Innate Immunity and the Failing Heart
The Cytokine Hypothesis Revisited

Douglas L. Mann

Abstract: Elevated levels of inflammatory mediators have been identified in patients with heart failure, including heart failure with reduced and preserved ejection fraction, as well as acute decompensated heart failure. Moreover, experimental studies have shown repeatedly that activation of inflammation in the heart provokes left ventricular remodeling and left ventricular dysfunction. Nonetheless, phase III clinical trials that have attempted to antagonize inflammatory mediators have been negative with respect to the primary end points of the trials, and in some patients, resulted in worsening heart failure or death. The following review will discuss how recent developments in the field of innate immunity have advanced our understanding of the role of inflammation in the pathogenesis of heart failure and will discuss the negative outcomes of the existing clinical trials in light of this new information. (Circ Res. 2015;116:1254-1268. DOI: 10.1161/CIRCRESAHA.116.302317.)

Key Words: clinical trials ▪ heart failure ▪ inflammation ▪ innate immunity

“First get your facts, then you can distort them at your leisure.”

Mark Twain

The link between heart failure and inflammation was first recognized in 1990 by Levine et al, who reported elevated levels of tumor necrosis factor (TNF) in patients with heart failure with a reduced ejection fraction (EF). Since this original report, there has been an exponential rise in the number of cytokines and chemokines that have been identified in the setting of heart failure with a reduced EF. Elevated levels of inflammatory mediators have also been identified in acute decompensated heart failure, as well as in patients with heart failure with a preserved ejection. Thus, there is evidence of an ongoing inflammatory response in all the manifestations of clinical heart failure.

The early clinical observations with respect to TNF prompted a series of experimental studies, which demonstrated that the sustained expression of TNF at levels that were observed in patients with heart failure was sufficient to provoke left ventricular (LV) dysfunction and LV remodeling. These and other preclinical studies formed the basis for several multicenter clinical trials that used targeted approaches to neutralize TNF in patients with moderate to advanced heart failure. As reported by the author in Circulation Research over a decade ago, the targeted anti-TNF approaches were negative with respect to the primary end points of the trial or resulted in worsening heart failure or death. Over the years, the ensuing debate over the negative outcome of these clinical trials has produced more questions than answers with respect to what role, if any, proinflammatory cytokines play in the pathogenesis of heart failure. One of the untoward consequences of the negative outcomes of these trials is that they have a profound chilling effect on further attempts to target inflammation in heart failure. Fortunately, during the past 10 years, there has been a much clearer appreciation of the importance of inflammation in the heart because of the pioneering efforts in the field of innate immunity by Charles Janeway (1943–2003) and Ruslan Medzhitov, as well as Bruce Beutler, Jules Hoffman, and Ralph Steinman who shared the Nobel Prize in Physiology/Medicine in 2011 for their work in innate immunity. In the following review, we will discuss how recent developments in the field of innate immunity have advanced our understanding of the role of inflammation in the pathogenesis of heart failure, and we will use these new insights to re-evaluate the clinical trials that have been conducted in this area.

Overview of Immune Responses in the Heart

Both innate and adaptive immune responses are activated in the heart in response to tissue injury that results from pathogens or environmental injury (eg, ischemia or hemodynamic overloading). Whereas the innate immune system provides a global, nonspecific defense against pathogens or tissue injury, the adaptive immune system provides a highly specific response that is mediated by B and T cells. Studies have shown that the ensuing inflammatory response induced by the innate immune system can be physiological and result in the upregulation of a portfolio of cytoprotective responses that provide the heart with a short-term adaptation to the stress.
Alternatively, the inflammatory response can become dysregulated (ie, pathophysiologic), leading to collateral myocardial damage that eventuates in progressive LV dysfunction and adverse LV remodeling. Although Ilya Metchnikoff first proposed in 1901 that the immune system had both physiologic and pathophysiologic roles,8 it has been challenging conceptually, to reconcile these 2 vastly different facets of the immunologic response to tissue injury. The relatively recent insight into the role of the innate immune responses in the heart has permitted a clearer understanding of physiological and pathophysiologic roles,8 it has been challenging conceptually, to reconcile these 2 vastly different facets of the immunologic response to tissue injury. The relatively recent insight into the role of the innate immune responses in the heart has permitted a clearer understanding of physiological and pathophysiologic roles,8 it has been challenging conceptually, to reconcile these 2 vastly different facets of the immunologic response to tissue injury.

Cardiac innate immune responses, which are essential for homeostatic responses and tissue repair, are initiated by the detection of pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by a fixed number of germ-line encoded pattern recognition receptors (PRRs; Figure 1). Classic examples of PAMPs include the lipopolysaccharides of Gram-negative organisms, the teichoic acids of Gram-positive organisms, the zymosans of yeast, the glycolipids of mycobacterium, or the double-stranded RNAs of viruses. More recently, it has become clear that cardiac PRRs also recognize the molecular patterns of endogenous host material released by dying or injured myocardial cells. Cells that die by accidental necrosis, regulated necrosis (necroptosis), or secondary apoptosis release their cytosolic contents into the extracellular space, thereby initiating a brisk inflammatory response through engagement of an ensemble of extracellular or intracellular PRRs. The time course of the inflammatory response that ensues after tissue injury is remarkably consistent, irrespective of the specific cause of cell injury, and is associated with the rapid influx of neutrophils and subsequently monocytes into the area of tissue injury. This inflammatory response has been referred to as sterile inflammation, insofar as the inflammation after tissue injury occurs in the absence of a known pathogenic infection. Thus, the innate immune system evolved not only to detect molecules that were nonself (eg, PAMPs) but also to detect a subset of intracellular molecules (eg, DAMPs) that were hidden by the plasma membrane (hidden-self) and not ordinarily found in extracellular fluids in the absence of cell death. This latter observation has provided a potentially important link between tissue injury, activation of proinflammatory mediators, and the resulting myocardial response to stress.

Many PRRs encountering PAMPs and DAMPs trigger signaling cascades that activate nuclear factor-κB, activator protein 1, and interferon regulatory factor transcription factors, that in turn regulate target genes that encode proinflammatory cytokines and interferons in the heart.10 The portfolio of cytokines implicated in the pathogenesis of heart failure has been the subject of numerous reviews and is summarized in the Online Data Supplement. Another subset of PRRs in the heart trigger a distinct proinflammatory mechanism that requires assembly of cytosolic protein complexes called inflammasomes.11 Canonical inflammasomes convert procaspase-1 into the catalytically active protease that is responsible for the production of interleukin (IL)-1β and IL-18, which are sufficient to trigger inflammatory responses in the heart.

PRRs can be subdivided into 2 major classes based on their subcellular localization. Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are found on plasma membranes or endosomes, where they can detect the presence of PAMPs or DAMPs. Messenger RNAs for TLRs 1 to 10 have been identified in the human heart.12 The relative expression levels for TLR mRNAs in the human heart is TLR4>TLR2>TLR3>TLR5>TLR1>TLR6>TLR7>TLR8>TLR9>TLR10.12 Although expression levels of TLRs have not been identified in human myocytes, TLR2, 3, 4, 6 mRNA has been identified in cardiac myocytes from neonatal rats.13 Although little is known with regard to the regulation of TLR expression in the heart, TLR4 seems to be upregulated in the failing human heart.14,15 Moreover, TLR2 and TLR4 have been shown to have a profound effect on cardiac remodeling in the context of ischemia reperfusion injury and myocardial infarction.10 The signaling pathways used by the TLRs are summarized in the Online Data Supplement. CLRs are calcium-dependent carbohydrate-binding receptors that contain ≥1 C-type lectin-like domains. Although various members of the CLR family have been demonstrated to play an important role in immune responses, little is known with respect to the role of CLRs in
the heart. Relevant to this discussion, CLRls are able to signal independently, as well as modulate the signaling through the TLRs. The relative expression levels of CLR mRNAs in the human heart, which is similar to the murine heart, is Bcl-10>Galectin-1>mamnose receptor 2>DC-SIGN (CD209)>Src, >mamnose receptor 1, >Dectin-1, triggering receptor expressed on myeloid cells 1 and Card-9.17

A second class of PRRs resides in intracellular compartments and includes retinoic acid inducible gene I–like receptors, also called RIG–I–like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)–like receptors (NLRs) and absent-in-melanoma 2 receptors.17,18

NLRs act as cytosolic sensors to intracellular DAMPs and PAMPs. In humans, the NLR family is composed of 22 intracellular pattern recognition molecules that share a central NACHT domain (domain present in NAIP, CIITA, HET-E, and TP1) and a carboxy-terminal leucine-rich repeat region.19 Analysis of human heart tissues has revealed that NOD (NOD2 [NLRC2]), NOD1 (NLRC1), and NLR family, pyrin domain–containing protein 2 (NLRP2 [NALP2]), NLRP3 (NALP3), also known as cryopyrin, are expressed. Both NOD1 and NLRP3 have been shown to activate canonical inflammasomes in the heart, and play an important role in adverse cardiac remodeling after ischemia reperfusion injury and myocardial infarction.11,20 The RLR family is composed of retinoic acid inducible gene I, melanoma differentiation–associated gene (MDA) 5, and LGP2. RLRs are localized in the cytoplasm and recognize the genomic RNA of dsRNA viruses and dsRNA generated as the replication intermediate of ssRNA viruses. The expression of RLRs is greatly enhanced in response to type I interferon stimulation or virus infection. At the time of this writing, little is known with respect to the RLRs in heart, although a recent study has shown that melanoma differentiation–associated gene 5 may play an important role in protecting the heart from direct viral injury during myocarditis.

When myocardial inflammation is induced by microbial or nonmicrobial sources, the primary purpose of the inflammatory response is to resolve the source of the disturbance, thereby allowing the heart to adapt to the abnormal conditions in the short-term, and ultimately to restore homeostasis and cardiovascular function in the long-term. If the abnormal conditions are sustained, then an ongoing inflammatory state persists in the tissue and leads to a state of chronic low-grade inflammation, which can contribute to further disease progression by virtue of the deleterious effects of sustained inflammation. Although speculative, it is probable that the chronic expression of proinflammatory cytokines and ongoing inflammation that have been demonstrated in the failing heart1 represent the inability of the myocardium to restore homeostasis, leading to a state of ongoing chronic inflammation that is intermediate between the baseline state and acute inflammation. This intermediate state has been termed as parainflammation,6 and does not require overt tissue injury or infection to be sustained, but instead represents a graded sustained inflammatory response that remains switched on in dysfunctional tissue in an attempt to restore homeostasis and tissue functionality (Figure 2). It should also be noted that activation of neurohormonal systems in heart failure, such as the renin–angiotensin–aldosterone system and the adrenergic nervous system, are capable of triggering inflammation in the heart, thereby leading to a state of low-grade inflammation.22,23 Unfortunately, at the time of this writing, it is not known whether down-modulating the level of parainflammation in the failing heart to prevent collateral damage can be accomplished without disrupting critical homeostatic responses that are provided by low levels of inflammation. Given that parainflammatory responses are graded, ranging from physiological levels of inflammation to a classic inflammatory response, it will be critically important to have a better understanding of when it is appropriate to target inflammation in the failing heart.

Role of Inflammation in the Pathogenesis of Heart Failure

The primary interest in deciphering the role of inflammation in heart failure arose from the observation that many of the biological effects of proinflammatory cytokines were sufficient to provoke a heart failure phenotype in experimental animals and in humans. The cytokine hypothesis24 for heart failure postulates that heart failure progresses, at least in part, as a result of the deleterious effects exerted by endogenous cytokine cascades on the heart and the peripheral circulation. Thus, analogous to sustained neurohormonal activation in heart failure, the chronic inflammation that occurs in heart failure may also contribute to worsening heart failure by virtue of the harmful effects of sustained inflammatory signaling.
It bears emphasis that at the time the cytokine hypothesis was proposed, the role of the innate immune system, as well as the concept that chronic inflammation was both beneficial and deleterious, were not at all well understood. Thus, although the biological underpinning for the cytokine hypothesis remains unchanged, the optimal approach to testing this hypothesis in patients with heart failure is far less certain because of the inherent complexity of chronic parainflammation.

**Effects of Cytokines on LV Function**

The pathophysiological effects of proinflammatory cytokines have been reviewed extensively,\(^2^,^3\) and will only be discussed here briefly. Proinflammatory cytokines were first shown to provoke LV dysfunction in the systemic inflammatory response that occurs during sepsis. Direct injections of TNF were shown to produce hypotension and rapid death within minutes, whereas injections of anti-TNF antibodies attenuated the hemodynamic collapse that occurs during endotoxin shock. Subsequent studies in dogs and rats showed that circulating levels of TNF produced negative inotropic effects in vivo and in vitro.\(^4\) More recent studies in transgenic mice with cardiac-restricted overexpression of TNF showed that forced overexpression of TNF resulted in depressed LV ejection performance that was dependent on TNF gene dosage.\(^2^,^6\)

With respect to the potential mechanisms for the deleterious effects of TNF on LV function, the literature suggests that TNF modulates myocardial function through an immediate pathway that is manifest within minutes and is mediated by activation of the neutral sphingomyelinase pathway. This is followed by a delayed response that requires hours to days to develop and is mediated by nitric oxide–mediated blunting of β-adrenergic signaling.\(^3\) Although the negative inotropic effects of IL-1 seem to be mediated, at least in part, through the production of nitric oxide (ie, the delayed pathway), the negative inotropic effects of IL-6 are less well understood. Recent studies suggested that TNF and IL-1 may produce negative inotropic effects indirectly through activation or release of IL-18. Remarkably, blockade of IL-18 using neutralizing IL-18–binding protein leads to an improvement in myocardial contractility in atrial tissue after ischemia reperfusion injury.\(^2^,^7\) Although the signaling pathways that are responsible for the IL-18–induced negative inotropic effects have not been delineated, thus far, it is probable that they will overlap those for IL-1, given that the IL-18 receptor complex uses components of the IL-1–signaling chain.

**Effects of Proinflammatory Cytokines on LV Remodeling**

LV ventricular remodeling refers to the multitude of changes that occur in cardiac shape, size, and composition in response to myocardial injury. Inflammatory mediators have several important biological effects that may play an important role in the process of LV remodeling, including cardiac myocyte hypertrophy, alterations in fetal gene expression, activation of collagenolytic matrix metalloproteinases, myocardial fibrosis, as well as progressive myocyte loss through apoptosis.\(^3\) Antagonism of innate immune receptors (TLR2 and TLR4), innate immune signaling pathways (MyD88, IRAK-1, IRAK-4, and NLRP3) and the proinflammatory cytokines downstream from these pathways (TNF, IL-1β, and IL-18) has been shown to attenuate adverse LV remodeling after acute myocardial infarction.\(^2^,^8\) Studies in chimeric mice, wherein it has been possible to separate the role of innate immune signaling in cells derived from the bone marrow from the effects in the myocardium have demonstrated that activation of innate immune signaling pathways in bone marrow–derived neutrophils and monocytes contributes to tissue damage, progressive fibrosis, and adverse cardiac remodeling, whereas activation of the same pathways in cardiac myocytes is beneficial through short-term mitochondrial stabilization, enhanced sarcolemma membrane integrity,\(^2^,^9\) and through conservation of energy secondary to the development of reversible LV dysfunction.\(^2^,^8\) Studies in experimental models wherein the inflammatory signaling is sustained have also provided important insights into the mechanisms for inflammation-induced adverse LV remodeling. For example, a study in rats showed that infusion of concentrations of TNF that overlap those observed in patients with heart failure led to a time-dependent change in LV dimension that was associated with progressive degradation of the extracellular matrix.\(^2\) Studies in transgenic mice with targeted overexpression of TNF have shown that these mice develop progressive LV dilation, and that TNF-induced activation of matrix metalloproteinases is responsible for collagen degradation and progressive LV dilation.\(^3^,^0\) These studies demonstrated that sustained myocardial inflammation leads to temporal changes in the balance between matrix metalloproteinase activity tissue inhibitor of matrix metalloproteinases and mast cell–mediated TGF-β signaling.\(^3^,^1\) Collectively, these time-dependent changes favor degradation of the extracellular matrix during the onset of inflammation and progressive myocardial fibrosis after sustained inflammation. Thus, the sustained activation of inflammatory signaling contributes to LV remodeling through a variety of different mechanisms that involve both the myocyte and the nonmyocyte components of the myocardium.
Clinical Applications

Inflammatory Biomarkers
The extant literature suggests that inflammatory biomarkers provide important diagnostic and prognostic information across the entire spectrum of heart failure syndromes. Table 1 shows that the proinflammatory cytokines that are elaborated in heart failure include members of the TNF superfamily, members of the IL-1 family (IL-1α, IL-1β), and IL-6. Soluble ST2, which is the receptor for IL-33 and is thus a member of the IL-1 superfamily (IL-1F) of cytokines, is the first inflammatory biomarker to be approved by the Food and Drug Administration for prognosis in heart failure. Importantly, soluble ST2 is secreted by cultured myocytes that are subjected to mechanical strain and is thus an integrated marker of mechanical strain and inflammation. In addition to cytokines and cytokine receptors, several inflammatory mediators that were originally identified in immune cells, most notably macrophages, have also been observed in patients with heart failure. The inflammatory mediators in this group that have garnered the most attention in heart failure include galectin-3 and pentraxin-3. Galectin-3, a member of the lectin family, is released by macrophages in response to tissue injury, as well as by damaged or dying cells. Galectin-3 is also approved by the Food and Drug Administration as a biomarker for determining heart failure prognosis. Pentraxin-3, a novel inflammatory marker and member of pentraxin superfamily of cytokines, has also recently been identified in patients with heart failure. In addition to providing information about patient prognosis, the measurement of inflammatory biomarkers in patients with heart failure may identify subsets of patients who are most likely to benefit from anti-inflammatory strategies.

Inflammation as a Therapeutic Target in Heart Failure
Given that elevated levels of proinflammatory cytokines mimic many aspects of the heart failure phenotype and that the deleterious effects of inflammatory mediators are potentially reversible once inflammation subsides, investigators have used a variety of different approaches to antagonize inflammatory mediators in heart failure (Table 2). These fall into 1 of 3 broad categories, such as anti-inflammatory therapies, immunomodulatory therapies, and autoimmune strategies.

Anti-Inflammatory Therapies
The biological effects of proinflammatory mediators can be antagonized through transcriptional or translational approaches, or by so-called biological response modifiers that bind or neutralize soluble mediators (eg, TNF or IL-1β). Many of these strategies have been explored in phases II to III clinical trials, as described below.

Transcriptional Suppression of Proinflammatory Cytokines
Pentoxifylline is a xanthine-derived agent that is known to inhibit TNF transcription and translation, as well as modulate a broad spectrum of inflammatory mediators. Pentoxifylline has been studied in several small randomized trials in patients with ischemic and dilated cardiomyopathy (Table 2). Treatment with pentoxifylline resulted in a significant improvement in New York Heart Association (NYHA) functional class or LVEF in each of these studies. Importantly, the beneficial effects were seen in all NYHA classes of heart failure, in patients with ischemic and nonischemic cardiomyopathy, and in patients treated with angiotensin-converting enzyme inhibitors and β-blockers. Apposite to the present discussion, the beneficial effects on cardiac function in some of the studies were accompanied by decreased circulating plasma levels of TNF. Given that pentoxifylline is a nonspecific phosphodiesterase inhibitor, it is possible that the salutary effects of this agent might be unrelated to its anti-inflammatory properties.

Thalidomide (α-N-pthalimidoglutarimide) suppresses TNF production, as well as the production of a spectrum of inflammatory mediators that are implicated in the pathogenesis of heart failure. The mechanism of action of thalidomide with respect to reducing TNF levels seems to be through enhancing mRNA degradation; however, the precise mechanism of action of thalidomide is unclear, and contradictory results have been reported about its effects on cytokine levels in vivo. Thalidomide was safe and potentially effective in a small open-label dose escalation study in patients with heart failure. There was a significant increase in the 6-minute walk distance and a trend (P=0.16) toward improvement in LVEF and quality of life (QoL) after 12 weeks of maintenance therapy with thalidomide. However, dose-limiting toxicity was observed in 2 patients (50 and 200 mg/d). In a larger placebo-controlled trial of 56 patients with NYHA classes II to III heart failure secondary to ischemic and nonischemic cardiomyopathy and an LVEF ≤40%, treatment with ≤200 mg/d of thalidomide was well tolerated, and significant improvements in QoL were noted (P<0.05).

Table 1. Inflammatory Biomarkers in Heart Failure

<table>
<thead>
<tr>
<th></th>
<th>HFrEF</th>
<th>HfPEF</th>
<th>ADHF</th>
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<tbody>
<tr>
<td><strong>Cytokines</strong></td>
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<tr>
<td>TNF (TNSF2), TWEAK (TNSF12), Fasl (TNFSF6), LIGHT (TNSF14), IL-1β (IL-1F2), IL-2, IL-6, IL-18 (IL-1F8), IL-33 (IL-1F11)</td>
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<td><strong>Cytokine receptors</strong></td>
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<tr>
<td>sTNFR1 (TNFRSF14A), sTNFR2 (TNFRSF1B), gp130 (IL6ST); IL-1ra (IL1F3), sST2 (IL-1RL1)</td>
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<tr>
<td><strong>Macrophage</strong></td>
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<tr>
<td>Galectin-3, Pentraxin-3</td>
<td></td>
<td>Galectin-3, Pentraxin-3</td>
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</table>

The parenthesis denote the nomenclature for the TNF and IL-1 superfamily of cytokines and cytokine receptors. ? indicates conflicting data; FasL, Fas ligand; gp130, soluble gp130; IL, interleukin; IL-1F, IL-1 family; IL-1RL1, IL-1–receptor-like-1; LIGHT, homologous to lymphotaxins, inducible expression, competes with HSV glycoprotein D for HVEM, a receptor expressed on T-lymphocytes; sST2, soluble ST2 receptor; sTNFR1, soluble TNF type 1 receptor; TNF, tumor necrosis factor; TNFSF, tumor necrosis factor superfamily; TNFSFR, tumor necrosis factor superfamily receptor; TNF-SF, TNF superfamily; and TWEAK, TNF–like weak inducer of apoptosis.

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Table 2. Clinical Trials Targeting Inflammation in Heart Failure

<table>
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<tr>
<th>Study</th>
<th>No. of Patients</th>
<th>NYHA Class</th>
<th>Agent</th>
<th>Category</th>
<th>Follow-up, mo</th>
<th>Mean Age, y</th>
<th>Mean LVEF %</th>
<th>% ACE-ARB/BB</th>
<th>Primary end point</th>
<th>Outcome</th>
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<td>ATTACH5</td>
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<td>7</td>
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<td>24</td>
<td>100/73</td>
<td>Clinical composite score</td>
<td>High dose had adverse effect on clinical outcomes</td>
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<td>73</td>
<td>ns</td>
<td>91/75</td>
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<td>63</td>
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<td>Clinical composite score</td>
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<td>33*</td>
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<td>Death, death and CV hospitalization</td>
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<td>56</td>
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<td>Thalidomide</td>
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<td>3</td>
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<td>LVEF, LV volumes, symptoms</td>
<td>Improved LVEF and LV remodeling</td>
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<tr>
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<td>405</td>
<td>III, IV</td>
<td>Oxypurinol</td>
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<td>...</td>
<td>65</td>
<td>26</td>
<td>95/01</td>
<td>Composite of HF mortality+morbidity+QoL</td>
<td>No overall effect; effect in those with elevated uric acid</td>
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<tr>
<td>Parrillo et al39</td>
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<td>NS</td>
<td>Prednisone</td>
<td>DCM</td>
<td>3</td>
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<td>Skudicky et al40</td>
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<td>NYHA class, exercise tolerance, and LVEF</td>
<td>Improved symptoms and LVEF</td>
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<td>Symptoms, cytokines, and LVEF</td>
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<td>3</td>
<td>59</td>
<td>35</td>
<td>85/84</td>
<td>6-MWT</td>
<td>No effect</td>
</tr>
</tbody>
</table>

6-MWT indicates 6-minute walk test; ACCLAIM, Advance Chronic Heart Failure Clinical Assessment of Immune Modulation; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker; ATTACH, Anti–TNF-α Therapy Against Congestive Heart Failure; BB, β-adrenergic receptor blocker; CORONA, Controlled Rosuvastatin Multinational Trial in Heart Failure; CV, cardiovascular; DCM, dilated cardiomyopathy; EXACT, Using Allopurinol to Relieve Symptoms in Patients With Heart Failure and High Uric Acid Level; GISSI-HF, Gruppo Italiano per lo Studio della Sopravvenza nell’ Insufficienza cardiaca-Heart Failure; HF, heart failure; IMAC, Intervention in Myocarditis and Acute Cardiomyopathy; IVG, intravenous immunoglobulin; LVEF, left ventricular ejection fraction; METIS, Methotrexate Therapy Effects in the Physical Capacity of Patients With Ischemic Heart Failure; MI, myocardial infarction; NA, not available; NS, not specified; NYHA, New York Heart Association; QoL, quality of life; RCT, randomized controlled trial; RECOVER, Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction; RENAISSANCE, Randomized Etanercept North American Strategy to Study Antagonism of CytokinEs; RENEWAL, Randomized Etanercept Worldwide Evaluation; and UNIVERSE, Rosuvastatin Impact on Ventricular Remodeling Cytokines and Neurohormones.

*10% of the patients in GISSI-HF has an ejection fraction >40%.

Data derived from Aukrust Gullestad et al.49
thalidomide for 12 weeks resulted in increased LVEF and decreased LV end-diastolic volume.37 These salutary changes were accompanied by a decrease in circulating levels of matrix metalloproteinase 2, but an increase in circulating levels of TNF. The effect of thalidomide on LVEF was observed to a greater degree in patients with dilated cardiomyopathy who were able to tolerate higher doses of thalidomide.37

**Translational Suppression of Proinflammatory Cytokines**

Dexamethasone is thought to suppress TNF biosynthesis at the translational level but may also block TNF biosynthesis at the transcriptional level. In an early study, Parrillo et al39 randomized 102 patients with dilated cardiomyopathy to treatment with prednisone (60 mg/d) or placebo. After 3 months of therapy, these investigators observed a ≥25% increase in EF in ≈ 50% of the prednisone-treated patients, whereas ≈ 25% of the controls had a significant improvement in LVEF (P=0.005). However, the mean increase in LVEF was not significantly (P=0.054) different in the prednisone-treated group (4.3±1.5%) when compared with controls (2.1±0.8%). When patients were divided into a reactive group (prespecified as a fibroblastic/lymphocytic infiltration or immunoglobulin deposition on endomyocardial biopsy, a positive gallium scan, or an elevated erythrocyte and nonreactive) and a nonreactive group, the authors noted that ≈ 65% of reactive patients had an improved LVEF at 3 months, whereas ≈ 25% of the reactive control patients had an improved LVEF (P=0.004). The prednisone-treated nonreactive patients did not have significantly improved LV function (P=0.51). This study was the first to demonstrate that patients with dilated cardiomyopathy benefit clinically from an anti-inflammatory therapy.

**Targeted Anticytokine Approaches Using Biological Response Modifiers**

Two different targeted approaches have been taken to selectively antagonize proinflammatory cytokines in the setting of heart failure (Table 2). The first approach used a genetically engineered TNF receptor (etanercept) that acts as a decoy to prevent TNF from binding to its TNF receptors on target cells, whereas the second approach used a chimeric monoclonal antibody that neutralizes circulating TNF.

Soluble TNF Receptors: Etanercept (Enbrel) is a genetically engineered humanized protein consisting of 2 human TNF p75 receptors coupled to a human IgG1:Fc fragment. Two small short-term studies in patients with stable heart failure showed that treatment with 25 mg BIW etanercept resulted in improved QoL, increased 6-minute walk distance, and improved LV ejection performance after 3 months of treatment.3 These early trials formed the basis for 2 moderate size multicenter trials that used parallel study designs, but differed in the dose of etanercept that was used. The Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE; n=900) trial was conducted in North America, whereas the Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER; n=900) trial was held in Europe and Australia. The primary end point for both the trials was a clinical composite score, in which patients were classified as improved, unchanged, or worsened at 24 weeks. In the RENAISSANCE study, patients were treated with placebo or subcutaneous etanercept 25 mg BIW or 25 mg TIW, whereas the RECOVER trial used doses of 25 mg QW or 25 mg BIW of subcutaneous etanercept. A third prespecified trial, the Randomized Etanercept Worldwide Evaluation (RENEWAL; n=1500) trial, used the pooled data from the RENAISSANCE (BIW and TIW dosing) and RECOVER (BIW dosing only). The primary end point for RENEWAL was all-cause mortality and hospitalization for heart failure. On the basis of prespecified stopping rules, both the trials were terminated prematurely because of the lack of benefit of etanercept on the clinical composite in RENAISSANCE (P=0.17) and RECOVER (P=0.34; Figure 3A). The prespecified analysis of RENEWAL showed that there was no effect of etanercept on the primary end point (Figure 3B) of death or chronic heart failure hospitalization (hazard ratio [HR], 1.1; 95% confidence interval [CI], 0.91–1.33; P=0.33).4 However, in a post hoc analysis of the RENAISSANCE trial, patients receiving BIW and TIW etanercept experienced, respectively, an increased 1.21 (P=0.17) and 1.23 (P=0.013) risk of death/heart failure hospitalization when compared with placebo. Further analysis of the components of the clinical composite score in the RENAISSANCE trial indicated that there was a significantly greater proportion of etanercept-treated patients (29%; P<0.04) in the worsened category at 24 weeks when compared with placebo-treated patients (20%). Increases in the risk of death/heart failure hospitalization and a worsening clinical composite were not observed in RECOVER, wherein the dose and duration of etanercept dosing was less. Patients in RECOVER received etanercept for a median time of 5.7 months, whereas patients in RENAISSANCE received etanercept for 12.7 months. On the basis of these findings, the prescribing information for etanercept has been updated and now suggests that physicians exercise caution in the use of etanercept in patients with heart failure.

Although the precise explanation for the worsening heart failure in the RENAISSANCE is not known, it bears emphasis that TNF receptor antagonists have intrinsic biological activity and, in certain settings, can act as agonists (referred to as a stimulating antagonist)39. We and others have reported that in some settings, etanercept can stabilize TNF and increase its bioactivity.1 Although the stabilizing effects of etanercept might not be problematic in rheumatoid arthritis, wherein TNF is encapsulated within a joint space and peripheral circulating TNF levels are relatively low (compared with heart failure) or are nonexistent, it is possible that an increase in the circulating levels of biologically active TNF in patients with heart failure might contribute to worsening heart failure.

Monoclonal Antibodies: Infliximab (Remicade) is a chimeric monoclonal antibody consisting of a genetically engineered anti-TNF murine Fab fragment fused to a human FC portion of human IgG1. Although infliximab had been shown to be effective in Crohn disease and rheumatoid arthritis, infliximab had never been tested in preclinical nor early phase I clinical studies in patients with heart failure. The Anti–TNF-α Therapy Against Congestive Heart Failure (ATTACH) trial was a phase II study in 150 patients with moderate to advanced heart failure (NYHA classes III, IV). The primary end point of the ATTACH trial was the clinical composite score that was also used in RENAISSANCE and RECOVER.56 Patients were
randomized to receive 3 separate intravenous infusions of infliximab (5 or 10 mg/kg) at baseline and at 2 and 4 weeks, followed by an assessment of the clinical composite score at 14 and 28 weeks. Because ATTACH was a pilot phase II study, there was no requirement for a formal Data Safety Monitoring Board to monitor ongoing clinical outcomes during the trial. Analysis of the completed data set revealed that there were increased rates of mortality and heart failure hospitalization, particularly in the group who was receiving the highest dose of infliximab (Figure 4A). On the basis of these findings, the prescribing information for infliximab has been changed and it is now recommended that treatment with infliximab be discontinued in patients with worsening heart failure and that infliximab treatment should not be initiated in patients with heart failure.

Analogous to the discussion above for the RENAISSANCE trial, it is not possible to precisely identify the mechanism for the untoward outcomes in ATTACH. However, the publication of the full trial results from ATTACH has allowed for some potential mechanistic insights that were not available previously. As shown in Figure 5A, one of the mechanisms of action of infliximab is to bind to cells expressing TNF on their membrane, and to lyse these cells through complement fixation. Although this type of biological activity is beneficial in eliminating clones of activated T cells in Crohn disease, it is predictable that infliximab might be deleterious in heart failure if infliximab bound to TNF that was expressed on the sarcolemma of failing cardiac myocytes (TNF is not expressed in the nonfailing heart), which would lead to complement fixation, lysis of cardiac myocyte cell membranes, and cell death. Analysis of the ATTACH trial indirectly supports this point of view. As shown in Figure 5B, plasma levels of immunoreactive TNF increased at 2 and 6 weeks after treatment with infliximab, as well as after the last dose of infliximab at 6 weeks. Although the increase in TNF levels was attributed to TNF that was bound to infliximab (and hence presumably neutralized), this explanation does not explain the striking 25-fold increase in TNF levels at 10 to 28 weeks, when the infliximab levels were declining below detectable levels (Figure 5B). Moreover, there was a progressive and paradoxical rise in C-reactive protein and IL-6 levels over the course of the ATTACH trial, consistent with ongoing tissue injury. Accordingly, one biologically plausible explanation for the increase in patient morbidity and mortality in the ATTACH trial is that infliximab was overtly toxic through complement fixation in the heart.

Since the completion of these 2 trials, there has been intense debate in the rheumatologic literature with regard to the safety of anti-TNF therapies in patients with rheumatoid arthritis, who are known to have higher risk for cardiovascular complications. Although some early studies showed an increased incidence of new onset heart failure in patients treated with infliximab or etanercept, other studies have not shown that these agents are associated with heart failure. Unfortunately, any meaningful interpretation of these conflicting clinical reports is fraught with difficulty and uncertainty, insofar as patients with rheumatoid arthritis are more likely to develop heart failure than age-matched subjects without rheumatoid arthritis. Furthermore, the large administrative data sets that are used to detect heart failure in these retrospective studies rely heavily on the use of International Classification of Diseases 9 codes, which are known to lack the requisite predictive accuracy to exclude the diagnosis of heart failure when present. The 2012 update of the American College of Rheumatology recommendations for the use of disease-modifying antirheumatologic drugs and biological agents for the treatment of rheumatoid arthritis recommends not using anti-TNF biologics in NYHA classes III to IV patients with heart failure with an EF <50%.

IL-1 Receptor Antagonist: Anakinra (Kineret) is an IL-1 receptor antagonist that blocks the biological activity of IL-1 by competitively inhibiting the binding of IL-1 to the IL-1 type receptor. Anakinra has been shown to prevent adverse cardiac remodeling after LAD ligation in mice, but did not have a significant effect on LV remodeling in small randomized study in patients with acute myocardial infarction. Although the experience with anakinra in heart failure has been limited, small studies have shown significant improvements in exercise performance in patients with heart failure with a depressed EF (n=7) and a preserved EF (n=12).
Other Anti-Inflammatory Agents: The biological effects of pro-inflammatory mediators can also be antagonized using pleiotropic drugs that have anti-inflammatory properties. Three of these therapeutics, statins, n-3 polyunsaturated fatty acids (PUFA), and oxypurinol, have been tested in phase III clinical trials.

Statins: Statins have a variety of pleiotropic effects, including inhibition of inflammatory responses, increased nitric oxide bioavailability, improved endothelial function, and antioxidant properties. On the basis of the results of several promising retrospective analyses of clinical trials and observational databases suggesting that statins decreased the incidence of heart failure or reduced mortality in patients with known heart failure, several large heart failure clinical trials were performed. The UNIVERSE (Rosuvastatin Impact on Ventricular Remodeling Cytokines and Neurohormones) trial examined the effects of rosuvastatin (40 mg/d) on LV remodeling in patients with ischemic and nonischemic dilated cardiomyopathy. Compared with placebo, rosuvastatin was associated with significant reduction of low-density lipoprotein cholesterol but had no effects on LV dimension, LVEF, nor circulating levels of neurohormones. Similar findings were reported in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA), in which 5011 patients (>60 years of age) with NYHA functional classes II to IV heart failure of ischemic cause were randomized to 10 mg/d of rosuvastatin versus placebo. In CORONA treatment with rosuvastatin did not confer a significant benefit with respect to the primary end point, which was a composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke (HR, 0.92; 95% CI, 0.83–1.02; P=0.12). Furthermore, there were no significant differences in several of the secondary end points, including all-cause mortality (HR, 0.95; 95% CI, 0.86–1.05; P=0.31) and coronary events (HR, 0.92; 95% CI, 0.82–1.04; P=0.18), despite a significant decrease in circulating levels of low-density lipoprotein, cholesterol, and C-reactive protein. It is worth noting that the rate of atherothrombotic events was relatively low in the CORONA study, and that the majority of deaths were because of sudden death or worsening heart failure, which reflects the fact that the patient population was composed of patients with symptomatic heart failure rather than symptomatic coronary artery disease. Thus, the primary composite end point of the CORONA study may not have captured the beneficial effects of rosuvastatin in this elderly group of patients with advanced heart failure. Importantly, treatment with rosuvastatin resulted in a significant decrease in heart failure events and improved quality of life.
failure hospitalizations, which was a prespecified secondary end point in the CORONA study, thus ending speculation that treatment with statins might lead to worsening heart failure. Moreover, a post hoc analysis of the CORONA trial demonstrated that rosuvastatin had beneficial effects among those patients with heart failure with evidence of increased inflammation at baseline, which was defined as a C-reactive protein >2.0 mg/dL.68

The Gruppo Italiano per lo Studio della Sopravvivenza nell’Insufficienza cardiaca-Heart Failure (GISSI-HF) investigated the efficacy and safety of rosuvastatin in patients with NYHA classes II to IV heart failure, irrespective of cause or LVEF.69 Patients were randomly assigned to rosuvastatin 10 mg daily (n=2285) or placebo (n=2289), and followed up for a median of 3.9 years. The primary end points of the trial were time to death or admission to hospital for cardiovascular reasons. There was no significant difference in the probability of all-cause death in patients who were treated with rosuvastatin when compared with the placebo group (adjusted HR, 1.00; 95.5% CI, 0.898–1.122; P=0.943). Furthermore, there was no significant difference in the composite end point of death or admission to hospital for cardiovascular reasons (adjusted HR, 1.01; 99% CI, 0.908–1.112; P=0.903).

n-3 Polyunsaturated Fatty Acids: There is a large body of experimental evidence suggesting that n-3 PUFA has favorable effects on inflammation, including a reduction of endothelial activation and production of inflammatory cytokines, platelet aggregation, autonomic tone, blood pressure, heart rate, and LV function. In a parallel arm of the GISSI-HF study, patients with NYHA classes II to IV heart failure were randomized to receive n-3 PUFAs or placebo. The GISSI-HF trial showed that long-term administration of 1 g/d of omega n-3 PUFA resulted in a significant reduction in both the all-cause mortality (adjusted HR, 0.91; 95.5% CI, 0.83–0.99; P=0.041) and the cardiovascular admissions (adjusted HR, 0.92; 99% CI, 0.849–0.999; P=0.009), in all the predefined subgroups, including patients with heart failure in nonischemic cardiomyopathy group.69 Although n-3 PUFA are not endorsed by current practice guidelines, the use of n-3 PUFA may be considered in patients who remain symptomatic, despite optimal medical therapy.

Oxypurinol/Allopurinol: Elevated levels of uric acid are known to predict mortality and the need for heart transplantation in patients with heart failure.70 Uric acid is a byproduct of the purine metabolism via the xanthine oxidase pathway. Serum uric acid levels may increase in heart failure because of increased generation, decreased excretion, or a combination of both the factors. Recent studies have shown that uric acid can trigger IL-1β–mediated inflammation via activation of the NLRP3 inflammasome, which is a large multimolecular complex that plays a critical role in the processing of immature IL-1β to mature secretable form of IL-1β. Monosodium urate crystals can also activate the innate immune system through engagement of TLR2 and TLR4.71

The OPT-CHF (Oxypurinol Therapy for Congestive Heart Failure) trial was a prospective randomized clinical trial that evaluated the effects of the xanthine oxidase inhibitor oxypurinol in patients with NYHA functional classes III to IV heart failure with a LVEF ≤40%.72 The end point of the trial was a clinical composite comprised morbidity, mortality, and QoL evaluated at 24 weeks. The percentage of patients characterized as improved, unchanged, or worsened did not differ between those receiving oxypurinol or placebo. In a subgroup analysis, patients with elevated serum uric acid level of >9.5 mg/dL responded favorably to oxypurinol, whereas oxypurinol patients with serum uric acid level of <9.5 mg/dL exhibited a trend toward worsening. The National Institutes of Health–sponsored EXACT (Using Allopurinol to Relieve Symptoms in Patients With Heart Failure and High Uric Acid Level Trial; NCT00987415) tested the hypothesis that treatment with allopurinol would lead to improvements in a composite clinical score in patients with heart failure with a reduced EF and a serum uric acid level of >9.5 mg. Overall, 253 patients were randomized in EXACT. Treatment with allopurinol significantly reduced serum uric acid levels versus placebo (P=0.001); however, the proportion of patients who worsened, stayed the same, or improved their heart failure classification was similar for allopurinol versus placebo (P=0.25). Moreover, the QoL (P=0.16) and submaximal exercise (P=0.64) was also similar between groups.

Immunomodulation

An alternative approach to targeting specific components of the inflammatory cascade is to use strategies that dampen the various components systemic inflammatory response. Given the recognition that cross-talk between innate and adaptive immune systems leads to progressive LV remodeling after acute myocardial infarction and that adverse LV remodeling is driven by activation of monocyte-derived macrophages, dendritic cells, and CD4+ T cells that interact with cardiac autoantigen–loaded dendritic cells,72–74 there has been interest in developing broad-based immunomodulatory strategies for patients with heart failure. Thus far, 3 different approaches have been used in heart failure studies, such as intravenous immunoglobulin (IVIG), methotrexate, and immune modulation therapy (IMT).

Intravenous Immunoglobulin
Therapy with IVIG has been tried in a wide range of immune-mediated disorders, such as Kawasaki syndrome, dermatomyositis, and multiple sclerosis, and most recently dilated cardiomyopathy, wherein the initial results have been encouraging. In a double-blind, placebo-controlled study of 20 ischemic and nonischemic NYHA classes II to IV patients with heart failure with an LVEF <40% IVIG treatment for 6 months resulted in a significant increase in LVEF from 26% to 31%, independent of heart failure cause.46 These improvements in functional class and LV function were accompanied by an increase in the anti-inflammatory mediators IL-10, IL-1 receptor antagonist (IL-1Ra), and soluble TNF receptors, as well as a slight decrease in plasma TNF suggesting that IVIG evoked a net anti-inflammatory effect. In contrast to these encouraging results, induction therapy with IVIG in the Intervention in Myocarditis and Acute Cardiomyopathy (IMAC) trial in patients with recent-onset cardiomyopathy (<6 months) and an LVEF <40% demonstrated no significant effect on LVEF when compared with placebo.47 However, it
bears emphasis that there was also an increase in LVEF from 23% to 42% in the placebo arm, which would have made it difficult to show a statistically significant increase in LVEF in the treatment arm. Moreover, there were important differences in the IVIG dosing strategies in IMAC and the study by Gullestad et al. That is, although both the studies used induction therapy (a total of 2 g/kg IVIG), in the study by Gullestad et al maintenance therapy (monthly infusions [0.4 g/kg] for a total of 5 months) was also given. Thus, 1 possible reason for the different outcomes in these 2 studies is that IVIG maintenance therapy is required for an extended period of time, as has been observed in other chronic inflammatory disorders.

**Methotrexate**

Epidemiological studies have shown that patients with rheumatoid arthritis have an increased incidence of heart failure, and that the heart failure that develops in elderly patients with rheumatoid arthritis cannot be explained entirely by traditional cardiovascular risk factors. Notably, the heart failure that develops in these patients is associated with a concomitant increase in circulating levels of TNF. Methotrexate, which was originally developed as a folate antagonist for the treatment of cancer, has become a mainstay of therapy in rheumatoid arthritis. Several mechanisms have been proposed, including inhibition of T-cell proliferation via its effects on purine and pyrimidine metabolism, inhibition of transmethylation reactions required for the prevention of T-cell cytotoxicity, interference with glutathione metabolism leading to alterations in recruitment of monocytes and other cells to the inflamed joint, and promotion of the release of the endogenous anti-inflammatory mediator adenosine. Of note, the use of methotrexate in rheumatoid arthritis has also been associated with reduced cardiovascular events, including heart failure hospitalization, especially in patients of 65 years. Methotrexate was evaluated in a small (n=71) prospective randomized clinical trial of patients with heart failure treated with 7.5 mg QW for 12 weeks. Compared with patients on optimal medical therapy, addition of low-dose methotrexate resulted in a significant reduction in the circulating levels of proinflammatory cytokines (TNF, IL-6, and MCP-1) and upregulation of the anti-inflammatory cytokines (IL-10 and soluble IL-1 receptor antagonist). There were also improvements in NYHA classification, 6-minute walk test distance, and QoL when compared with baseline values. However, methotrexate had no effect of LV remodeling nor LVEF after 12 weeks of therapy. The main adverse effects reported for low-dose methotrexate were related to gastrointestinal symptoms. Importantly, there were no severe drug toxicities recorded, such as bone marrow suppression or alopecia. The Methotrexate Therapy Effects in the Physical Capacity of Patients With Ischemic Heart Failure (METIS) trial evaluated low-dose methotrexate in 50 patients with chronic ischemic heart disease. Patients were given methotrexate (7.5 mg) or placebo, plus folic acid (5 mg), for 12 weeks. The primary end point was the difference in 6-minute walk test distance before and after treatment. There was no significant difference between groups in distance covered in the 6-minute walk test, nor NYHA classification. The effects of methotrexate on the rate of heart failure hospitalization (secondary outcome measure) are being evaluated in the ongoing Cardiovascular Inflammation Reduction Trial (CIRT; NCT 1594333), which examines whether low-dose methotrexate reduces heart attacks, strokes, or death in people with type 2 diabetes mellitus or metabolic syndrome that have had a heart attack or known coronary artery disease.

**Immune Modulation Therapy**

IMT (Celacade; Vasogen, Inc) used a medical device that exposes a sample of blood to a combination of physio-chemical stressors ex vivo. The treated blood sample is administered intramuscularly along with local anesthetic into the same patient from whom the sample is obtained. The physio-chemical stresses to which the autologous blood sample is subjected are known to initiate or facilitate apoptotic cell death. The uptake of apoptotic cells by macrophages results in a downregulation of proinflammatory cytokines, including TNF, IL-1β, and IL-8, and an increase in production of the anti-inflammatory cytokines, including TGF-β and IL-10. Given the imbalance between pro and anti-inflammatory cytokines in patients with heart failure, it was hypothesized that IMT would restore this balance toward normal. In a pilot study using Celacade in 73 patients with moderate heart failure, the investigators noted that the group receiving Celacade experienced significantly fewer hospitalizations or deaths, when compared with the placebo group. The decrease in event rate in the treatment arm was accompanied by improvements in QoL and NYHA clinical classification. On the basis of the encouraging results of the early studies, the Advance Chronic Heart Failure Clinical Assessment of Immune Modulation (ACCLAIM) pivotal study was conducted in 2426 patients with NYHA classes II to IV heart failure patients with ischemic and non-ischemic dilated cardiomyopathy. Patients were randomly assigned to receive Celacade (n=1213) or placebo (n=1213) by intragluteal injection on days 1, 2, 14, and every 28 days thereafter. The primary end point was an event-driven composite of time to death from any cause or first hospitalization for cardiovascular reasons. There was no significant difference between the Celacade and placebo-treated patients with respect to the primary end point of the trial (HR, 0.92; 95% CI, 0.80–1.05; P=0.22). However, in a prespecified subgroup analysis of patients with NYHA II heart failure and patients without a history of previous myocardial infarction, it was noted that treatment with Celacade was associated with a 39% (HR, 0.61; 95% CI, 0.46–0.80; P=0.0003) and 26% (HR, 0.74; 0.57–0.95; P=0.02) reduction in the risk death from any cause or first hospitalization for cardiovascular reasons, respectively, suggesting that IMT may have benefited patients with nonischemic cardiomyopathy or patients with milder heart failure (NYHA class II).

**Autoimmunity**

Autoimmunity triggered by microbial infections or tissue injury has been implicated in the pathogenesis of dilated cardiomyopathy. Although a full discussion of autoimmunity in heart failure is beyond the intended scope of this review, this topic is mentioned briefly herein insofar as direct communication between the innate and adaptive immune systems has been shown to lead to myocarditis and a dilated cardiomyopathy through a mechanism that involves self-recognition.
of autoantigens. Of several causal pathways of heart failure, anti-inflammatory therapy targets a pathway that contributes to the clinical outcomes. Of several causal pathways of heart failure, anti-inflammatory therapies worsened clinical outcomes in patients with heart failure who are placed on evidence-based medical or device therapies. Although plausible, these possibilities do not necessarily explain the observation that anti-TNF therapies worsened clinical outcomes in patients who received the highest doses, or who had the longest duration of therapy. Figure 6C illustrates a third possibility, in which anti-inflammatory strategies might favorably influence the biology of inflammation in the heart and the outcomes of clinical heart failure trials targeting inflammation is not known, Figure 6 illustrates several possibilities that warrant discussion.

The first and most obvious question raised by the neutral or negative clinical trials reviewed above, is whether inflammation is a correlate of heart failure, or whether instead it is disease causing. The extant literature shows that circulating levels of inflammatory biomarkers correlate with disease progression (ie, NYHA class), as well as with clinical outcomes. One possibility depicted in Figure 6A is that although inflammatory markers are elevated in heart failure, they may not be directly responsible for the pathophysiological process(es) that lead to untoward clinical outcomes; that is, inflammation correlates with disease severity, but does not contribute to disease progression in heart failure. Although this possibility cannot be formally excluded, the wealth of preclinical data demonstrating that clinically relevant levels of proinflammatory cytokines faithfully mimic the heart failure phenotype in a variety of different species, including humans, argues against this possibility as the primary or sole explanation. Figures 6B illustrates a clinical scenario, in which there may be several disease-causing pathways in heart failure, with 1 pathway predominately driving clinical outcomes, and the other pathway(s) contributing to but not primarily responsible for clinical outcomes (ie, disease modifying). In this situation, the effects of anti-inflammatory therapies could be substantially offset by the effects of therapies (eg, neurohormonal antagonists) that are more directly antagonistic to the pathways driving clinical outcomes. Figure 6B might also explain why circulating levels of proinflammatory cytokines decrease in patients with heart failure who are placed on evidence-based medical or device therapies. Although plausible, these possibilities do not necessarily explain the observation that targeted anti-TNF therapies worsened clinical outcomes in patients who received the highest doses, or who had the longest duration of therapy. Figure 6C illustrates a third possibility, in which anti-inflammatory strategies might favorably influence...
Institutes of Health (RO1 HL89543, RO1 111094).

cited in this review because of the imposed space limitations.

We apologize in advance to colleagues whose work was not directly
during the past 2 decades.

from the striking advances that have been made in this field
flammation, despite optimal medical and device therapy, rais-
subsets of patients with heart failure who have ongoing in-
therapies in general, as well as the specific difficulties in
targeting chronic inflammation in the setting of heart failure, is there a foreseeable future for developing anti-inflammatory
Figure
(eg, disruption of homeostatic parainflammatory responses; Figure 2) of the anti-inflammatory strategy. It is also completely possible that some combination of Figure 6A–6C may
explain the disappointing results of the clinical trials that have been conducted thus far.

Given the inherent difficulties in developing new heart fail-
ure therapies in general, as well as the specific difficulties in

Acknowledgments
We apologize in advance to colleagues whose work was not directly
cited in this review because of the imposed space limitations.

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None.

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Innate Immunity and the Failing Heart: The Cytokine Hypothesis Revisited

Douglas L. Mann

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TOLL-LIKE RECEPTOR SIGNALING PATHWAYS

A, Toll-like receptors and interleukin-1 receptors have a conserved cytoplasmic domain, that is known as the Toll/IL-1 R domain. The TIR domain is characterized by the presence of three highly homologous regions (known as boxes 1, 2 and 3). Despite the similarity of the cytoplasmic domains of these molecules, their extracellular regions differ markedly: TLRs have tandem repeats of leucine-rich regions (known as leucine rich repeats, LRR), whereas IL-1 Rs have three immunoglobulin (Ig)-like domains. B, Stimulation of TLRs triggers the association of MyD88, which in turn recruits IRAK4, thereby allowing the association of IRAK1. IRAK4 then induces the phosphorylation of IRAK1. TRAF6 is also recruited to the receptor complex, by associating with phosphorylated IRAK1. Phosphorylated IRAK1 and TRAF6 then dissociate from the receptor and form a complex with TAK1, TAB1 and TAB2 at the plasma membrane (not shown), which induces the phosphorylation of TAB2 and TAK1. IRAK1 is degraded at the plasma membrane, and the remaining complex (consisting of TRAF6, TAK1, TAB1 and TAB2) translocates to the cytosol, where it associates with the ubiquitin ligases UBC13 and UEV1A. This leads to the ubiquitylation of TRAF6, which induces the activation of TAK1. TAK1, in turn, phosphorylates both MAP kinases and the IKK complex, which consists of IKK-α IKK-β and IKK-γ (also known as IKK1, IKK2, NEMO, respectively). The IKK complex then phosphorylates IκB, which leads to its ubiquitylation and subsequent degradation. This allows NF-κB to translocate to the nucleus and induce the expression of its target genes. (Reproduced with permission from Mann DL, The role of innate immunity in heart failure, in Heart Failure: A Companion to Braunwald's Heart Disease, edited by Mann DL and Felker GM, 2015, pp 109-126, Elsevier/Saunders, Philadelphia, Pennsylvania).
As shown in panel A of the Figure, the signaling pathway that is used by the TLR family of receptors is highly homologous to that of the IL-1 receptor (IL-1R) family (see below). TLRs are type 1 membrane-spanning receptors that have a leucine-rich repeat extracellular motif and an intracellular signaling motif that is similar to interleukin (IL-1). With the exception of TLR3, all TLRs interact with an adaptor protein termed MyD88 (myeloid differentiation factor 88) via their Toll Interleukin Receptor (TIR) domains (panel B). MyD88-dependent signaling through TLR2 and TLR4 requires an adaptor protein termed TIRAP (TIR domain-containing adaptor protein) to initiate signaling. When stimulated, MyD88 sequentially recruits IL-1 receptor associated kinases 4, 1 and 2 (IRAK4, IRAK1 and IRAK2) to the receptor complex. Phosphorylation of IRAK1 on serine/threonine residues by IRAK4 results in recruitment of tumor necrosis receptor associated factor 6 (TRAF6) to the complex, which is responsible for early responses in response to TLR signaling (see reference 1 for more details on TLR signaling).

**PROINFLAMMATORY CYTOKINES**

**Tumor Necrosis Factor Superfamily**

The tumor necrosis factor (TNF) superfamily consists of 19 well-characterized ligands (TNFSF) and 34 TNF superfamily receptors (TNFRSF). Members of the TNF superfamily of ligands and receptors are expressed in a broad variety of cell types, including myocardial cells. Notably, all members of the TNF superfamily exhibit pro-inflammatory activity. Of note, recent studies have identified a potential role for TNF superfamily ligands/receptors in terms of mediating inflammatory responses in the heart, including TNF/TNFR1, TNFR2 (TNFSF2/TNFRSF1A, TNFRSF1B), FasL/Fas (TNFSF6/TNFRSF6), TWEAK (tumor necrosis factor-like weak inducer of apoptosis)/TWEAKR (TNFSF12/TNFRSF12), and RANKL (Receptor activator of NF-κB ligand)/RANK (TNFSF11/TNFRSF11A). In contrast to FasL, TNF, TWEAK and RANKL signal through TNFSF receptors that engage a common scaffolding protein.
termed TNF receptor associated factor 2 (TRAF2), that is recruited to the TNFSF receptors following engagement of their cognate ligands. In contrast to TNF, TWEAK and RANKL, cardiac-restricted overexpression of FasL (TNFSF6) does not lead to dilated cardiomyopathy. Notably, TNF, TWEAK and RANKL signal through TNFSF receptors that engage a common scaffolding protein termed TNF receptor associated factor 2 (TRAF2), that is recruited to the TNFSF receptors following engagement of their cognate ligands. Cardiac restricted expression of TRAF2 provokes a dilated cardiac phenotype, that phenocopies cardiac restricted overexpression of TNF, and is mediated, at least in part, though NF-κB.\textsuperscript{5}

**Interleukin-1 Family**

Although the original IL-1 family (IL-1F) consisted of IL-1\textsubscript{α} (IL-1F1) and IL-1\textsubscript{β} (IL-1F2), the IL-1 family has now expanded to include seven ligands with agonist activity (IL-1α and IL-1β, IL-18 (IL-1F4), IL-33 (IL-1F11), IL-36α (IL-1F6), IL-36β (IL-1F7), IL-36γ (IL-1F8), three receptor antagonists (IL-1Ra [IL-F3], IL-36Ra [IL-F5], IL-38 [IL-F10]), and an anti-inflammatory cytokine (IL-37 [IL-10]). Members of the IL-1 Receptor (IL-1R) family include six receptor chains forming four signaling receptor complexes, two decoy receptors (IL-1R2, IL-18BP), and two negative regulators (TIR8 or SIGIRR [IL-1R8], IL-1RAcPb).\textsuperscript{6} Similar to TNF, IL-1β and IL-18 appears to synthesized within the myocardium in response to stressful environmental stimuli, and both IL-1β mRNA and protein have been detected in the hearts of patients with dilated cardiomyopathy.\textsuperscript{7} Moreover, in vivo studies have shown specific blockade of IL-18 using IL-18 binding protein improves contractile function in human atrial tissue following ischemia reperfusion injury,\textsuperscript{8} as well as lipopolysaccharide-induced LV dysfunction in experimental animals.\textsuperscript{8}

**Interleukin-6 (IL-6) Family**

Based on their functional redundancy, structural similarity, and use of a common signaling receptor, interleukin-6 (IL-6), leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1),
ciliary neurotrophic factor (CNTF), interleukin-11 (IL-11), and oncostatin M (OSM) are considered to represent the "IL-6 family" of cytokines. Inclusion in the IL-6 family is based on a helical cytokine structure and receptor subunit makeup. The IL-6 family of cytokines triggers downstream signaling pathways in multiple cell types, including cardiac myocytes, either through the homodimerization of the gp130 receptor or through the heterodimerization of gp130 with a related transmembrane receptor. All IL-6 type cytokines potentially activate STAT3, and to a lesser extent STAT1 through their common gp130 subunits. The specificity of cytokine signaling within the IL-6 family is determined by the composition of the cytoplasmic domains associated with the signal-competent receptor complex. The suppressor of cytokine signaling (SOCS) (also referred to as cytokine-inducible SH2 proteins [CIS]) are a family of specific negative regulatory feedback elements of JAK/STAT signaling. Expression of some SOCS family members is regulated transcriptionally by STATs, thereby acting as a negative feedback loop for JAK-STAT signaling. Both SOCS-1 and SOCS-3 interact with the kinase domain of various JAK proteins, thereby preventing STAT phosphorylation. Previous clinical studies showed that the plasma level of IL-6, CT-1, LIF and gp130 are elevated in patients with advanced heart failure and that high levels are associated with a poor prognosis for heart failure patients.

REFERENCES


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