

# Inflammatory Cytokines in Heart Failure: Mediators and Markers

Lars Gullestad<sup>a, e</sup> Thor Ueland<sup>b, c</sup> Leif Erik Vinge<sup>a, b</sup> Alexandra Finsen<sup>a, b</sup>  
Arne Yndestad<sup>b</sup> Pål Aukrust<sup>b, d, e</sup>

<sup>a</sup>Department of Cardiology, <sup>b</sup>Research Institute for Internal Medicine, <sup>c</sup>Section of Endocrinology, and <sup>d</sup>Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, and <sup>e</sup>Faculty of Medicine, University of Oslo, Oslo, Norway

## Key Words

Chronic heart failure · Inflammatory mediators · Cardiac remodeling · Immunomodulating treatment · Markers

## Abstract

Evidence from both experimental and clinical trials indicates that inflammatory mediators are of importance in the pathogenesis of chronic heart failure (HF) contributing to cardiac remodeling and peripheral vascular disturbances. Several studies have shown raised levels of inflammatory cytokines such as tumor necrosis factor (TNF) $\alpha$ , interleukin (IL)-1 $\beta$  and IL-6 in HF patients in plasma and circulating leukocytes, as well as in the failing myocardium itself. There is strong evidence that these mediators are involved in processes leading to cardiac remodeling such as hypertrophy, fibrosis and apoptosis. Some of these cytokines can also give useful prognostic information as reliable biomarkers in this disorder. In general, immunomodulating treatments have, with a few exceptions, been neutral or even harmful. However, the negative results of anti-TNF studies, for instance, do not necessarily argue against the 'cytokine hypothesis'. These studies just underscore the challenges in developing treatment modalities that can modulate the cytokine network in HF patients and result in beneficial net effects. Future studies should identify the crucial actors and their mechanisms of action in the immunopathogenesis of chronic HF and, in par-

ticular, clarify the balance between adaptive and maladaptive effects of these molecules. Such studies are a prerequisite for the development of new treatment strategies that target inflammatory and immunopathogenic mechanisms in HF. In this review article, these issues are thoroughly discussed, and we also argue for the possibility of future therapeutic targets such as mediators in innate immunity, chemokines and mediators in matrix remodeling.

Copyright © 2012 S. Karger AG, Basel

## Introduction

Heart failure (HF) is a highly complex multistep disorder in which a number of physiological systems participate. Until a few decades ago, HF was considered a hemodynamic disorder. In recent years, both experimental and clinical studies have clearly demonstrated the involvement of neurohormones in the progression of HF, leading to new treatment modalities such as angiotensin-converting enzyme inhibitors, angiotensin receptor blockers,  $\beta$ -blockers and aldosterone blockers in chronic systolic HF. However, additional blockade of other hormonal systems has been disappointing (i.e. endothelin, vasopressin and sympathomimetics). Thus, in spite of several improvements in the management of chronic HF, this disorder is still characterized by high

**Table 1.** Pathophysiological effects of inflammatory mediators

---

LV dysfunction
Negative inotropic effect
Hypertrophy
Fibrosis
Apoptosis
Endothelial dysfunction
Cachexia
Anemia
Activation of fetal gene program
Promotion of thromboembolism
$\beta$ -receptor uncoupling from adenylate cyclase
Abnormalities of mitochondrial energetics
Muscular weakness

---

mortality and morbidity, suggesting that important pathogenic mechanisms remain active and unmodified by the present treatment modalities. Cytokines and other inflammatory mediators may contribute to these. However, although several lines of evidence suggest a role for inflammation in the development and progression of HF, these issues are still unclear.

### Role of Inflammation in Systolic HF

Several reports have demonstrated enhanced expression and release of inflammatory cytokines such as tumor necrosis factor (TNF) $\alpha$ , interleukin (IL)-1, IL-6, IL-18, cardiotrophin-1 and Fas ligand, as well as several chemokines [e.g. monocyte chemoattractant peptide (MCP)-1/CCL2, IL-8/CXCL8 and macrophage inflammatory protein-1 $\alpha$ /CCL3] in HF patients [1–5]. Plasma levels of inflammatory cytokines and chemokines appear to be elevated in direct proportion to deterioration of functional class (i.e. New York Heart Association classification) and cardiac performance [i.e. left ventricular ejection fraction (LVEF)] [1, 3, 6]. Moreover, enhanced expression of inflammatory mediators has also been demonstrated within the failing myocardium (e.g. adhesion molecules, TNF $\alpha$ , IL-6-related cytokines and chemokine receptors) [7, 8]. Moreover, several of these mediators have been found to give prognostic information beyond that of traditional risk markers [9].

A series of experimental studies have revealed that the biological effects of cytokines may explain several aspects of the syndrome of chronic HF. The pathogenic role of inflammatory cytokines in chronic HF is supported by

various transgenic mouse models. Notably, systemic administration of TNF $\alpha$  in concentrations comparable to those found in the circulation of HF patients has been shown to induce a dilated cardiomyopathy-like phenotype in animal models [10], and cardiac-specific overexpression of TNF $\alpha$  has been found to promote a phenotype mimicking several features of clinical HF such as cardiac hypertrophy, ventricular dilation and fibrosis, as well as several biochemical and cellular dysfunctions [11]. More recent studies in gene-modified mice have also shown a link between IL-6 and its receptor subunit glycoprotein (gp)130, which is common to several cytokines in the IL-6 family, as well as various chemokines (e.g. MCP-1 and CXCL13) and the development of HF [12]. Inflammatory cytokines may modulate myocardial functions by a variety of mechanisms including stimulation of hypertrophy and fibrosis through direct effects on cardiomyocytes and fibroblasts, impairment of myocardial contractile function through direct effects on intracellular calcium transport and signal transduction through  $\beta$ -adrenergic receptors, induction of apoptosis and stimulation of genes involved in myocardial remodeling [13]. Inflammatory mediators could also contribute more indirectly to the progression of HF through impairment of bone marrow function with secondary anemia, inappropriate endothelial cell activation and impairment of peripheral muscle with secondary induction of systemic inflammation and reflex abnormalities in HF [13] (table 1). However, as will be discussed below, while ‘too much’ of these mediators is maladaptive, ‘too little’ could also be harmful, illustrating the challenges for immunomodulating therapy in HF.

### Inflammation in Post-Myocardial Infarction Remodeling

During the acute phase of myocardial infarction (MI), the production of cytokines represents an intrinsic response to injury. The triggers of cytokine release during MI include ischemia, reactive oxygen species that are released during ischemia-reperfusion, membrane injury and mechanical stress, as well as the release of so-called danger-associated molecular patterns (DAMPs; e.g. ATP, uric acid, mitochondrial DNA and heat shock proteins), promoting an inflammatory cytokine response including the release of prototypical inflammatory cytokines such as TNF $\alpha$ , IL-6 and IL-1 $\beta$  [14]. This inflammatory process is a prerequisite for wound repair, scar formation and compensatory hypertrophy. Recent data have also

shown that these inflammatory cytokines initiate a cardioprotective signaling cascade, also called the survival-activating factor enhancement pathway [15]. However, while a moderate cytokine response could be protective, an inappropriate and persistent inflammatory reaction could lead to maladaptive responses [13]. Besides that, animal data have shown that when the infarct size is large, the cytokine gene expression may remain significantly elevated for a long time, especially in the noninfarcted region. Moreover, the levels of late cytokine activation have been shown to correlate with left ventricular (LV) end-diastolic diameter 20 weeks after infarction in rats [16]. Thus, the magnitude of the injury and inflammatory response as well as the time of activation appear to be of importance and need to be taken into account when treatment strategies are planned.

### **Inflammation in HF with Preserved Ejection Fraction**

Approximately half of the patients with HF have preserved ejection fraction (EF) (HFpEF), while the remainder has reduced EF (HFrEF). Although HFpEF and HFrEF share common symptoms such as dyspnea and fatigue, hemodynamic strain with elevated filling pressures and reduced cardiac output, as well as a clinical course with frequent hospitalizations and reduced survival, they are thought to have different underlying disease mechanisms. The strongest argument for different pathophysiologies is probably that therapies with proven benefit in HFrEF have failed to improve outcome in HFpEF [17]. Moreover, the pattern of ventricular remodeling is different in HFpEF, characterized by normal to near-normal chamber size, increased wall thickness, greater ratio of wall thickness to chamber dimensions and preserved EF. In HFpEF, the myocytes have increased diameter and stiffness, and changes in the extracellular matrix (ECM) consisting of increased collagen volume, collagen isoform shift and increased stiffness are also seen [18]. Another feature of HFpEF is a more subtle, slow process in the transition towards overt HF, with hypertension as an important contributing process, whereas HFrEF is often characterized by distinct pathophysiological perturbations such as MI. The question is whether inflammatory processes could be involved in the pathophysiology of HFpEF.

While increased inflammation is a well-known feature of HFrEF, this has been less well studied in HFpEF. However, recent data show that patients with diabetes mellitus and hypertension with evidence of diastolic dys-

function or patients with overt HFpEF frequently have increased blood levels of TNF $\alpha$ , IL-1 $\beta$  and IL-6 [19]. Recent studies demonstrate an important role for T cells in myocardial remodeling, involving increased activity of the enzyme lysyl oxidase, which contributes to increased collagen cross-linking and increased myocardial stiffness [20]. In mouse models, hypertension and metabolic syndrome, both risk factors for the development of HFpEF, are associated with induction of an inflammatory T helper (Th)1 phenotype (i.e. increased levels of TNF $\alpha$ , interferon- $\gamma$  and IL-18) and increased levels of the profibrotic cytokine transforming growth factor (TGF)- $\beta$ , potentially contributing to an inflammatory-driven myocardial fibrosis [21]. Moreover, IL-6 infusion in rats results in concentric LV hypertrophy, increased collagen volume fraction and increased myocardial stiffness [22]. Additionally, IL-18 has been demonstrated to increase the expression and production of osteopontin, which stimulates interstitial fibrosis [23], and TGF- $\beta$ , which stimulates collagen synthesis, and inhibit matrix degradation by reducing matrix metalloproteinases (MMPs) [24]. The increased production and reduced degradation of collagen and increased activation of lysyl oxidase-1, resulting in a cross-linked and insoluble collagen network, may in turn result in diastolic dysfunction over time. Importantly, the interaction between inflammatory cytokines and ECM remodeling is not specific for HFpEF, and these interactions could also be of importance in adverse remodeling during HFrEF. Moreover, the findings regarding cytokine levels in HFpEF point more towards a complex dysregulation of the cytokine network rather than a simple imbalance between Th1 and Th2 cytokines. This could include activation of mediators involved in both inflammation and fibrogenesis such as galectin-3 [25], enhanced Th17 activation, which also involves IL-6 and has been linked to myocardial fibrosis [26], as well as a lack of overall regulation of the immune response by impaired function of regulatory T cells [27].

### **Inflammatory Variables as Biomarkers**

Biomarkers are now widely used for risk stratification and evaluation of therapeutic responses in cardiovascular disease, and in HF, N-terminal pro-hormone of brain natriuretic peptide (NT-proBNP) has been most extensively studied both as a prognostic marker and recently also to guide therapy [28]. In addition to being involved in the pathogenesis of HF as mediators, inflammatory cytokines and related mediators could therefore also be

suitable markers for risk stratification and prognostication in patients with HF. However, the most important mediators may not necessarily be the best biomarkers. The leading role of C-reactive protein (CRP) as an inflammatory biomarker in cardiovascular disease is not primarily based on its pathogenic role in these disorders, but rather on its ability to reflect upstream inflammatory activity. Several small studies have shown elevated CRP levels in HF, and a few have shown associations with HF development and adverse outcome. Most of these are limited in size or do not adjust for state-of-the-art biomarkers such as NT-proBNP. Recently, however, Anand et al. [29] demonstrated that in 4,202 patients with HF, increased CRP was associated with features of more severe HF and was independently associated with adverse outcome. Moreover, in a substudy of the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA), the authors found a significant interaction between CRP and the effect of rosuvastatin on adverse events, indicating that inflammatory biomarkers may be useful in identifying patients who will benefit from statin therapy [30]. Interestingly, another pentraxin (pentraxin 3), which unlike CRP is produced at the site of inflammation, has recently been found to be associated with an increased risk for cardiac events in HF patients [31].

A variety of inflammatory cytokines are upregulated in chronic HF, and several studies suggest that TNF $\alpha$ , IL-6 or IL- $\beta$  can predict adverse outcome in these patients, although these studies are often limited in sample size and lack full adjustment [9, 32]. Furthermore, these cytokines circulate at low levels, thus increasing analytical variation, and therefore require expensive high-sensitivity assays that need a large sample volume. Still, assessing these cytokines in large populations of well-characterized HF patients may provide important pathophysiological information.

While soluble ligands often circulate at low levels, their corresponding soluble receptors are frequently detected at high levels in serum and plasma with the potential to be more reliable biomarkers. Thus, soluble receptors for TNF $\alpha$  [i.e. soluble TNF receptor (sTNFR) 1 and sTNFR2] and several other members of the TNF receptor superfamily such as CD27, Fas and osteoprotegerin (OPG) are present in the circulation at relatively high levels and are further elevated in HF [33]. These soluble receptors may be considered stable and reliable markers of activity in their ligand/receptor system [33]. Both sTNFR1 and sTNFR2 have been shown to give prognostic information in HF, although the studies are of limited size and lack proper multivariable adjustment [9, 34]. The

association between OPG and long-term outcome in patients with chronic HF was recently studied in subpopulations of the GISSI-HF trial (n = 1,229) [35] and CORONA trial (n = 1,464) [36]. In GISSI-HF, OPG was independently associated with all-cause mortality and hospitalization due to cardiovascular causes, although this study did not adjust for NT-proBNP [35]. In the CORONA trial, OPG was associated with hospitalization due to worsening of HF, also after adjustment for conventional risk markers including measures of LV dysfunction and NT-proBNP [36]. As for IL-6 signaling, circulating levels of the common receptor subunit gp130 could potentially reflect the activity of the whole IL-6 family, but information on the prognostic relevance of gp130 in HF is still limited [37].

Galectins are a family of soluble  $\beta$ -galactoside-binding lectins that play regulatory roles in inflammation, immunity and cancer. Recently, it has been suggested that galectin-3 may play a role in the pathophysiology of HF through promotion of myocardial fibrosis and inflammation, two related and interacting processes involved in myocardial remodeling [38]. Therefore, increased galectin-3 may be a marker for patients with a poor prognosis related to excessive and potentially irreversible myocardial fibrosis, which again may be related to enhanced inflammation. Thus, we and others have recently reported an association between elevated circulating galectin-3 and poor clinical outcomes in patients with HF [39–41]. However, the study population in these studies is rather small, and further studies are needed to confirm that galectin-3 could provide additional prognostic information in HF patients, beyond that of established risk markers.

The ability of these inflammatory markers to predict the development of HF and adverse outcome supports the notion that they reflect important pathogenic pathways that are activated during HF. However, as shown in the GISSI-HF trial, although significant associations were found between OPG and an adverse outcome, even after multivariable adjustment, reclassification analyses suggested that the association was unlikely to improve risk stratification of HF patients in a clinically meaningful way. Still, although NT-proBNP is a strong biomarker in HF, it is unlikely that it reflects all the pathogenic processes involved in this complex disorder. Thus, it has been suggested that the measurement of global patterns of cytokines and other biomarkers may yield more relevant biological information than assays of individual proteins. As several mediators are involved in the development and progression of HF, at least partly through different mech-

**Table 2.** Randomized trials of immunomodulating therapy in HF

Study	Patient number/design	Agent	Follow-up	Mean age years	Mean EF	ACE-RB/BB %	Primary end point	Outcome
RENEWAL [46]	1,500/RDB	etanercept	RECOVER: 5.7 months RENAISSANCE: 12.9 months	63	23	98/62	clinical composite score	no effect on clinical status, death or HF hospitalization
ATTACH [47]	150/RDB	infliximab in 2 doses	28 weeks	61	24	100/73	clinical composite score	high dose had adverse effect on clinical outcome
Sliwa et al. [103]	38/RDB	pentoxifylline	6 months	55	25	100/100	NYHA class and LVEF	improved symptoms and LVEF
Muller et al. [50]	34/prospective cases, controlled	immuno-adsorption	12 months	48	23	100/100	NYHA class and LVEF	improved symptoms and LVEF
Staudt et al. [104]	25/prospective cases, controlled	immuno-adsorption + IVIg	3 months	50	20	100/64	NYHA class, myocardial inflammation and LVEF	improved symptoms and LVEF, reduced myocardial inflammation
Gullestad et al. [49]	40/RDB	IVIg	6 months	61	27	100/75	NYHA class and LVEF	improved clinical status and LVEF
McNamara et al. [105]	62/RDB	IVIg	12 months	43	25	90/18	LVEF and symptoms	no effect
Gullestad et al. [52]	56/RDB	thalidomide	3 months	66	25	100/91	LVEF, LV volume, symptoms	improved LVEF and LV remodeling
Torre-Amione et al. [54]	75/RDB	immuno-modulating therapy	6 months	62	22	89/51	6-min walk test, symptoms, QoL, LVEF	no change in symptoms, QoL, LVEF; reduced hospitalization and mortality
ACCLAIM [55]	2,216/RDB	nonspecific immuno-modulating therapy	10.2 months	64	23	94/91	composite: death CV hospitalization	no overall effect; effect in subgroups (nonischemic HF, NYHA II)
Hare et al. [62]	405/RDB	oxypurinol	24 weeks	65	26	95/91	composite: HF mortality + morbidity + QoL	no effect overall; effect in those with elevated uric acid
CORONA [59]	5,011/RDB	rosuvastatin 10 mg	32.8 months	73	31	92/75	composite: CV death + nonfatal MI and stroke	no effect overall; reduction in CV hospitalization
GISSI-HF [60]	4,631/RDB	rosuvastatin 10 mg	45 months	68	33	94/62	composite: CV death + hospitalization	no effect overall

RENEWAL = Randomized Etanercept Worldwide Evaluation, incorporating the two clinical trials Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER) and Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE); ATTACH = Anti-

TNF $\alpha$  Therapy Against Congestive HF; ACCLAIM = Advanced Chronic Heart Failure Clinical Assessment of Immune Modulation Therapy; RDB = randomised double blinded; ACE = angiotensin-converting enzyme; RB = receptor blocker; BB = beta blocker; NYHA = New York Heart Association; QoL = quality of life.

anisms and at different levels, a combination of circulating levels of multiple markers could potentially identify the subjects with a clinically significant risk with a high degree of accuracy. Such an approach could also be beneficial for the selection of individualized therapy. However, at present the methodology for measurements of several inflammatory markers is not available on a routine basis.

### Immunomodulating Therapy I: Clinical Studies So Far

While there are several studies in animal models suggesting anti-inflammatory effects of angiotensin-converting enzyme inhibitors and  $\beta$ -blockers, the anti-inflammatory effects of these medications in clinical HF seem rather modest. Thus, while high-dose enalapril significantly reduced IL-6 bioactivity in clinical HF, associ-

ated with a reduction in LV septum thickness, there was no effect on other inflammatory cytokines [42]. Moreover, the effect of  $\beta$ -blockers on inflammation in human HF is mixed and uncertain [43, 44]. Thus, it is conceivable that treatments targeting immune activation and inflammation will require the development of new treatment modalities in patients with HF. Studies using immunomodulating therapy are summarized in table 2. Given the central role of TNF $\alpha$  in the pathogenesis of HF, therapeutic modulation targeting this cytokine has received a particularly large amount of attention. Preliminary reports suggested that TNF $\alpha$  inhibition with recombinant chimeric sTNFR2 (etanercept) had a beneficial clinical as well as antiremodeling effect in HF patients [45], but the large Randomized Etanercept Worldwide Evaluation program examining the effect of etanercept on morbidity and mortality in a population of 1,500 patients with symptomatic HF and LVEF <30% demonstrated no effect on mortality, hospitalizations or a clinical composite score [46]. Subanalysis of the trial has suggested an interaction between dose and outcome, as there were fewer hospitalizations/deaths among patients taking the lowest active dose compared with those who received the higher active dose. A second approach targeting TNF $\alpha$  was a trial with an anti-TNF $\alpha$  chimeric monoclonal antibody that binds to human TNF $\alpha$  (infliximab), the Anti-TNF $\alpha$  Therapy Against Congestive HF trial, which was a placebo-controlled phase II trial in 150 patients with symptomatic HF and LVEF <35%. However, the trial was stopped early because of higher rates of mortality and hospitalization in particular in the active high-dose group [47]. A possible explanation for this unfavorable result is that infliximab binds directly to the transmembrane form of TNF, resulting in damage to TNF-expressing cells by antibody-dependent cellular toxicity, complement-dependent cytotoxic effector mechanisms and induction of apoptosis [48]. While such mechanisms may be beneficial in inflammatory disorders such as inflammatory bowel disease [48], they may result in deleterious effects in HF leading to damage to TNF $\alpha$ -expressing cardiomyocytes. On the other hand, it remains to be determined if anti-TNF $\alpha$  therapy could have a beneficial effect in patients with HFpEF, which is characterized by myocardial hypertrophy and fibrosis.

Failure of anti-TNF therapy led to interest in a more general immunomodulating approach with the aim not only to block the detrimental effects of the inflammatory cytokines but also to increase anti-inflammatory cytokines in order to restore an inflammatory imbalance. We demonstrated in a small, double-blinded, placebo-con-

trolled study that intravenous immunoglobulin (IVIg) increased LVEF significantly by 5 EF units, whereas no significant change was seen in the placebo group, and this improvement in LVEF was associated with an anti-inflammatory net effect on the cytokine network [49]. Immunoadsorption has also been shown to have beneficial effects on myocardial function in a relatively small study, potentially by removing pathogenic autoantibodies directed against  $\beta$ -adrenergic receptors within the myocardium [50]. Although there are some similarities between the mechanisms of action of IVIg and immunoadsorption, IVIg did not have any effect on these autoantibodies [51], suggesting that the combination of IVIg and immunoadsorption could be a therapeutic approach in HF, representing overlapping but also distinct mechanisms of action. Thalidomide has been viewed as an anti-TNF agent, and we showed a beneficial effect of this medication on LVEF in a small randomized, double-blinded, placebo-controlled study in patients with chronic HF [52]. However, rather than decreasing TNF $\alpha$ , thalidomide increased plasma levels of this cytokine, and the potential beneficial effect of thalidomide seems to be related to an effect on matrix remodeling through inhibitory effects on MMP-2. Nonetheless, the mechanism of action of thalidomide in HF is still unclear, and larger studies that also include clinical end points are needed to make any firm conclusions. Another approach that has been used is re-administration of autologous blood, exposed *ex vivo* to oxidative stress. Such an approach induces several biological responses including a decrease in inflammation in animal experiments [53], and pilot trials in humans suggested a beneficial effect in HF [54]. However, a larger placebo-controlled trial (Advanced Chronic Heart Failure Clinical Assessment of Immune Modulation Therapy) involving 2,414 patients with HF was recently stopped due to lack of effect [55]. A beneficial effect was reported in patients with mild HF (i.e. New York Heart Association class II) and nonischemic cardiomyopathy [55], but these results may only be viewed as hypothesis generating, and it remains to be determined if this approach really modifies the inflammatory response and is beneficial in clinical HF.

Several studies have shown a remarkable improvement in the prognosis of coronary artery disease after treatment with 3-hydroxy-3-methyl-glutaryl coenzyme A reductase inhibitors (statins) [56, 57]. Recent data suggest that statins have pleiotrophic effects in addition to their cholesterol-lowering properties. Various studies have demonstrated that these so-called pleiotrophic effects of statins include immunomodulatory and anti-in-

flammatory effects as well as antithrombotic effects [58]. Such effects could clearly be of interest in HF, but recently, two large placebo-controlled trials, the CORONA and GISSI-HF trials [59, 60], failed to improve outcome in patients with HF, despite lowering CRP. However, further analysis from the CORONA trial has demonstrated that rosuvastatin has beneficial effects among those patients with evidence of increased inflammation at baseline, i.e. those with CRP >2.0 mg/dl [30].

Several studies suggest that enhanced lipid oxidation and damage induced by reactive oxygen species play a pathogenic role in HF [61]. Oxidative stress can increase cytokine levels and vice versa, involving nuclear factor- $\kappa$ B-related mechanisms, possibly representing a vicious cycle in HF [61]. It was therefore disappointing that a medium-sized placebo-controlled study in HF patients did not show benefit of allopurinol, an agent shown to reduce uric acid and regarded as a marker of enhanced oxidative stress and inflammation [62]. Notably, there was an effect in the subgroup with elevated uric acid at baseline [62].

Thus, in general, clinical trials attempting to modulate inflammation in HF have been largely disappointing except for some small studies with a broad anti-inflammatory approach and subanalyses demonstrating effects in subgroups. This may not necessarily mean the end of the cytokine era; it just underscores the challenges in this therapeutic approach of modulating a cytokine network representing both beneficial and harmful effects on myocardial remodeling. Moreover, the failure of previous trials also emphasizes the need for better understanding the pathogenic roles of inflammation in HF in order to define the most important targets for therapy. Such mechanistic studies have delineated some interesting new targets for therapy in HF.

## **Immunomodulating Therapy II: New Therapeutic Targets**

### *The Innate Immune System in HF*

Infection, as well as tissue damage and cellular stress, is sensed by the innate immune system through pattern recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMP, e.g. lipopolysaccharide, peptidoglycans and microbial nucleic acids) or DAMPs (e.g. heat shock proteins, reactive oxygen species and extracellular ATP) [63]. Upon activation, PRRs initiate defense and repair programs that also include an acute inflammatory response. In the vast majority of cases, this response is terminated once the triggering insult

is cleared and the damaged tissue is repaired. However, if the inflammatory response is not terminated, either because of excessive tissue damage or because of inappropriate regulation of the inflammatory response, the inflammatory state may proceed into chronic inflammation. A state of persistent or nonresolving inflammation with simultaneous destruction and healing of tissue is seen in a number of diseases including arthritis, diabetes and inflammatory bowel diseases [64], in subgroups of patients with MI and in chronic HF.

Several classes of PRRs have been identified [63]. The two major families are the transmembrane toll-like receptors (TLRs) and the cytosolic nucleotide oligomerization domain (NOD)-like receptors (NLRs). In humans, 11 different TLRs have been cloned, and importantly, TLRs are not just expressed and functional in immune cells. In fact, the human heart expresses all known TLRs [65, 66]. Moreover, several TLRs have also been found in cardiac myocytes and fibroblasts [67].

The central role of TLRs in cardiovascular disease was first demonstrated in sepsis, where it was shown that TLR4 deficiency protected against lipopolysaccharide-induced cardiac dysfunction [68]. Various TLRs have also been implicated in the development and progression of experimental atherosclerosis [69, 70]. More recently, a role for innate immune mechanisms in HF has also been suggested. Several experimental studies have shown that TLR4 and TLR2 may play a role in the development of myocardial failure [69]. Thus, TLR4-deficient mice develop less myocardial hypertrophy than controls after aortic banding, directly linking innate immune mechanisms to the response to hemodynamic overload [69]. Additionally, a growing body of studies link TLR signaling to adverse cardiac remodeling secondary to ischemia-reperfusion injury or MI [69]. Mice lacking functional TLR2 or TLR4 have reduced infarct sizes and are therefore less prone to HF development following MI. These TLRs may represent novel targets for intervention in ischemic heart disease, as exemplified by experimental studies showing attenuated myocardial ischemia-reperfusion injury during treatment with the TLR4 antagonist eritoran and the anti-TLR2 antibody OPN-301 [71, 72]. Apart from studies showing a role for TLR3 and TLR9 in viral myocarditis, the role of other TLRs in the pathogenesis of HF is largely unknown.

While most of the TLRs are transmembrane receptors, intracellular cytosolic sensors of DAMPs and PAMPs have recently been identified [73]. These include NOD1, NOD2 and the NLRs. A subgroup of NLRs, together with the adaptor protein ASC and caspase-1, form large cyto-

plasmic complexes called inflammasomes [73]. When active, the inflammasomes catalyze the proteolytic activation of the inflammatory cytokines IL-1 $\beta$  and IL-18. Thus, in most cells, increased gene expression of IL-1 $\beta$  and IL-18 is not sufficient for release of these inflammatory cytokines, and a second signal leading to inflammasome activation with subsequent caspase-1-mediated cleavage of pro-IL-1 $\beta$  and pro-IL-18 is necessary for the release of active cytokines. NLRP3 seems to be particularly important in the initiation of 'sterile inflammatory responses', i.e. innate immune responses primarily caused by cellular stress or injury. Several endogenous danger signals can activate the NLRP3 inflammasome, including urate crystals, extracellular ATP and oxidative stress, and all of these factors have been implicated in the pathogenesis of HF [74, 75]. Recently, studies have shown an important role for the inflammasome in myocardial ischemia-reperfusion injury [76], suggesting that targeting of the inflammasome may represent a new therapeutic approach for limiting post-MI HF development. However, to this end the role of NLRP3 and other NLRs in the development of HF has to be further unraveled.

#### *The Pathogenic Role of Chemokines in HF*

Several lines of evidence suggest that chemokines may be involved in the pathogenesis of cardiac failure. Chemokines are a family of chemotactic cytokines causing directed migration of leukocytes into inflamed tissue, but importantly, chemokines have also been shown to have effects beyond that of chemotaxis. Accordingly, chemokines enhance the release of inflammatory cytokines and reactive oxygen species in various leukocyte subsets and may also interact with 'nonimmune' cells such as fibroblasts, endothelial cells and vascular smooth muscle cells. They have also been shown to directly modulate the function of cardiomyocytes. Hence, these inflammatory mediators could both indirectly (e.g. via recruitment and activation of infiltrating leukocytes) and directly (e.g. through modulation of apoptosis, fibrosis and angiogenesis within the failing myocardium) contribute to myocardial failure [8, 77]. Thus, interstitial monocyte infiltration in the myocardium associated with cardiac hypertrophy, ventricular dilatation and depressed contractile function is found in transgenic mice with myocardial overexpression of the CC chemokine MCP-1 [78]. Conversely, gene therapy with an MCP-1 antagonist was recently found to attenuate the development of ventricular remodeling in a mouse model for post-MI HF [79]. Also, macrophage inflammatory protein-1 $\alpha$  knockout mice do not develop cardiac lesions after Coxsackie B vi-

rus infection because of attenuated recruitment of activated monocytes into the myocardium [80]. Moreover, the observation of high embryonic mortality and organ defects, including cardiac ventricular septum defects, in CXC chemokine receptor 4 knockout mice indicates a crucial and direct dependence on chemokines in the development and function of the myocardium [81]. Furthermore, we have shown increased CX3C chemokine ligand (CX3CL1) production in both experimental and clinical HF [82], and very recently, Xuan et al. [83] showed that neutralizing CX3CL1 antibody treatment improved HF induced by MI or pressure overload. Other recent studies have also suggested a direct role for chemokines in ECM remodeling. Dahl et al. [84] showed enhanced myocardial expression of CXCL16 in both clinical and experimental HF and in vitro CXCL16 promoted proliferation and impaired collagen synthesis in myocardial fibroblasts and increased MMP activity in both cardiac myocytes and fibroblasts [84]. Finally, lack of chemokine signaling through CXC chemokine receptor 5 was recently shown to cause increased mortality, ventricular dilatation and deranged matrix during cardiac pressure overload in experimental HF, at least partly involving altered expression of several small leucine-rich proteoglycans that are of importance for collagen cross-linking [85]. Several experimental models are promising for 'antichemokine therapy' in various inflammatory conditions, and these approaches could potentially also result in novel treatment strategies in clinical HF.

#### *The Role of TNF-Related Molecules in HF*

Whereas the results from anti-TNF trials in HF were disappointing, recent studies have suggested that other TNF-related molecules could be of interest as targets for therapy in HF. In particular, several studies have suggested a potential role for the OPG/receptor activator of nuclear factor- $\kappa$ B (RANK)/RANK ligand (RANKL) system in the pathogenesis of HF. OPG is a soluble decoy receptor which inhibits the ligation of RANKL to its transmembrane receptor RANK, with antagonizing effects on RANKL. These cytokines were first identified as mediators for paracrine signaling in bone metabolism. In addition to its role in bone homeostasis, RANKL seems to be involved in immune responses. RANKL is an important regulator of dendritic cell function by prolonging dendritic cell survival and increasing the release of inflammatory cytokines such as IL-15 and IL-1 $\beta$  [86]. It has been suggested that interactions between T cells expressing RANKL and dendritic cells may represent a link between adaptive and innate immune responses in failing myo-



cardial cells, leading to persistent and increased inflammation in HF. Also, the inflammatory cytokine IL-17, produced almost exclusively by activated T cells, has recently been shown to regulate myocardial fibrosis in experimental models of HF through a RANKL-dependent mechanism [87]. In cultured cardiac fibroblasts, the OPG/RANK/RANKL axis was one of the intermediaries for IL-17-induced MMP-1 production in cardiac fibroblasts [87]. Evidence that the OPG/RANKL/RANK axis can promote matrix degradation and remodeling within the failing myocardium has also been demonstrated in human cells in vitro, where RANKL was found to induce MMP expression in fibroblasts and cardiomyocytes [88]. Also, while OPG at high concentrations inhibits RANKL activity, OPG may, at least at low OPG/RANKL ratios, enhance the MMP-inducing effect of RANKL, and at high concentrations, OPG may have MMP-inducing and chemotactic effects of its own, illustrating the complexity of this system [89]. Moreover, TNF-related apoptosis-inducing ligand has been related to enhanced apoptosis within the myocardium following MI [90], and in addition to being a soluble decoy receptor for RANKL, OPG also binds to TNF-related apoptosis-inducing ligand, potentially preventing apoptosis within cardiac cells during HF development. From a therapeutic point of view, a chimeric OPG-Fc fusion protein was developed to antagonize RANKL [90], but the formation of neutralizing antibodies against OPG after administration of the fusion protein led to the development of denosumab, a humanized monoclonal antibody against RANKL [91]. This attracted major attention as it was shown to be successful in the treatment of postmenopausal osteoporosis by efficiently blocking RANK/RANKL signaling [91]. However, whether this medication could benefit HF patients is at present unknown and should first be investigated in experimental HF.

#### *ECM and Inflammation during HF*

The ECM is not a mere static scaffold for dysfunctional cardiac cells in HF. The ECM undergoes both quantitative and qualitative alterations in HF as both the amounts and intrinsic composition of the various ECM components are altered [92]. The net effect of these alterations is stiffness translated into recoil/relaxation impairments and/or slippage, ultimately visualized as chamber dilatation. These ECM alterations are consequences of a shift in the equilibrium with both altered biosynthesis and degradation of ECM components. Inflammatory mediators have been shown to influence both aspects. Furthermore, the ECM can provide storage and up-concentrat-

ing capabilities of inflammatory mediators, as well as proteolytic activation of proinflammatory cytokines [93]. In addition, it is evident that the influx of inflammatory cells is heavily dependent on the ECM milieu [94]. Overall, there appears to be a clear link between inflammatory signaling and ECM remodeling in HF. Even so, the dynamic regulation of the ECM is multifactorial and complex, involving interaction between inflammatory and anti-inflammatory mediators and synthesis of the various ECM components, ECM quality (e.g. the degree of collagen cross-linking) and matrix degradation activity involving regulation of the major degrading enzyme system (i.e. the MMP family) and their endogenous inhibitors [i.e. the tissue inhibitor of metalloproteinase (TIMP) family].

A total of 25 MMPs are recognized, all with specific ECM targets, and approximately one third of these have been suggested to be of importance within the myocardium [95]. Studies on gene-targeted mice suggest that inhibition of cardiac MMP-2 and -9 may be advantageous as it decreases myocardial inflammation and the incidence of cardiac rupture associated with MI [94]. On the other hand, similar gene-targeting strategies were shown to worsen inflammatory cardiomyopathies [96], illustrating the complexity of the MMP system potentially mediating both adaptive and maladaptive responses, at least partly depending on the etiology of HF. Besides transcriptional and translational control and intrinsic regulation by other MMPs, a core regulatory system is provided by TIMPs. A total of four TIMPs have been characterized, and their key function in maintaining ECM homeostasis is supported by studies in various TIMP-deficient mice resulting in worsening or even development of cardiomyopathies [97]. Both MMPs and TIMPs are subject to regulation by classic inflammatory mediators. Thus, several lines of evidence suggest that one core mechanism by which TNF $\alpha$  might influence HF is via its regulation of collagen production and ECM remodeling through regulation of MMP activities [98]. Furthermore, inhibition of TNF $\alpha$  and MMP-2 abolished the cardiomyopathic phenotype in TIMP-3 knockout mice [99]. However, several other inflammatory cytokines may also regulate the balance between MMPs and TIMPs (e.g. various chemokines, IL-1 and TNF-related molecules such as RANKL). More importantly, developing therapeutic approaches that directly inhibit MMP activity is a major challenge in cardiovascular research as well as in other medical disciplines.

TGF- $\beta$  is a classical profibrotic cytokine and considered a central player in the regulation of fibroblast func-

tion and ECM remodeling in HF, and increased levels of TGF- $\beta$  are seen in HF. TGF- $\beta$  resides in the ECM in a latent form but is proteolytically activated by proteases like MMPs due to increments of a wide array of signals generally associated with tissue damage and cellular stress [100]. Stimulation of fibroblasts by TGF- $\beta$  induces secretion of several ECM components [101]. Several studies have explored the functional consequence of TGF- $\beta$  in HF, and results from these studies have shown that TGF- $\beta$  antagonism inhibits fibrotic processes and provides salutary cardiac effects in HF. However, the timing of TGF- $\beta$  antagonism seems to be of great importance as attenuation of TGF- $\beta$  signaling in the immediate early phases after MI increased mortality [102]. Moreover, while TGF- $\beta$  enhances fibrogenesis, it also has potent anti-inflammatory properties, suggesting that the net effect of TGF- $\beta$  inhibition may be uncertain.

Overall, previous investigations clearly outline a significant role of inflammatory mediators and regulation of the cardiac ECM. However, this interplay has not been extensively studied in the context of HF. Future studies may reveal specific interactions that may pave the road to new treatment modalities.

## Conclusions

Chronic HF appears to be accompanied by a persistent rise in inflammatory cytokines. While a sustained overexpression of these cytokines may contribute to progressive LV remodeling and the syndrome of HF, a balanced incidental response may be beneficial, illustrating the challenges in cytokine-modulating therapy. Future studies should identify the crucial actors and their mechanism of action in the immunopathogenesis of chronic HF and in particular, clarify the balance between adaptive and maladaptive effects of these molecules. Forthcoming studies should also try to characterize the immunophenotype in HF more precisely and whether the dysregulation of the cytokine network includes altered homeostatic control, for example by impaired function of regulatory T cells. Such studies are a prerequisite for the development of new treatment strategies in HF which target inflammatory and immunopathogenic mechanisms in this disorder.

## References

- 1 Aukrust P, Ueland T, Lien E, Bendtzen K, Muller F, Andreassen AK, Nordøy I, Aass H, Espevik T, Simonsen S, Frøland SS, Gullestad L: Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 1999; 83:376–382.
- 2 Testa M, Yeh M, Lee P, Fanelli R, Loperfido F, Berman JW, LeJemtel T: Circulating levels of cytokines and their endogenous modulators in patients with mild to severe congestive heart failure due to coronary artery disease or hypertension. *J Am Coll Cardiol* 1996;28:964–971.
- 3 Torre-Amione G, Kapadia S, Benedict C, Oral H, Young JB, Mann DL: Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). *J Am Coll Cardiol* 1996;27:1201–1206.
- 4 Aukrust P, Ueland T, Muller F, Andreassen AK, Aass H, Kjekshus J, Simonsen S, Frøland SS, Gullestad L: Elevated circulating levels of C-C chemokines in patients with congestive heart failure. *Circulation* 1998;97:1136–1143.
- 5 Damàs JK, Gullestad L, Ueland T, Solum NO, Simonsen S, Frøland SS, Aukrust P: CXC-chemokines, a new group of cytokines in congestive heart failure – possible role of platelets and monocytes. *Cardiovasc Res* 2000;45:428–436.
- 6 Kapadia S: Cytokines and heart failure. *Cardiol Rev* 1999;7:196–206.
- 7 Deveaux B, Scholz D, Hirche A, Kløverkorn WP, Schaper J: Upregulation of cell adhesion molecules and the presence of low grade inflammation in human chronic heart failure. *Eur Heart J* 1997;18:470–479.
- 8 Damàs JK, Eiken HG, Øie E, Bjerkeli V, Yndestad A, Ueland T, Tønnesen T, Geiran O, Aass H, Simonsen S, Christensen G, Frøland SS, G. Attramadal H, Gullestad L, Aukrust P: Myocardial expression of CC- and CXC-chemokines and their receptors in human end-stage heart failure. *Cardiovasc Res* 2000;47:778–787.
- 9 Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL: Cytokines and cytokine receptors in advanced heart failure. *Circulation* 2001;103:2055–2059.
- 10 Bozkurt B, Kribbs SB, Clubb FJ, Michael LH, Didenko VV, Hornsby PJ, Seta Y, Oral H, Spinale FG, Mann DL: Pathophysiologically relevant concentrations of tumor necrosis factor-alpha promote progressive dysfunction and remodelling in rats. *Circulation* 1998;97:1382–1391.
- 11 Kubota T, McTernan CF, Frye CS, Slawson SE, Lemster BH, Koretsky AP, Demetris AJ, Feldman AM: Dilated cardiomyopathy in transgenic mice with cardiac-specific overexpression of tumor necrosis factor-alpha. *Circ Res* 1997;81:627–635.
- 12 Fischer P, Hilfiker-Kleiner D: Role of gp130-mediated signalling pathways in the heart and its impact on potential therapeutic aspects. *Br J Pharmacol* 2008;153:(suppl 1):S414–S427.
- 13 Mann DL: Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res* 2002;91:988–998.
- 14 Pomerantz BJ, Reznikov LL, Harken AH, Dinarello CA: Inhibition of caspase 1 reduces human myocardial ischemic dysfunction via inhibition of IL-18 and IL-1beta. *Proc Natl Acad Sci USA* 2001;98:2871–2876.

- 15 Lecour S, James RW: When are pro-inflammatory cytokines SAFE in heart failure? *Eur Heart J* 2011;32:680–685.
- 16 Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S: Cytokine gene expression after myocardial infarction in rat hearts. *Circulation* 1998;98:149–156.
- 17 Borlaug BA, Redfield MM: Diastolic and systolic heart failure are distinct phenotypes within the heart failure spectrum. *Circulation* 2014;123:2006–2013.
- 18 van Heerebeek L, Borbely A, Niessen HW, Bronzwaer JG, van der Velden J, Stienen GJ, Linke WA, Laarman GJ, Paulus WJ: Myocardial structure and function differ in systolic and diastolic heart failure. *Circulation* 2006;113:1966–1973.
- 19 Dinh W, Futh R, Nickl W, Krahn T, Ellinghaus P, Scheffold T, Bansemir L, Bufe A, Barroso MC, Lankisch M: Elevated plasma levels of TNF-alpha and interleukin-6 in patients with diastolic dysfunction and glucose metabolism disorders. *Diabet Med* 2011;28:311–318.
- 20 Zibadi S, Vazquez R, Larson DF, Watson RR: T lymphocyte regulation of lysyl oxidase in diet-induced cardiac fibrosis. *Cardiovasc Toxicol* 2010;10:190–198.
- 21 Yu Q, Horak K, Larson DF: Role of T lymphocytes in hypertension-induced cardiac extracellular matrix remodeling. *Hypertension* 2006;48:98–104.
- 22 Melendez GC, McLarty JL, Levick SP, Du Y, Janicki JS, Brower GL: Interleukin 6 mediates myocardial fibrosis, concentric hypertrophy, and diastolic dysfunction in rats. *Hypertension* 2010;56:225–231.
- 23 Yu Q, Vazquez R, Khojeini EV, Patel C, Venkataramani R, Larson DF: IL-18 induction of osteopontin mediates cardiac fibrosis and diastolic dysfunction in mice. *Am J Physiol Heart Circ Physiol* 2009;297:H76–H85.
- 24 Westermann D, Lindner D, Kasner M, Zietsch C, Savvatis K, Escher F, von SJ, Skurk C, Steendijk P, Riad A, Poller W, Schultheiss HP, Tschope C: Cardiac inflammation contributes to changes in the extracellular matrix in patients with heart failure and normal ejection fraction. *Circulation Heart Fail* 2011;4:44–52.
- 25 de Boer RA, Voors AA, Muntendam P, van Gilst WH, van Veldhuisen DJ: Galectin-3: a novel mediator of heart failure development and progression. *Eur J Heart Fail* 2009;11:811–817.
- 26 Baldeviano GC, Barin JG, Talor MV, Srinivasan S, Bedja D, Zheng D, Gabrielson K, Iwakura Y, Rose NR, Cihakova D: Interleukin-17A is dispensable for myocarditis but essential for the progression to dilated cardiomyopathy. *Circ Res* 2010;106:1646–1655.
- 27 Yndestad A, Finsen AV, Ueland T, Husberg C, Dahl CP, Øie E, Vinge LE, Sjaastad I, Sandanger Ø, Ranheim T, Dickstein K, Kjekshus J, Damås JK, Fiame A, Hilfiker-Kleiner D, Lipp M, Gullestad L, Christensen G, Aukrust P: The homeostatic chemokine CCL21 predicts mortality and may play a pathogenic role in heart failure. *PLoS One* 2012;7:e33038.
- 28 Lainchbury JG, Troughton RW, Strangman KM, Frampton CM, Pilbrow A, Yandle TG, Hamid AK, Nicholls MG, Richards AM: N-terminal pro-B-type natriuretic peptide-guided treatment for chronic heart failure: results from the BATTLESCARRED (NT-proBNP-Assisted Treatment To Lessen Serial Cardiac Readmissions and Death) trial. *J Am Coll Cardiol* 2009;55:53–60.
- 29 Anand IS, Latini R, Florea VG, Kuskowski MA, Rector T, Masson S, Signorini S, Mocarelli P, Hester A, Glazer R, Cohn JN; Val-HeFT Investigators: C-reactive protein in heart failure: prognostic value and the effect of valsartan. *Circulation* 2005;112:1428–1434.
- 30 McMurray JJ, Kjekshus J, Gullestad L, Dunselman P, Hjalmarsen A, Wedel H, Lindberg M, Waagstein F, Grande P, Hradec J, Kamenisky G, Korewicki J, Kuusi T, Mach F, Ranjith N, Wikstrand J; CORONA Study Group: Effects of statin therapy according to plasma high-sensitivity C-reactive protein concentration in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA): a retrospective analysis. *Circulation* 2009;120:2188–2196.
- 31 Suzuki S, Takeishi Y, Niizeki T, Koyama Y, Kitahara T, Sasaki T, Sagara M, Kubota I: Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure. *Am Heart J* 2008;155:75–81.
- 32 Orus J, Roig E, Perez-Villa F, Pare C, Azqueta M, Filella X, Heras M, Sanz G: Prognostic value of serum cytokines in patients with congestive heart failure. *J Heart Lung Transplant* 2000;19:419–425.
- 33 Dempsey PW, Doyle SE, He JQ, Cheng G: The signaling adaptors and pathways activated by TNF superfamily. *Cytokine Growth Factor Rev* 2003;14:193–209.
- 34 Ferrari R, Bachetti T, Confortini R, Opasich C, Febo O, Corti A, Cassani G, Visioli O: Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure. *Circulation* 1995;92:1479–1486.
- 35 Roysland R, Masson S, Omland T, Milani V, Bjerre M, Flyvbjerg A, Di Tano G, Misuraca G, Maggioni AP, Tognoni G, Tavazzi L, Latini R; GISSI-HF Investigators: Prognostic value of osteoprotegerin in chronic heart failure: the GISSI-HF trial. *Am Heart J* 2010;160:286–293.
- 36 Ueland T, Dahl CP, Kjekshus J, Hulthe J, Böhm M, Mach F, Goudev A, Lindberg M, Wikstrand J, Aukrust P, Gullestad L: Osteoprotegerin predicts progression of chronic heart failure: results from CORONA. *Circ Heart Fail* 2011;4:145–152.
- 37 Gwechenberger M, Pacher R, Berger R, Zorn G, Moser P, Stanek B, Huelsmann M: Comparison of soluble glycoprotein 130 and cardiac natriuretic peptides as long-term predictors of heart failure progression. *J Heart Lung Transplant* 2005;24:2190–2195.
- 38 Henderson NC, Sethi T: The regulation of inflammation by galectin-3. *Immunol Rev* 2009;230:160–171.
- 39 Ueland T, Aukrust P, Broch K, Aakhus S, Skårdal R, Muntendam P, Gullestad L: Galectin-3 in heart failure: high levels are associated with all cause mortality. *Int J Cardiol* 2011;150:361–364.
- 40 van Kimmenade RR, Januzzi JL Jr, Ellinor PT, Sharma UC, Bakker JA, Low AF, Martinez A, Crijns HJ, MacRae CA, Menheere PP, Pinto YM: Utility of amino-terminal pro-brain natriuretic peptide, galectin-3, and apelin for the evaluation of patients with acute heart failure. *J Am Coll Cardiol* 2006;48:1217–1224.
- 41 Lok DJA, Van Der Mer P, de la Porta PW-A, Lipsic E, Hillege HL, van Veldhuisen DJ: Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. *Clin Res Cardiol* 2010;99:323–328.
- 42 Gullestad L, Aukrust P, Ueland T, Espevik T, Yee G, Vagelos R, Fowler MB: Effect of high-versus low-dose angiotensin converting enzyme inhibition on cytokine levels in chronic heart failure. *J Am Coll Cardiol* 1999;34:2061–2067.
- 43 Gullestad L, Ueland T, Brunsvig A, Kjekshus J, Simonsen S, Frøland SS, Aukrust P: Effect of metoprolol on cytokine levels in chronic heart failure – a substudy in the Metoprolol Controlled-Release Randomized Intervention Trial in Heart Failure (MERIT-HF trial). *Am Heart J* 2001;141:418–421.
- 44 Ohtsuka T, Hamada M, Hiasa G, Sasaki O, Suzuki M, Hara Y, Shigematsu Y, Hiwada K: Effects of beta-blockers on circulating levels of inflammatory and anti-inflammatory cytokines in patients with dilated cardiomyopathy. *J Am Coll Cardiol* 2001;37:412–417.
- 45 Deswal A, Bozkurt B, Seta Y, Pariltili-Eiswirth S, Hayes FA, Blosch CB, Mann DL: Safety and efficacy of a soluble p75 tumor necrosis factor receptor (Enbrel, Etanercept) in patients with advanced heart failure. *Circulation* 1999;99:3224–3226.

- 46 Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, Drexler H, Feldman A, Kober L, Krum H, Liu P, Nieminen M, van Veldhuisen DJ, Waldenström A, Warren M, Westheim A, Zannad F, Fleming T: Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). *Circulation* 2004; 109:1594–1602.
- 47 Chung ES, Packer M, Lo KH, Fasanmade AA, Willerson JT: Anti-TNF Therapy Against Congestive Heart Failure Investigators: Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor- $\alpha$ , in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. *Circulation* 2003;107:3133–3040.
- 48 Luger A, Schmidt M, Luger N, Pauels HG, Domschke W: Infliximab induces apoptosis in myocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. *Gastroenterology* 2001; 121:1145–1157.
- 49 Gullestad L, Aass H, Fjeld JG, Andreassen AK, Ihlen H, Simonsen S, Kjekshus J, Nitter-Hauge S, Ueland T, Lien E, Frøland SS, Aukrust P: Effect of immunomodulating therapy with intravenous immunoglobulin in chronic congestive heart failure. *Circulation* 2001; 103:220–225.
- 50 Muller J, Wallukat G, Dandel M, Bieda H, Brandes K, Spiegelsberger S, Nissen E, Kunze R, Hetzer R: Immunoglobulin adsorption in patients with idiopathic dilated cardiomyopathy. *Circulation* 2000;101:285–391.
- 51 Larsson L, Mobini R, Aukrust P, Gullestad L, Wallukat G, Waagstein F, Fu M: Beneficial effect on cardiac function by intravenous immunoglobulin treatment in patients with cardiomyopathy is not due to neutralization of anti- $\beta$  adrenoceptor antibody. *Autoimmunity* 2004;37:489–493.
- 52 Gullestad L, Ueland T, Fjeld J, Holt E, Gundersen T, Breivik K, Følling M, Hodt A, Skårdal R, Kjekshus J, Andreassen AK, Kjekshus E, Wergeland R, Yndestad A, Frøland SS, Semb AG, Aukrust P: The effect of thalidomide on cardiac remodeling in chronic heart failure: results of a double-blind placebo-controlled study. *Circulation* 2005;112:3408–3414.
- 53 Nolan Y, Minogue A, Vereker E, Bolton AE, Campbell VA, Lynch MA: Attenuation of LPS-induced changes in synaptic activity in rat hippocampus by Vasogen's Immune Modulation Therapy. *Neuroimmunomodulation* 2002;10:40–46.
- 54 Torre-Amione G, Sestier F, Radovancevic B, Young J: Effects of a novel immune modulation therapy in patients with advanced chronic heart failure: results of a randomized, controlled, phase II trial. *J Am Coll Cardiol* 2004;44:1181–1186.
- 55 Torre-Amione G, Anker SD, Bourge RC, Colucci WS, Greenberg BH, Hildebrandt P, Keren A, Motro M, Moye LA, Otterstad JE, Pratt CM, Ponikowski P, Rouleau JL, Sestier F, Winkelmann BR, Young JB: Advanced Chronic Heart Failure CLinical Assessment of Immune Modulation Therapy Investigators: Results of a non-specific immunomodulation therapy in chronic heart failure (ACCLAIM trial): a placebo-controlled randomized trial. *Lancet* 2008;371:228–236.
- 56 Scandinavian Simvastatin Survival Study Group: Randomized trial of cholesterol lowering in 4444 patients with coronary artery disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1995;344:1383–1389.
- 57 The Long-Term Intervention with Pravastatin in Ischemic Disease (LIPID) Study Group: Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of cholesterol levels. *N Engl J Med* 1998;339: 1349–1357.
- 58 Kwak B, Mulhaup F, Mylt S, Mach F: Statins as a newly recognized type of immunomodulator. *Nat Med* 2000;6:1399–1402.
- 59 Kjekshus J, Apetrei E, Barrios V, Bohm M, Cleland JG, Cornel JH, Dunselman P, Fonseca C, Goudev A, Grande P, Gullestad L, Hjalmarsen A, Hradec J, Janosi A, Kamensky G, Komajda M, Korewicki J, Kuusi T, Mach F, Mareev V, McMurray JJ, Ranjith N, Schaufelberger M, Vanhaecke J, van Veldhuisen DJ, Waagstein F, Wedel H, Wikstrand J; CORONA Group: Rosuvastatin in older patients with systolic heart failure. *N Engl J Med* 2007;357:2248–2261.
- 60 GISSI-HF Investigators, Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, Lucci D, Nicolosi GL, Porcu M, Tognoni G: Effect of rosuvastatin in patients with chronic heart failure (the GISSI-HF trial): a randomized, double-blind, placebo-controlled trial. *Lancet* 2008;372:1231–1239.
- 61 Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, Jeejeebhoy KN: Increased oxidative stress in patients with congestive heart failure. *J Am Coll Cardiol* 1998;31:1352–1356.
- 62 Hare JM, Mangal B, Brown J, Fisher C Jr, Freudenberger R, Colucci WS, Mann DL, Liu P, Givertz MM, Schwarz RP; OPT-CHF Investigators: Impact of oxypurinol in patients with symptomatic heart failure. Results of the OPT-CHF study. *J Am Coll Cardiol* 2008; 51:2301–2309.
- 63 Takeuchi O, Akira S: Pattern recognition receptors and inflammation. *Cell* 2010;140: 805–820.
- 64 Nathan C, Ding A: Nonresolving inflammation. *Cell* 2010;140:871–882.
- 65 Nishimura M, Naito S: Tissue-specific mRNA expression profiles of human toll-like receptors and related genes. *Biol Pharm Bull* 2005;28:886–892.
- 66 Frantz S, Kobzik L, Kim YD, Fukazawa R, Medzhitov R, Lee RT, Kelly RA: Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. *J Clin Invest* 1999; 104:271–280.
- 67 Frantz S, Kelly RA, Bourcier T: Role of TLR-2 in the activation of nuclear factor kappaB by oxidative stress in cardiac myocytes. *J Biol Chem* 2001;276:5197–5203.
- 68 Nemoto S, Vallejo JG, Knuefermann P, Misra A, DeFreitas G, Carabello BA, Mann DL: *Escherichia coli* LPS-induced LV dysfunction: role of toll-like receptor-4 in the adult heart. *Am J Physiol Heart Circ Physiol* 2002; 282:H2316–H2323.
- 69 Mann DL: The emerging role of innate immunity in the heart and vascular system: for whom the cell tolls. *Circ Res* 2011;108:1133–1145.
- 70 Frantz S, Ertl G, Bauersachs J: Mechanisms of disease: Toll-like receptors in cardiovascular disease. *Nat Clin Pract Cardiovasc Med* 2007;4:444–454.
- 71 Shimamoto A, Chong AJ, Yada M, Shomura S, Takayama H, Fleisig AJ, Agnew ML, Hampton CR, Rothnie CL, Spring DJ, Pohlman TH, Shimpo H, Verrier ED: Inhibition of Toll-like receptor 4 with eritoran attenuates myocardial ischemia/reperfusion injury. *Circulation* 2006;114(1 suppl):I270–I274.
- 72 Arslan F, Smeets MB, O'Neill LA, Keogh B, McGuirk P, Timmers L, Tersteeg C, Hoefer IE, Doevendans PA, Pasterkamp G, de Kleijn DP: Myocardial ischemia/reperfusion injury is mediated by leukocytic toll-like receptor-2 and reduced by systemic administration of a novel anti-toll-like receptor-2 antibody. *Circulation* 2010;121:80–90.
- 73 Martinon F, Mayor A, Tschopp J: The inflammasomes: guardians of the body. *Ann Rev Immunol* 2009;27:229–265.
- 74 Bergamini C, Cicoira M, Rossi A, Vassanelli C: Oxidative stress and hyperuricaemia: pathophysiology, clinical relevance, and therapeutic implications in chronic heart failure. *Eur J Heart Fail* 2009;11:444–452.
- 75 Vassort G: Adenosine 5'-triphosphate: a P2-purineric agonist in the myocardium. *Physiol Rev* 2001;81:767–806.
- 76 Kawaguchi M, Takahashi M, Hata T, Kashima Y, Usui F, Morimoto H, Izawa A, Takahashi Y, Masumoto J, Koyama J, Hongo M, Noda T, Nakayama J, Sagara J, Taniguchi S, Ikeda U: Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. *Circulation* 2011;123:594–604.
- 77 Sasayama S, Okada M, Matsumori A: Chemokines and cardiovascular diseases. *Cardiovasc Res* 2000;45:267–269.

- 78 Kolattukudy PE, Quasch T, Berges S: Myocarditis induced by targeted expression of the MCP-1 gene in murine cardiac muscle. *Am J Pathol* 1998;152:101–111.
- 79 Hayashidani S, Tsutsui H, Shiomi T, Ikeuchi M, Matsusaka H, Suematsu N, Wen J, Egashira K, Takeshita A: Anti-monocyte chemoattractant protein-1 gene therapy attenuates left ventricular remodeling and failure after experimental myocardial infarction. *Circulation* 2003;108:2134–2140.
- 80 Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, Smithies O: Requirement of MIP-1 alpha for an inflammatory response to viral infection. *Science* 1995;269:1583–1585.
- 81 Tachibana K, Hirota S, Ilzasa H, Yoshida H, Kawabata K, Kataoka Y, Kitamura Y, Matsushima K, Yoshida N, Nishikawa S, Nagasawa T: The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. *Nature* 1998;393:524–525.
- 82 Husberg C, Nygard S, Finsen AV, Damas JK, Frigessi A, Oie E, Waehre A, Gullestad L, Aukrust P, Yndestad A, Christensen G: Cytokine expression profiling of the myocardium reveals a role for CX3CL1 (fractalkine) in heart failure. *J Mol Cell Cardiol* 2008;45:261–269.
- 83 Xuan W, Liao Y, Chen B, Xu D, Liu Y, Kitakaze M: Detrimental effect of fractaline on myocardial ischemia and heart failure. *Cardiovasc Res* 2011;92:361–362.
- 84 Dahl CP, Husberg C, Gullestad L, Waehre A, Damas JK, Vinge LE, Finsen AV, Ueland T, Florholmen G, Aakhus S, Halvorsen B, Aukrust P, Oie E, Yndestad A, Christensen G: Increased production of CXCL16 in experimental and clinical heart failure: a possible role in extracellular matrix remodeling. *Circ Heart Fail* 2009;2:624–632.
- 85 Waehre A, Halvorsen B, Yndestad A, Husberg C, Sjaastad I, Nygard S, Dahl CP, Ahmed MS, Finsen AV, Reims H, Louch WE, Hilfiker-Kleiner D, Vinge LE, Roald B, Attramadal H, Lipp M, Gullestad L, Aukrust P, Christensen G: Lack of chemokine signaling through CXCR5 causes increased mortality, ventricular dilatation and deranged matrix during cardiac pressure overload. *PLoS One* 2011;6:e18668.
- 86 Walsh MC, Choi Y: Biology of the TRANCE axis. *Cytokine Growth Factor Rev* 2003;14:251–263.
- 87 Feng W, Li W, Liu W, Wang F, Li Y, Yan W: IL-17 induces myocardial fibrosis and enhances RANKL/OPG and MMP/TIMP signaling in isoproterenol-induced heart failure. *Exp Mol Pathol* 2009;87:212–218.
- 88 Ueland T, Yndestad A, Oie E, Florholmen G, Frøland SS, Simonsen S, Christensen G, Gullestad L, Aukrust P: Dysregulated osteoprotegerin/RANK ligand/RANK axis in clinical and experimental heart failure. *Circulation* 2005;111:2461–2468.
- 89 Sandberg WJ, Yndestad A, Oie E, Smith C, Ueland T, Ovchinnikova O, Robertson A-KL, Semb AG, Scholz H, Andreassen AK, Gullestad L, Damås JK, Frøland SS, Hansson GK, Halvorsen B, Aukrust P: Enhanced T cell expression of RANK ligand in acute coronary syndrome – possible role in plaque progression. *Arterioscler Thromb Vasc Biol* 2006;26:857–863.
- 90 Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR: The effect of a single dose of osteoprotegerin in postmenopausal women. *J Bone Miner Res* 2001;16:348–360.
- 91 Rachner TD, Khosla S, Hofbauer LC: Osteoporosis: now and the future. *Lancet* 2011;377:1276–1287.
- 92 Spinale FG: Myocardial matrix remodeling and the matrix metalloproteinases: influence on cardiac form and function. *Physiol Rev* 2007;87:1285–1342.
- 93 Murray DB, Levick SP, Brower GL, Janicki JS: Inhibition of matrix metalloproteinase activity prevents increases in myocardial tumor necrosis factor-alpha. *J Mol Cell Cardiol* 2010;49:245–250.
- 94 Matsumura S, Iwanaga S, Mochizuki S, Okamoto H, Ogawa S, Okada Y: Targeted deletion or pharmacological inhibition of MMP-2 prevents cardiac rupture after myocardial infarction in mice. *J Clin Invest* 2005;115:599–609.
- 95 Vanhoutte D, Heymans S: TIMPs and cardiac remodeling: 'Embracing the MMP-independent-side of the family'. *J Mol Cell Cardiol* 2010;48:445–453.
- 96 Cheung C, Marchant D, Walker EK, Luo Z, Zhang J, Yanagawa B, Rahmani M, Cox J, Overall C, Senior RM, Luo H, McManus BM: Ablation of matrix metalloproteinase-9 increases severity of viral myocarditis in mice. *Circulation* 2008;117:1574–1582.
- 97 Fedak PW, Smookler DS, Kassiri Z, Ohno N, Leco KJ, Verma S, Mickle DA, Watson KL, Hojilla CV, Cruz W, Weisel RD, Li RK, Khokha R: TIMP-3 deficiency leads to dilated cardiomyopathy. *Circulation* 2004;110:2401–2409.
- 98 Li YY, Feng YQ, Kadokami T, McTiernan CF, Draviam R, Watkins SC, Feldman AM: Myocardial extracellular matrix remodeling in transgenic mice overexpressing tumor necrosis factor alpha can be modulated by anti-tumor necrosis factor alpha therapy. *Proc Natl Acad Sci USA* 2000;97:12746–12751.
- 99 Kassiri Z, Oudit GY, Sanchez O, Dawood F, Mohammed FF, Nuttall RK, Edwards DR, Liu PP, Backx PH, Khokha R: Combination of tumor necrosis factor-alpha ablation and matrix metalloproteinase inhibition prevents heart failure after pressure overload in tissue inhibitor of metalloproteinase-3 knock-out mice. *Circ Res* 2005;97:380–390.
- 100 Annes JP, Munger JS, Rifkin DB: Making sense of latent TGFbeta activation. *J Cell Sci* 2003;116:2–24.
- 101 Desmouliere A, Geinoz A, Gabbiani F, Gabbiani G: Transforming growth factor-beta 1 induces alpha-smooth muscle actin expression in granulation tissue myofibroblasts and in quiescent and growing cultured fibroblasts. *J Cell Biol* 1993;122:103–111.
- 102 Ikeuchi M, Tsutsui H, Shiomi T, Matsusaka H, Matsushima S, Wen J, Kubota T, Takeshita A: Inhibition of TGF-beta signaling exacerbates early cardiac dysfunction but prevents late remodeling after infarction. *Cardiovasc Res* 2004;64:526–535.
- 103 Sliwa K, Woodiwiss A, Kone VN, Candy G, Badenhorst D, Norton G, Zambakides C, Peters F, Essop R: Therapy of ischemic cardiomyopathy with the immunomodulating agent pentoxifylline: results of a randomized study. *Circulation* 2004;109:750–755.
- 104 Staudt A, Schaper F, Stangl V, Plagemann A, Böhm M, Merkel K, Wallukat G, Wernecke KD, Stangl K, Baumann G, Felix SB: Immunohistological changes in dilated cardiomyopathy induced by immunoadsorption therapy and subsequent immunoglobulin substitution. *Circulation* 2001;103:2681–2686.
- 105 McNamara DM, Holubkov R, Starling RC, Dec GW, Loh E, Torre-Amione G, Gass A, Janosko K, Tokarczyk T, Kessler P, Mann DL, Feldman AM: Controlled trial of intravenous immune globulin in recent-onset dilated cardiomyopathy. *Circulation* 2001;103:2254–2259.