redox-sensitive signaling pathway activation, as TNF-α was reported to activate multiple targets, including p38, JNK, and ERK1/2 MAP kinases, as well as stimulating the phosphorylation of c-jun and ATF2 (16, 32, 33, 93, 96, 97). Activation of these factors can result in a diverse number of intracellular events, including alterations in gene expression and apoptosis. Furthermore, both TNF-α and ROS can induce activation of transcription factors such as NF-κB and AP-1 (20). In addition to TNF-α, there have only been a few studies on the interaction between chemokines and ROS. One such study demonstrated that MCP-1 enhanced the generation of superoxide in monocytes from unstable angina patients and glutathione modestly suppressed the production of IL-8 and MCP-1 in these cells, demonstrating an interaction between MCP-1 and ROS and its possible pathogenic role in plaque rupture (6). Lipase activation and liberation of arachidonic acid and prostaglandins are also responsible for the production of ROS and oxidative stress by TNF-α (21).

Interleukin-10 and Oxidative Stress

While many studies have analyzed the function of inflammatory mediators in the pathogenesis of heart failure, relatively few have examined the expression levels and role of anti-inflammatory cytokines such as IL-10, which is produced by antigen presenting cells (101). Despite limited knowledge regarding its role in downstream signaling pathways in the heart, IL-10 is known to suppress cytokine synthesis via induction of suppressors of cytokine synthesis via the signal transducer and activator of transcription 3 (STAT3) pathway (18) as well as by inducing the expression of heme oxygenase 1 (83). Circulating IL-10 levels are altered in heart failure patients (143, 158, 165). IL-10 inhibits the synthesis of a number of cytokines such as IFN-γ, IL-12, and, importantly, TNF-α, providing the basis upon which IL-10 was initially described as a cytokine synthesis inhibitory factor (50, 144). IL-10 acts by enhancing the release of soluble TNFR, contributing to the reduction of TNF-α activity. In septic shock models, IL-10 was found to inhibit the secretion of TNF-α and protect against endotoxicity (12, 43). Decreased serum levels of IL-10 were reported in patients with unstable angina, further supporting its protective role in cardiac conditions (142). IL-10-mediated protection against autoimmune myocarditis (161), rheumatoid arthritis (69), and atherosclerosis (118) has also been reported. Furthermore, elevated serum levels of IL-10 are associated with a favorable prognosis in patients with acute coronary syndromes, further supporting the importance of the balance between pro- and anti-inflammatory markers in cardiac conditions (56).

In contrast to TNF-α, IL-10 has been reported to have an antioxidant role, and has been shown to inhibit the formation of ROS as well as the production of NO (34, 50) (Fig. 3). The initial suggestion that IL-10 might act as an antioxidant was reported by Bogdan *et al*. (11), wherein recombinant IL-10 was found to suppress both TNF-α expression and ROS production in mouse peritoneal macrophages. Consistent with these findings, IL-10 was also suggested to modulate TNF-α mediated, oxidative stress-induced lung injury, a condition found to be augmented by the application of IL-10 antibody (104, 127). In a murine renal model of I/R injury, IL-10 treatment reduced lipid peroxidation, while increasing the redox ratio and antioxidant enzyme activity (78). IL-10 was previously shown to activate the ERK1/2 MAP kinase pathway, and we have recently provided evidence of p38 and ERK1/2 MAP kinase-mediated regulation of TNF-α/IL-10 interaction (33). Here, we also found that TNF-α significantly increased ROS levels and cardiomyocyte apoptosis, whereas these changes were prevented by the inclusion of IL-10 (33).

IL-10 can inhibit a broad array of immune and inflammatory responses. Indeed, IL-10 has been shown to have potent anti-inflammatory actions that operate both with and without oxidative stress-mediated mechanisms. For instance, it has been reported that IL-10 protects against inflammation by decreasing superoxide anion and hydroxyl radical production while also inhibiting NF-κB signaling through the preservation of IκB (32, 34, 49, 124). In a murine model of acute visceral ischemia, IL-10 pretreatment resulted in a dose-dependent reduction in neutrophil infiltration and TNF-α expression (57). In an IL-10-deficient mouse model of MI, an enhanced inflammatory response was observed, including increased TNF-α, NO, ICAM, and infarct size, further supporting a protective role for IL-10 in suppressing the inflammatory process (166). It has been shown that the molecular mechanism of IL-10 anti-inflammatory effect involves activation of ERK pathway (89).

In addition to its antioxidant and anti-inflammatory properties, IL-10 is suggested to play a significant role in matrix remodeling by modifying the expression of MMPs and their inhibitors (82). Interestingly, gene transfer of human IL-10 in rats was found to stabilize post-MI-associated hypothalamic inflammation, thereby reducing progression towards heart failure and suggesting a potentially important role therein for the balance of cytokine expression in the brain (168). In summary, IL-10 has significant anti-inflammatory and antioxidant properties. Its known interactions with pro-inflammatory cytokines such as TNF-α, position it as a key factor for consideration in the analysis of redox-cytokine dynamics and standalone therapeutic utility.

Cytokine Interplay: The Potential Significance of IL-10:TNF-α

Recently, we have shown that in a rat model of MI, TNF-α exhibits a biphasic response, featuring an increase in its levels in the early and moderate stages of heart failure and a return to normal levels during the severe stage of heart failure (65–67). However, there was a steady decrease in IL-10 levels from an early state to a severe state of heart failure. Thus, IL-10:TNF-α ratio decreased with the progression of heart failure (65). An increase in TNF-α:IL-10 was also reported in patients with advanced congestive heart failure (143), and an imbalance between IL-10 and TNF-α has also been suggested to play a role in atherosclerotic lesions (160). We have further demonstrated that treatment with losartan, an angiotensin II

type 1 receptor blocker, improved the ratio of membrane-bound as well as soluble fractions of IL-10:TNF-α protein in a rat model of MI (66). Post-MI losartan-treated rats showed improved antioxidant status and hemodynamic function (66, 74). Taken together, these studies strongly suggest that the two cytokines may interact in influencing oxidative stress and that an appropriate balance between them may be of crucial importance for mitigating conditions leading to heart failure (65- 67) (Fig. 3). These findings are further supported by the report that a common regulatory mechanism—via p38 and ERK 1/2 MAP kinases as well as NF-κB pathway—govern the interplay of TNF-α and IL-10 in regulating oxidative stress and cardiomyocyte apoptosis (32, 33) (Fig. 4). Moreover, we have recently reported that IL-10 prevents TNF-α-induced NF-κB activation and pro-apoptotic changes in cardiomyocytes by inhibiting IKK phosphorylation through the activation of ERK 1/2 MAP kinase (32).

Thus, despite many examples of altered TNF-α, IL-10, and ROS levels in the literature, we highlighted the importance of the IL-10 to TNF-α ratio in the failing heart, and were the first to demonstrate a common regulatory pathway and its significant effects on oxidative stress (65). We believe that these recent findings bolster the argument for a renewed interest in anti-cytokine and antioxidant therapies in heart failure. Indeed, our emphasis on co-investigation of redox-cytokine interplay is keenly in step with the widely held view that progress in this area is possible only via a broader consideration of the cytokine network; a sensibility underscored by the success of the recent broad-spectrum immunomodulatory trial, ACCLAIM (19, 152).

The Effect of Low-Dose Ionizing Radiation on Inflammation and Oxidative Stress

Intriguing recent reports indicate that low-dose ionizing radiation (LDIR) may not only be harmless but may also be beneficial to biological systems. Total body irradiation (TBI) of animals with LDIR has been found to promote the antioxidant defense system to variable extents in different organs within 24 h of irradiation (9, 115). TBI with LDIR (<0.5 Gy) has also been reported to activate the immune system (120), delayed development of tumor growth in experimental animals (76), prevent type 1 diabetes in NOD mice (149), suppress chemically-induced hepatotoxicity (77) and autoimmune diseases (150), enhance cytotoxic activity of macrophages, suppress tumor metastases (111), and activate dendritic cells (132).

Nakatsukasa *et al.* (107) reported suppression of the pro-inflammatory cytokines, TNF-α, INF-γ, and IL-6 in splenocytes obtained from collagen-induced arthritic DBA/IJ mice after TBI with low-dose gamma rays (0.5 Gy/week for 5 weeks). Recently, Tsukimoto *et al.* (156) reported that exposure to 0.5 Gy gamma ray radiation induced upregulation of MAPK phosphatase-1, leading to inactivation of p38 MAPK and suppression of TNF-α production in mouse macrophages. However, no change in the expression of mRNA of IL-10 was observed in the splenocytes obtained from animals exposed to TBI at a dose of 0.2 Gy (52). Similarly, expression of IL-10 was not altered in dendritic cells enriched from the spleens of B6 mice on exposure to a radiation dose of 0.05 Gy (132).

Given that we have found TNF-α and IL-10 protein and mRNA to be altered in rat models of MI (66), it is tempting to suggest that TBI of animals with LDIR (<0.5 Gy) immediately prior to and/or after induction of MI will increase cardiac function and improve the outcome. These and similar studies offer both novel avenues to investigate the potential significance of IL-10:TNF-α as well as promising noninvasive, non-pharmacological modalities for treatment of cardiovascular disease.

The Impact of Redox–Cytokine Interaction in Cardiac Remodeling and Failure

Complex physiologic systems are loaded with regulators and counter-regulators and the disease conditions analyzed in this review are no different; multifactorial in nature, with novel 'checks and balances' discovered upon each experiment and trial. As we endeavor to learn more about manipulating such relationships, it should not be surprising that more molecular 'players', and thus layers of complexity are discovered to have concerted roles to play in determining the ultimate phenotype. Clearly, a broader approach is required to fully understand these processes.

Cytokines are a complex network of molecules within which significant crosstalk occurs between pro- and anti-inflammatory members, and, as per this review, between cytokines and ROS, during signal transduction. Indeed, while an excess of pro-inflammatory cytokines or ROS may be harmful, a paucity of these mediators may also have adverse effects, underscoring their importance in adaptive and maladaptive processes alike. While, when balanced, the redox and cytokine networks play important physiological roles in maintaining cardiac function, disturbances may lead to pathophysiological processes. As borne out by the failures of both the anti-TNF-α and antioxidant therapies, an important message in this review is that no single gene, protein, pathway, or even process acts independently to produce the ultimate phenotype. From a clinical standpoint, the focus among investigators has thus shifted from anti-TNF-α- and antioxidant-based therapies toward a more holistic approach to understanding the basis of inflammatory cytokine expression and its role in remodeling and heart failure. This 'bigger picture' view of inflammation will undoubtedly include the contributions of oxidative stress, given that these processes are demonstrably interconnected throughout the cardiac remodeling process.

The implication of oxidative stress and inflammation in pathophysiological states has been recognized for some time, both in cardiac and noncardiac conditions, including sepsis, autoimmune processes, and malignancy alike. As the oxidative stress–inflammatory cytokine cycle concept matures, new lines of investigation as well as novel perspectives on existing data may become available, hopefully bringing with them new therapeutic avenues. In the future, consideration of the tenuous balance that exists not only at the 'local' level of inflammatory cytokine network and redox status, but also at the 'global' level of these pathways' interaction, will surely provide valuable insight into the nature of cardiac remodeling and failure.

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Address correspondence to:
Dr. Pawan K. Singal
Institute of Cardiovascular Sciences
St. Boniface General Hospital Research Center
351 Tache Avenue
Winnipeg, MB R2H 2A6
Canada

E-mail: psingal@sbrc.ca

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Abbreviations Used

ACE = angiotensin converting enzyme
AP-1 = activator protein 1
ATF = activating transcription factor
$CD8^+$ = cluster of differentiation 8
CHF = congestive heart failure
CT-1 = cardiotropin-1
DNA = deoxyribonucleic acid
ERK = extracellular signal regulated kinase
GDF = growth differentiation factor
GSH = glutathione
GSHPx = glutathione peroxidase
Gy = gray
ICAM = intercellular adhesion molecule 1
IFN-γ = interferon-gamma
IκB = inhibitory kappa B
IL = interleukin
iNOS = inducible nitric oxide synthase
I-R = ischemia-reperfusion
JNK = jun N-terminal kinase
LDIR = low-dose ionizing radiation
LVEF = left ventricular ejection fraction
MAPK = mitogen activated protein kinase
MCP = monocyte chemoattractant peptide
MDA = malondialdehyde
MI = myocardial infarction
MIP-1α = macrophage inflammatory protein-1 alpha
MMP = matrix metalloproteinase
mRNA = messenger ribonucleic acid
NADH = nicotinamide adenine dinucleotide
NADPH = nicotinamide adenine dinucleotide phosphate
NF-κB = nucleur factor-kappa B
NO = nitric oxide
NYHA = New York Heart Association
$\cdot O_2^-$ = superoxide radical
$\cdot OH^-$ = hydroxyl radical
$ONOO^-$ = peroxynitrite
RNS = reactive nitrogen species
ROS = reactive oxygen species
SMase = sphingomyelinase
SOD = superoxide dismutase
STAT3 = signal transducer and activator of transcription 3
TBARS = thiobarbituric acid reactive substances
TGF = transforming growth factor
TBI = total body irradiation
TNF-α = tumor necrosis factor-alpha
TNFR = tumor necrosis factor receptor
TRAF = TNF receptor associated factor

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