Heart failure (HF) is primarily a clinical diagnosis that develops secondary to either left ventricular (LV) systolic and diastolic dysfunctions. Despite significant advancements in medical therapies designed to both prevent HF development and treat HF once established, the prognosis of patients after their first hospital admission is poor. Worldwide, 2% to 17% of patients die during their first admission, with 17% to 45% mortality within 1 year of admission, and >50% mortality within 5 years.1 Given the staggering burden chronic HF exerts on society, in terms of not only mortality but also morbidity related to repeated and prolonged hospitalization, a greater understanding of the pathophysiological mechanisms at play merits accelerated investigation.

For decades, the activation of neurohormonal and sympathetic systems dominated research in established HF, both in experimental animals and clinically in patients. Blockade of these pathways demonstrated significant beneficial outcomes in a variety of patient populations, in particular those with reduced LV systolic function.2 However, ≈50% of all HF admissions are in patients with HF with a preserved ejection fraction (HFpEF), and unfortunately, none of the clearly efficacious therapies that focus on neurohormonal blockade (such as angiotensin-converting enzyme inhibitors, angiotensin receptor blocker, and β-blockers) have a beneficial effect in HFpEF.3,4 Importantly, what is common to both HF with reduced EF (either from an ischemic or nonischemic cause), and patients...
with HFpEF is a correlation between elevated serum proinflammatory cytokines and adverse clinical outcomes.\textsuperscript{5,6} The magnitude in the elevation of proinflammatory cytokines in chronic HF is significantly less than what would be observed in cases of autoimmune diseases or acute infections, suggesting that low-grade chronic inflammation may be an important contributor to the maintenance or clinical deterioration of patients with established chronic HF.\textsuperscript{9,10} Here, we will review what is known about the role of chronic inflammation in established HF. Importantly, we will highlight that although we have come to understand some of the intricate and context-dependent mechanisms in which inflammatory cells and pathways contribute to the development of HF in the acute setting, few of those insights have been extended to assessing the role of inflammation once chronic HF has been established.

### Causes of Chronic HF

Important to the discussion of HF is the underlying cause, which defines the mechanisms that drive the development of HF. These can be divided into 4 broad categories. The first is based on traditional risk factors, such as ischemic injury, hypertension, and metabolic syndrome (diabetes mellitus, central obesity, and hyperlipidemia), which include the majority of patients with HF.\textsuperscript{12,13} The second is genetic cardiomyopathies. Our greatest understanding comes from autosomal dominant mutations/family clusters with rare allele frequency but major pathophysiological effects (ie, hypertrophic cardiomyopathy), many of which have been modeled in the mouse.\textsuperscript{14–16} The third is mechanical, due to valve dysfunction, most commonly aortic stenosis in the elderly leading to pressure overload of the LV, which initially leads to cardiac hypertrophy, but can eventually progress into LV dysfunction, which has also been modeled in the mouse.\textsuperscript{12,13} Common to these first 3 causes is that the initial insult was not immune based, and activation of the immune system was a secondary response. The last category is immune based, which include autoimmune and infectious (viral and bacterial) triggers, where the innate and adaptive immune systems are activated to coordinate a primary response.\textsuperscript{17,18} Each of these causes contributes to the development of both HF with a reduced EF and HFpEF.

### Defining the Study of Chronic HF: An Important Distinction From Acute Injury and Repair

As reviewed in this issue, both tissue injury and tissue repair are orchestrated to a great degree by resident and infiltrating immune cells that activate subsequent inflammatory/reparative pathways. The vast majority of animal studies are focused on this acute window after injury.\textsuperscript{17} We believe that the relative balance between pathological inflammatory pathways and tissue reparative processes (physiological inflammation) define the trajectory of HF development. That is, to create a new baseline of cardiac function with the potential to develop only mildly impaired diastolic function or severe systolic and diastolic dysfunction; the latter being associated with LV/right ventricular dilatation and adverse cardiac remodeling (Figure 1). Unfortunately, there is a real paucity of mechanistic animal studies that examine what occurs after HF is established, which reveals a major gap in our knowledge base. This is of particular clinical and therapeutic importance because the vast majority of patients who are diagnosed with HF are those who most likely had HF for some time, and presented to hospital only when they became symptomatic.\textsuperscript{12,18} In these patients, the trajectory of disease from the initial insult (whether it be from acute ischemic injury or long-standing chronic hypertension) has for the most part already been set, with little ability to modify that trajectory at the time of first presentation. For example, we have ample evidence in animal studies that recruited blood monocytes modify ischemic myocardial injury, with excessive infiltration associated with worsening cardiac function in the acute setting.\textsuperscript{20} In patients, elevated circulating CD14\textsuperscript{+} monocyte counts 3 days post–myocardial infarction (MI) predict a failure to recover LV systolic function 3 months post MI.\textsuperscript{21} This highlights how monocytes can set the trajectory of injury; however, we have no knowledge in animals or patients, what role monocytes play once chronic ischemic cardiomyopathy (ICM) has set in and has become established independent of their role during the initial phases. We have framed our discussion of the literature, current limitations, and potential avenues for future exploration by highlighting the few human trials and mechanistic animal studies that examine chronic HF. Interestingly, in this regard, there may be more evidence in humans, where the initial inflammatory/cytokine hypothesis as a mediator of HF was born, and where we will begin our discussion.

### Clinical Trials Targeting Inflammation in Established Chronic HF

#### Relationship Between Inflammatory Biomarkers and Chronic HF: The Proinflammatory Cytokine Hypothesis

Elevated inflammatory biomarkers is a hallmark feature of chronic HF, yet whether inflammation is causative to disease progression is not yet clear. Measurement of biomarkers in patients with systolic HF (either ischemic or nonischemic)
and animal studies has convincingly demonstrated an elevation in numerous proinflammatory cytokines (such as tumor necrosis factor [TNF]-α, interleukin [IL]-1, IL-6, galectin 3, TNF receptor 1 and TNF receptor 2) during HF progression supporting the hypothesis that inflammation may contribute to HF. Similarly, elevated serum proinflammatory cytokines have been observed in HFpEF, however, we still lack an understanding of how multiple individual risk factors for HFpEF, including hypertension, diabetes mellitus, and coronary artery disease, individually contribute to the inflammatory milieu. Unfortunately, therapeutic interventions aimed at limiting inflammation in the chronic setting have been controversial. This is likely because of our lack of understanding how inflammatory cytokines mediate cardiac remodeling and whether these represent a cause or consequence of disease progression. The role of acute and chronic viral infection as a mechanism for the initiation and propagation of chronic inflammation in patients with non-ICM (NICM) may highlight important aspects of the inflammatory response where clinical interventions have been attempted. Below we will summarize the clinical trials that have been conducted targeting either specific molecules or inflammation in general. Given the heterogeneous nature of HFpEF, the majority of these trials have been focused on patients with reduced EF.

Figure 1. Schematic defining acute injury and the balance between tissue damage and tissue repair that sets the trajectory of the healing response. After an initial insult (ischemia, hypertension, diabetic cardiomyopathy, infection, genetic, etc) manipulations at the time of injury or shortly thereafter can alter cardiac function and pathological remodeling. The vast majority of studies published in the literature examine this injury phase (first 4 wk in mice). The initial response and trajectory set by the experimental conditions influence the degree to which inflammation is active in response to injured or stressed cells. For example, if the autophagy pathway is impaired in cardiomyocytes in heart failure (HF), it may elicit an inflammatory response, as a compensatory mechanism, that in the long term leads to pathology. Alternatively, an injured heart that initially responds poorly may, through the re-establishment of homeostasis without significant inflammation, reverse-remodel and improve cardiac function. An example of this may be in the neonate, which has the same injurious response as an adult heart, but can completely regenerate, a process that is dependent on embryonic-derived macrophages.

Question: How do immune cells control the ongoing balance between inflammatory and reparative pathways at different stages of chronic HF development?
Myocarditis: The Viral Perspective

Host defense against invading pathogens is the primary responsibility of the immune system during the acute stages of infection. Viral infection is an important contributor to the development of NICM, and the immune response (innate and adaptive) activated during acute infection. Importantly, there is compelling clinical evidence that we are underestimating the clinical impact of viral myocarditis and viral-induced cardiomyopathy.23,24 Coxackievirus B is the best known primary infectious agent associated with viral myocarditis; however, in the past decade, parvovirus and adenovirus have become more prevalent.25 Viral genomes can be detected within the myocardium of ≈64% of patients with dilated cardiomyopathy, suggesting a high prevalence of infection.26 In a large cohort of ≈500 patients, when specifically examining a single virus (parvovirus B19), 67% of patients with acute myocarditis had high levels of the viral genome, 35% of those with chronic cardiomyopathy had significant viral DNA, whereas only 7% of control samples had detectable virus. This suggests that chronic cardiac disease may develop from previous myocarditis in a relatively large subset of patients.27 Up to ≈40% of individuals aged <35 years with unexpected death have evidence of myocarditis on histology.28 T cells are required to eliminate virus-infected host cells, and nearly half of patients with dilated cardiomyopathy have cardiac T-cell infiltrates, suggesting a chronic inflammatory process.29 Interestingly, patients who resolve myocardial viral infection spontaneously improve LV function, whereas those who have viral persistence within the myocardium have progressively impaired LV function.25 Antiviral therapy may improve LV function; however, this is likely dependent on the particular viral genome. Clinical trials have had mixed results but suggest that there may be some clinical improvement in patients with persistent detection of viral genomes.30,31 Interestingly, although there are clear associations between the presence of viral genomes within the myocardium and cardiac dysfunction, the presence of virus does not predict whether a particular individual will develop chronic HF.25–27,32,33 It is unclear why a subset of patients with severe viral myocarditis and LV dysfunction develop chronic HF, whereas others fully recover. However, host susceptibility has been hypothesized to play a role.23

Unfortunately, trials using immunosuppressive therapies have been ineffective in patients with evidence of chronic myocarditis, and few used robust genotyping to parse chronically infected from uninfected subjects. The Myocarditis Treatment Trial enrolled 111 subjects who were diagnosed with HF (LVEF<45%) over the previous 2 years without evidence of coronary artery disease, and that had confirmed myocarditis on biopsy. Subjects were assigned to conventional therapy or generalized immunosuppression with prednisone and either cyclosporine or azathioprine.34 Immunosuppression did not change the primary end point, LV systolic function. Irrespective of immunosuppressive therapy, those patients who had a more robust inflammatory response at the time of diagnosis (elevated white blood cell count, increased macrophage/natural killer cell numbers on biopsy, elevated cardiac IgG) improved LV function and other clinical indices, suggesting that a robust early inflammatory response may be protective, and that nonspecifically targeting inflammation based on biopsy alone in chronic HF may not be beneficial. Importantly, this trial did not distinguish between viral and nonviral causes. Nor did it distinguish between patients with more recent onset of HF and those with more established HF were enrolled together.

Additional trials assessed the role of immune suppression in more defined populations. In one such trial, 88 subjects were enrolled to receive either standard therapy with or without immunosuppression (3-month treatment with prednisone and azathioprine). Patients were excluded if they were more apt to recover on their own (<6 months since diagnosis), and included only those patients who had evidence of additional histological markers of inflammation, such as upregulation of human leukocyte antigen (major histocompatibility complex II) on biopsy. Improvement in LV function and other clinical indices was seen in ≈72% of patients treated with immunosuppression versus 21% of controls (P<0.001) after 3 months, and this benefit was maintained after 3 years.35

A recent meta-analysis was performed on the role of corticosteroids in myocarditis (with and without viral genomes detected), grouping adults and children together. The study found significant heterogeneity although a trend to improved LV function with treatment, without any affect on mortality.36 Together, these data in chronic viral and nonviral myocarditis suggest that ongoing inflammation plays a role in a subset of patients with HF after viral infection; however, we lack the ability to accurately predict which patients will respond.37 This is likely because of many factors, including understanding the basic mechanisms and roles of different immune populations during chronic HF and nonspecific targeting of multiple pathways using corticosteroids, rather than pathological pathways that may be activated.

Myocarditis: Circulating Autoantibodies and the Autoimmune Perspective

For decades, there has been a known clinical association between autoantibodies and chronic HF, suggesting recognition of host antigens and deposition of antibodies in a subset of patients could trigger or maintain inflammation and thus promote HF.38 Autoantibodies that target intracellular cardiomyocyte antigens, such as cardiac troponin I, cardiac actin, Na/K ATPase, β1 receptor, and M2 muscarinic receptor, are elevated in patients with ischemic or NICM, and during viral myocarditis. One might speculate that corticosteroids, used to treat conditions in which autoantibodies are causative, such as traditional autoimmune diseases (ie, Lupus), would have an equally dramatic effect in these cases of HF. However, as mentioned above, trials using corticosteroids in chronic HF have had little success, and autoantibodies have been detected in patients without cardiac disease, which suggests an additional level of specificity may be required to demonstrate whether autoantibodies are causative factors in chronic HF.39 New-generation detection systems, such as for the β1 receptor, may help facilitate detection of true autoantibodies and reduce the false-positive rate.39 Furthermore, designer molecules that block autoantibody recognition of its target (such as the β1 receptor) have been demonstrated in Phase I trials to lower...
autoantibody titers in patients with chronic HF. These data establish an approach in which specific autoantibodies can be targeted to determine whether they contribute to chronic HF in the setting of a clinical trial.40

Complicate Case of TNF-α in Chronic HF

Likely the best known examples of immunomodulation therapy in chronic HF were a group of trials that focused on decreasing TNF-α levels as a means to improve clinical end points. These trials were based on numerous clinical studies that linked elevated TNF-α levels to a worsening prognosis in patients with HF.4 Furthermore, animal studies demonstrated that administration or overexpression of TNF-α leads to worsening HF, and blockade of TNF-α improves cardiac function in models of HF.41–43 Unfortunately, the net result of these trials was negative; however, it is worth exploring each trial specifically to help give context.

Pathological inflammation and physiological inflammation are 2 different concepts that were initially proposed by Ilya Metchnikoff. Inappropriate targeting of physiological inflammation, which may be required for homeostatic or reparative activities, can lead to deleterious results, which is a context the TNF-α trials can be viewed through. TNF-α has been targeted by either soluble receptor infusion (etanercept) or by using humanized neutralizing antibodies (infliximab).

Small-scale studies using etanercept demonstrated a clinical benefit to TNF-α blockade with improved LV function,44,45 which led to 2 large clinical studies. The Randomized Etanercept North American Strategy to Study Antagonism of Cytokines (RENAISSANCE) and the Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER), each enrolled 900 subjects (total 1800). The trials themselves were similar except for the dosing of etanercept, which was more frequent and lasted for a longer period of time in RENAISSANCE. Most of the subjects (n=1500) from these 2 trials were pooled and analyzed together in a study called Randomized Etanercept Worldwide Evaluation (RENEWAL), which importantly excluded the subjects with the lowest dose of etanercept (once weekly injection).10 Both clinical trials were stopped because of lack of clinical benefit, and those patients receiving the highest dosing (twice or thrice weekly) had increased adverse cardiac outcomes. Similar results were seen with infliximab in the Anti-TNF-α in Congestive Heart Failure (ATTACH) trial, which also demonstrated increased death in patients with HF at higher doses, presumably through the lysis of cells expressing TNF-α, such as cardiomyocytes.46 However, one wonders, if examining the data carefully, there was a nonsignificant trend to reduced deaths in the lowest dose of etanercept group (P=0.17). This is supported by recent animal data that argue that TNF-α may be cardioprotective through its ability to form stress-induced cytoskeleton filaments that prevent deterioration of cardiac function and potentially removal of damaged mitochondria, through the process of autophagy.47,48 Thus, the wrong therapeutic threshold may have been applied to TNF-α blockade. Although unlikely to occur, should future trials of TNF-α blockade proceed, they should be focused on modestly lowering TNF-α levels using low-dose pharmacological inhibition.

Targeting General Inflammation in Chronic HF

Beyond the TNF-α trials, the largest trial that directly targeted inflammation was the Advance Chronic Heart Failure Clinical Assessment of Immune Modulation (ACCLAIM) trial, which enrolled 2426 patients with New York Heart Association II-IV symptoms, mixed between ischemic and nonischemic causes. The hypothesis of this trial was that an anti-inflammatory stimuli could produce an endogenous anti-inflammatory state in the recipient HF patient, which would suppress ongoing low-grade pathological chronic inflammation—a concept supported by animal data.49 Although nonspecific in nature, ACCLAIM utilized a technology called Immune Modulation therapy (celdecade), where the patients’ own blood would be stressed to induced cell death, and then the mixture of apoptotic cells was injected intramuscularly into the same patient (average follow-up of 10.2 months). Although there was no difference in the primary end point (all-cause mortality or cardiovascular admission) in the entire cohort, there was improvement in patients with NICM and with more mild (New York Heart Association-II) symptoms. In a subtotal of 919 subjects, there was a 39% reduction in all-cause mortality (hazard ratio, 0.61; P=0.0003) and a 26% reduction in cardiovascular admission (hazard ratio, 0.74; P=0.02).50 Although these data were derived from a particular subset of patients, and therefore must be replicated in prospective trials, if true, the study would suggest that de novo production and release of anti-inflammatory mediators at remote sites (in this case, gluteal injection) can have a direct cardioprotective effect. It has been well established that macrophages that phagocytose apoptotic cells (termed efferocytosis) downregulate proinflammatory cytokines and upregulate anti-inflammatory cytokines, which suggest a possible mechanism.51,52 Interestingly, although these data were confirmed by a smaller phase II trial by the same group, in the smaller study, there was no effect on circulating cytokines (IL-6, interferon[IFN]-γ, IL-10, TNF-α, or C-reactive protein), suggesting that these cytokines were either not modulated or there is as of yet unexplained mechanisms at work.53

Numerous other small trials suggest potential promise, but larger studies are necessary. For example, a pooled meta-analysis of small trials (total n=221) using pentoxifylline, a nonselective phosphodiesterase inhibitor thought to suppress inflammation, demonstrated a decrease in all-cause mortality in a mix of patients with NICM and ICM.54 Other smaller trials using intravenous immunoglobulin and methotrexate have yielded either mixed results or no benefit.55–57 Another agent, colchicine, was used (n=277) in a blinded study of stable patients with HF, and although it reduced serum proinflammatory cytokines, there was no effect on any clinical indices.58 With the exception of perhaps pentoxifylline, these non specific anti-inflammatory compounds showed little efficacy in chronic HF. Importantly, all these studies were limited by a small sample size.

Stem Cell Therapy in Chronic HF: An Immunomodulatory Mechanism

The initial goal of mesenchymal stem cell (MSC) therapy was to deliver cells with elements of multipotential lineage differentiation, so that they could differentiate into cardiomyocytes/
endothelial cells to regenerate injured myocardium. Clinical trials have targeted patients with primarily ICM, secondarily NICM, using a variety of cellular sources such as CD34+ stem cells, MSCs, and cardiac progenitor-derived cardiospheres. Subjects that received cell-based therapies had varied benefits in quality of life, exercise capacity, improvement in LV function, and in some trials, improved survival. Interestingly, the mechanisms by which stem cells operate seem to have little to do with engraftment, as studies have shown little long-term persistence of infused or intramyocardially injected cells. Rather, mechanistic studies in animals demonstrate that stem cells (and cardiospheres) seem to be taken up by resident cardiac macrophages, and that macrophages are required for the cardioprotective effects of stem cell therapy (Figure 2). In parallel to this association between stem cells and macrophages, a recent clinical trial expanded bone marrow–derived MSCs and activated macrophages (CD45+ CD14+ autofluorescent) ex vivo (Ixmelyocel-T) for subsequent intramyocardial injection in patients with chronic ischemic dilated cardiomyopathy. This combined cell therapy produced significant injection in patients with chronic ischemic dilated cardiomyopathy.63 This combined cell therapy produced significant improvement in their primary end point (all-cause mortality, cardiac admissions, and HF admissions) and a modest improvement in LV function. It remains to be determined how the interaction between macrophages and stem cell–based therapies operates mechanistically and if therapy reduces systemic or local inflammatory processes that drive inflammation. Bone marrow mononuclear cells have been shown to express the anti-inflammatory cytokine IL-10 and intramyocardial injection of these cells post MI improved LV function in an IL-10–dependent manner.64 The specific role of IL-10 in the acute setting in which it was studied was found to exert its protective role by limiting T-cell recruitment to the damaged myocardium. Importantly, stem cell therapy represents an exciting avenue of study, and it will be critical to gain a better understanding of how these transplanted cells interact with local immune cell subsets to modulated cardiac inflammation at different times during disease progression.

**Animal Studies of Established Chronic HF**

As mentioned, the majority of animal studies focus on the combined outcome of the acute tissue injury and initial reparative response in various models where individual cell types, cytokines, or other pathways are modulated through either genetic or pharmacological means. Relatively few studies strictly address the role of inflammation once HF is established. We will briefly outline the major factors that govern how the immune system controls the development of HF based on initiating insult and mechanism of injury, and focus on the majority of the discussion on those few studies that give insight into the role of inflammation during established HF (Table), with the understanding that this field is not well investigated.

From studies of chronic inflammation in other tissues, we know a reduction in inflammation is a precursor for tissue repair to occur. Many approaches have been successfully used for their ability to limit inflammation and thus minimize cardiac damage and prevent HF development. These range from the blockade of proinflammatory cytokines and chemokines (TNF-α, IL-1β, monocyte chemoattractant protein [MCP] 1), enhancing phagocytosis of dying cells (efferocytosis), inhibition of inflammatory receptors, alterations in the recruitment and expansion of cardiac myeloid cells (monocytes, macrophages, and neutrophils) to stem cell–based therapies, all of which alter net balance between inflammation and repair, setting the trajectory of HF development (Figure 1).

**ICM Development**

The most common initiating factor in the development of chronic HF is ischemic injury after an MI. Post MI, there is a cascade of signals that lead to recruitment of inflammatory cells into the infarcted territory that is driven by inflammatory cytokines and chemokines, which ultimately transition into a tissue repair process characterized by fibroblast activation and scar formation. This time course has been relatively well defined in a mouse model of MI (ligation of the left anterior descending coronary artery). The initial inflammatory response
is dominated by neutrophil accumulation, followed by the infiltration of mononuclear phagocytes (monocytes, macrophages, and dendritic cells [DCs]) around day 3 post MI, which further mediate the inflammatory response. By 1 week, the majority of macrophages present are anti-inflammatory (express IL-10) and participate in the resolution of inflammation, angiogenesis, myofibroblast proliferation, and ultimately mature scar formation.\(^8\) An increase in lymphocytes (T cells, B cells, and natural killer cells) is also noted around this time point, and these cells are implicated in the resolution of inflammation and LV remodeling.\(^8\) The resultant thinning and fibrosis of the myocardial wall leads to a decrease in regional LV contractility, and as an initial compensatory mechanism, hypertrophy of the remote myocardium ultimately becomes pathological. In the following weeks, the surviving myocardium undergoes adverse remodeling that can ultimately lead to varying degrees of chronic HF. This initial phase of injury and repair has been highly investigated and marks the beginning of the chronic HF phase, which is our interest.

**Inflammation in Chronic ICM**

As previously noted, few studies have examined the role of immune cells in the chronic setting of ischemic-induced chronic HF. Recent evidence has implicated a role for Toll-like receptor 4 (TLR4) in the progression of chronic HF.\(^8\) TLR4 is a member of the TLRs and is expressed on the cell surface of cardiomyocytes and myeloid cells.\(^8,8\) TLRs recognize specific ligands, termed damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), which are derived from damaged host cells and pathogens, respectively. Signaling via these receptors activates the transcription factor nuclear factor-κB (NF-κB) leading to the expression of a wide range of inflammatory genes.\(^9\) Many stressors, including ischemia, can stimulate cardiomyocytes to release DAMPs such as heat shock protein (HSP) 60. Notably, HSP60 has been found in the circulation of rats with ICM (>9 weeks post MI) and humans with dilated ICM.\(^9\) Furthermore, TLR4 upregulation persists in chronic HF, and blockade of TLR4 during chronic ICM improved LV function.\(^4\) It is tempting to speculate that chronic low-grade stimulation through TLR4 perpetuates chronic HF, presumably through binding and sensing DAMPs (Figure 3).

**Table. Role of Immune Cell Subsets in Chronic HF**

<table>
<thead>
<tr>
<th>Immune Cell Targeted</th>
<th>HF Cause Studied</th>
<th>Intervention</th>
<th>Time of Intervention (Acute vs Chronic)</th>
<th>Observation in the Chronic Setting</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes/ macophages</td>
<td>Ischemic cardiomyopathy</td>
<td>CSF-1 expressing skeletal myoblasts</td>
<td>Chronic</td>
<td>Increased recruitment of macrophages to the infarct border zone, increased angiogenesis, improved cardiac function</td>
<td>65</td>
</tr>
<tr>
<td>AngII</td>
<td>Cl-Lip depletion</td>
<td>Acute*</td>
<td>Decrease in myofibroblast numbers/cardiac fibrosis</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td>Cl-Lip depletion</td>
<td>Acute*</td>
<td>Decreased LV hypertrophy and cardiac fibrosis</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>High-salt diet</td>
<td>Cl-Lip depletion</td>
<td>Chronic</td>
<td>Preservation of cardiac function</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Diabetic cardiomyopathy</td>
<td>Cl-Lip depletion</td>
<td>Acute*</td>
<td>Partially protected LV systolic function</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>EAM</td>
<td>CCR2 siRNA</td>
<td>Chronic</td>
<td>Decreased fibrosis and increased cardiac function</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>T-lymphocytes</td>
<td>TAC</td>
<td>RA62 K0 mouse</td>
<td>Acute*</td>
<td>Fail to develop ventricular dilation, macrophage recruitment, fibrosis and adverse cardiac remodeling</td>
<td>70</td>
</tr>
<tr>
<td>Regulatory T-cells</td>
<td>EAM</td>
<td>IL-17 KO mouse</td>
<td>Acute*</td>
<td>No effect on acute myocarditis but protected from developing dilated cardiomyopathy</td>
<td>71</td>
</tr>
<tr>
<td>EAM</td>
<td>IL-17–neutralizing antibodies</td>
<td>Chronic</td>
<td>Preserved LV systolic function</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>

AngII indicates angiotensin II; CCR2, C-C motif chemokine receptor 2; CSF-1, colony-stimulating factor 1; Cl-Lip, clodronate liposome; EAM, experimental autoimmune myocarditis; HF, heart failure; IL, interleukin; LV, left ventricle; RAG2 KO, recombination-activating gene 2 knockout; and TAC, transverse-aortic constriction.* Intervention during the acute phase does not give a true understanding of the pathophysiology that is occurring during the chronic phase of HF. These studies only address how altering immune cell composition sets the trajectory of disease.
Murf-1 and MAFbx, 2 E3 ubiquitin ligases involved in protein degradation. Mechanistically, the authors suggested that chronic TNF-α production leads to a degradation of troponin I, and therefore a decline in cardiac contractility.92

The potential for stem cells to heal damaged myocardium has gained much attention over the years because of the success seen using MSCs in both animal models and clinical trials (as discussed above). Because MSCs do not engraft, their therapeutic success (albeit limited at this stage) has been attributed to autocrine mechanisms. An unexpected role for colony-stimulating factor 1 (CSF-1) may prove important in this regard (Figure 2). CSF-1 is a cytokine expressed by many cell types, including MSCs, and is a known regulator of tissue resident macrophage proliferation and survival.93

Despite the low success observed using skeletal myoblast autologous transplant to treat cardiac injury, 1 study showed improved cardiac function after intermyocardial injection of myoblasts transfected with a CSF-1 expression plasmid in animals with chronic ischemic HF.65 The cell treatment was given 3 weeks post MI (day 21) to allow for the establishment of HF in their rat model, and cardiac function was assessed at day 52 and 86 post MI. They concluded that CSF-1 enhanced myoblast proliferation and survival leading to better engraftment. In addition, they observed enhanced macrophage recruitment to the border zone of the infarcted area and increased angiogenesis and matrix metalloproteinase 2 (MMP-2) protein levels. This translated to improved cardiac function and increased infarct wall thickness. Inhibition of CSF-1 signaling has been shown to reduce cardiac macrophage expansion 2 weeks post MI, corresponding with an increase in inflammatory cytokines and worsening cardiac function.94

Cardiac progenitor cells are stem cell–like populations that are produced from cardiac biopsies and used in clinical trials; however, their mechanism of action, given their lack of engraftment is not clear. Intracoronary infusion of cardiac progenitor cells into rats 30 days post MI (chronic ICM) improved LV function and reduced cardiac fibrosis.95 Interestingly, cardiac progenitor cells changed the inflammatory state of macrophages within the myocardium (from proinflammatory to anti-inflammatory), and depletion of all macrophages ablated the cardioprotective effects of cardiac progenitor cells post MI.62 Delivery of cardiac progenitor cells results in long-term improvement in cardiac function through the enhancement of endogenous progenitor proliferation leading to additional

![Diagram](https://via.placeholder.com/150)

**Figure 3. Regulation of chronic inflammation in heart failure (HF).** Expression of inflammatory cytokines in chronic HF may arise from several mechanisms. Activation of cardiomyocytes may occur in response to damage-associated molecular patterns (DAMPs; eg, HSP60 [heat shock protein 60], HMGB1 [high-mobility group box 1]) in the extracellular space released by immune cells (macrophages/monocytes), splenocytes or stressed/necrotic cardiomyocytes. Signal in response to DAMPs in chronic HF is via Toll-like receptor 4 (TLR4) that activates the NOD-like receptor family pyrin domain–containing protein 3 (NLRP3) inflammasome leading to the release of proinflammatory cytokines. The NLRP3 inflammasome can also be activated in response to oxidative stress and reactive oxygen species (ROS) production in the setting of diabetes mellitus. Proinflammatory cytokines can be released in response to free mitochondrial DNA (mtDNA) from damaged mitochondria via TLR9 signaling, in the presence of impaired mitophagy. The inflammatory state drives macrophage recruitment (in an monocyte chemoattractant protein 1 [MCP-1]–dependent manner), which can further participate in the release of these cytokines. Cardiac macrophages are also responsible for the recruitment/differentiation of myofibroblasts driving cardiac fibrosis in the setting of chronic HF. This is suggested to be transforming growth factor (TGF)-β–dependent, yet remains to be conclusively demonstrated in animal models of chronic HF (represented by the dashed arrow). IL indicates interleukin; and TNF, tumor necrosis factor.
formation of endothelial cells, smaller scars, and more viable myocardium. Together, these data suggest that macrophages are critical to stem cell–based therapies, and that chronic manipulation of macrophage function may be an important stem cell–based outcome.

**Chronic ICM and the Spleen**

A recent article by Ismahil et al.96 examined a role for the spleen during the chronic phase of ischemic HF, 8 weeks post MI. This represents a time at which the initial inflammatory response would have stabilized and chronic cardiac remodeling would be the driving force behind disease progression. Splenectomy 8 weeks post MI significantly improved LV systolic function and reduced LV hypertrophy, with a reduction in cardiac myeloid cell numbers. Adoptive transfer of splenocytes isolated from mice with chronic HF (8 weeks post MI) demonstrated that they homed to the myocardium of naïve mice, causing LV dilation and LV dysfunction. This suggests that in chronic HF, a population of cells reside in the spleen that can directly cause cardiac injury. The authors attributed the pathological effects to the increased expression of several alarmins in HF splenocytes, such as high-mobility group box 1 (HMGB1). Alarmins are DAMPs, typically expressed by immune cells, which signals trauma when they have been activated by either DAMPs or PAMPs, to work in an autocrine fashion. These data argue in favor of the hypothesis that the incomplete resolution of inflammation, and the progression to chronic HF, is due to ongoing signaling via the pattern-recognition receptors (ie, TLRs).

These data can also be linked with human studies that use positron emission tomography (PET)-based fluoroxyglucose (FDG) imaging as a measure of inflammation. In the acute setting post MI, there is increased spleen, bone marrow, and arterial FDG uptake, and in patients without risk factors for coronary disease, increased splenic uptake alone was predictive of arterial inflammation and increased risk for future MI.97 Together, these data suggest that although we do not have any insight into the mechanism, there is a relationship between the spleen, systemic inflammation, and ongoing cardiac injury in chronic HF that requires further investigation.

**Hypertensive Cardiomyopathy and the Role of Inflammation**

Hypertensive heart disease is typically associated with LV hypertrophy, systolic/diastolic dysfunction, and decreased EF in response to increased pressure overload in the heart. The contribution of inflammation to the development of hypertension is well established, as is the identification of inflammatory cytokines in the setting of chronic hypertension. Unfortunately, mechanistic insight into the role the immune system plays in chronic hypertension comes from studies in which the interventions are placed early on in the development of the disease. These highlight how the immune system is able to alter the trajectory of disease; however, their role once HF is established is not known. Below we will highlight key aspects of the acute/subacute role of inflammation to the development of hypertensive cardiomyopathy in the 2 main mouse models currently used to study this phenomenon, angiotensin II (AngII) infusion and transverse-aortic constriction (TAC).

**AngII Infusion and TAC: Acute Hemodynamic Changes and Inflammation**

AngII is an activator of the renin-angiotensin system and leads to hemodynamic strain through an acute rise in blood pressure but can also act directly on cardiomyocytes and fibroblasts to induce hypertrophy.96,98 AngII is a central mediator of cardiovascular disease clinically and is the target of a wide range of therapeutically successful pharmaceuticals.100 Acutely, AngII infusion results in dramatic and complex changes in cardiac macrophage composition. Recently, distinct cardiac macrophage subsets have been identified, which have their own separate origins and function. After AngII infusion, total cardiac macrophage numbers expanded via parallel pathways involving C-C motif chemokine receptor 2 (CCR2)–monocyte recruitment and local proliferation of resident macrophages. Resident macrophages are derived primarily from embryonic development and self-maintain in adult animals.78 Functionally, embryonic-derived cardiac macrophages are enriched in reparative genes, whereas recruited CCR2+ macrophages are enriched in inflammatory genes, in particular, the NOD-like receptor family pyrin domain–containing protein 3 (NLRP3) inflammasome pathway, regulating IL-1β production.78 A role for macrophages in the development of cardiac fibrosis can be gleaned from studies done in the acute setting of AngII infusion. Depletion of macrophages with clodronate liposomes resulted in a reduction in α-smooth muscle actin (SMA) myofibroblasts in the heart and reduced cardiac fibrosis.46 Similarly, the MCP-1 knockout mouse (chemokine ligand for CCR2) has a decrease in macrophages and bone marrow–derived fibroblasts in the heart with a decrease in AngII-induced cardiac fibrosis.101,102 Macrophages likely play an important role in the transdifferentiation of fibroblasts to myofibroblasts through expression of the profibrotic cytokine transforming growth factor (TGF)-β. Increased expression of TGF-β has been demonstrated in alternatively activated macrophages and in CD11b+ myeloid cells in humans with HFP EF.103,104 Addition of TGF-β to fibroblasts in vitro induces the expression of α-SMA, collagen type I, and fibronectin, in a Smad3-dependent manner.105 Inflammatory pathways also control, in part, the development of cardiac hypertrophy during AngII infusion. TIR-domain–containing adapter–inducing interferon-β (TRIF) is an important cellular mediator in TLR4 signaling, and mice deficient in TRIF were protected from developing cardiac hypertrophy and fibrosis in the acute setting. These data suggest that DAMPs, released by either resident or recruited cells, may contribute to pathology via TLR4 or other pattern-recognition receptors.106,107

Experimental TAC produces abrupt pressure overload on the LV and is a tool to study compensatory cardiac hypertrophy leading to maladaptive remodeling that eventually leads to cardiac dilatation and HF. Similar to what has been shown in the AngII system, post TAC, macrophage numbers increases and fibrosis develops. Depletion of monocytes/macrophages using clodronate liposomes immediately after TAC resulted in decreased expression of TGF-β, COL3A1, and atrial natriuretic peptide at day 3.67 Moreover, continuous depletion over a 3-week period resulted in decreased LV hypertrophy
and cardiac fibrosis at day 21, suggesting that the net effect of pan monocyte/macrophage depletion is protective.

The pathological effect of macrophages is in part dependent on cytokine release, as macrophage specific knockout of miR-155, a regulator of suppressor of cytokine signaling 1 (Socs1), abolishes TAC-induced cardiac hypertrophy. The role of cytokines in the regulation of hypertrophy, however, remains controversial. Overexpression of the profibrotic growth factor TGF-β, for example, induced cardiac hypertrophy, whereas inhibition of TGF-β with neutralizing antibodies showed no effect on the hypertrophic response. Furthermore, inhibition of IL-6 has been shown to reduce cardiac hypertrophy and fibrosis after AngII infusion, yet this had no effect after TAC. In addition, the protective versus detrimental role of cytokine signaling pathways have been debated. For example, cardiac-specific deletion of the NF-κB subunit, p65, showed reduced cardiac hypertrophy after TAC, reduced pathological remodeling, and preserved contractile function, whereas other reports argue for a protective role for the NF-κB pathway. These differential effects, some of which are downstream of TLR signaling, but also many other cell surface receptors, reflect that the role of traditional proinflammatory signaling cascades, such as NF-κB, are dependent on the cell type and microenvironment in which they are engaged. This emphasizes the importance of cause and the temporal aspect of disease progression after different initial insults. It will be interesting to see whether these interventions, administered late during true chronic hypertension in either model, will have the same effects on (or potentially reverse) cardiac hypertrophy, fibrosis, and transition into systolic HF.

A role for the adaptive immune system in TAC-mediated hypertension has been documented in the recombination-activating gene (RAG) 2 knockout mouse model, which lacks both B and T lymphocytes. In this study (assessed at 6 weeks post TAC), we may observe mechanisms at play both during acute injury and in the chronic phase; however, the chronic phase was not assessed in isolation. RAG-deficient mice and major histocompatibility class II–deficient mice (CD4 T-cell defect) developed cardiac hypertrophy but failed to develop ventricular dilation, macrophage accumulation, fibrosis, and adverse cardiac remodeling 6 weeks post TAC. Recent studies in mice that lack the T-cell receptor, or depletion of total T cells, have demonstrated similar findings 4 weeks post TAC. Data from these mouse models suggest that the role of T cells in atherogenesis is both cell type and microenvironment dependent. Macrophages may be a direct target of statin therapy, which are known to demonstrate that macrophages are a direct target of statins, it will be interesting to determine which cardiac macrophage populations (those derived from embryonic progenitors or those recruited to the myocardium) are pathological in the chronic state. Although in vitro studies demonstrate that macrophages are a direct target of statins, and statins reduce inflammation in vivo, the full range of effects of statin use are likely to include other cell types beyond macrophages.

**Diabetic Cardiomyopathy and Chronic Inflammation**

Despite the amount of evidence supporting the role of inflammation and macrophage infiltration in the development of insulin resistance and diabetes mellitus in obesity, the effect on HF development/progression in this setting is just beginning to be understood. Although HF could develop secondary to increased susceptibility to atherosclerosis, dyslipidemia, hypertension, and the prothrombotic state, epidemiological studies have shown increased development of cardiomyopathy even in the absence of underlying coronary artery disease or hypertension. This susceptibility to impaired LV function in diabetic patients has been termed diabetic cardiomyopathy. The characteristics observed in both mouse and human models of diabetic cardiomyopathy, such as lipid accumulation, oxidative stress, mitochondrial dysfunction, and cardiomyocyte cell death, have been shown to trigger inflammation.

Cardiac inflammation and leukocyte recruitment, as observed in the setting of diabetic cardiomyopathy, seems to be mediated in part through the NLRP3 inflammasome in a similar fashion to MI. It has been hypothesized that the mechanism of inflammasome activation in diabetic cardiomyopathy is dependent on the combination of oxidative stress and reactive oxygen species production, as a result of mitochondrial dysfunction. Damaged mitochondria are removed from the cell in a process known as mitophagy, and if this system is overwhelmed or ineffective, increased reactive oxygen species ensues. Diabetic (db/db) mice deficient in Smad3, a mediator of TGF-β expression, were shown to have a reduction in reactive oxygen species levels (compared with wild-type db/db mice), which was associated with a decrease in cardiac fibrosis and reduced cardiac macrophage numbers. Although the relationship between reactive oxygen species and macrophage recruitment requires further investigation, this represents an interesting link between Smad3/TGF-β signaling and oxidative stress in the diabetic myocardium.
It is established that decreased insulin resistance leads to lipid accumulation in myocardial tissue and this results in cardiotoxicity. Using a transgenic mouse model of cardiomyocyte-specific lipid excess, Schilling et al. found early induction of proinflammatory cytokines in myocardial tissue (IL-6, MCP-1, and MCP-2, but not TNF-α) and increased cardiac macrophage accumulation at ~3 weeks of age, whereas mice had normal cardiac function. By 8 weeks of age, lipid accumulation led to decreased LV function independent of systemic abnormalities in insulin or glucose metabolism. Early nonselective monocyte/macrophage depletion with clodronate liposomes improved LV systolic function. Similar to some other studies of macrophage depletion in HF, cardiac hypertrophy (as measured by LV wall thickness and LV mass index) was unaffected by macrophage depletion, suggesting that other factors are at play in the development of cardiac hypertrophy in the setting of lipid overload. Whether macrophage depletion would improve or restore cardiac function in established diabetic cardiomyopathy has yet to be determined.

**Autophagy, Chronic HF, and Inflammation**

Central to tissue homeostasis is the ordered degradation and recycling of cellular proteins and organelles in a process known as autophagy. Disruption of this process leads to the accumulation of misfolded protein aggregates. In adult mice, inducible cardiomyocyte-specific loss of autophagy-related 5 (Atg5), a protein required for autophagy, led to rapid accumulation of mitochondria, disorganized sarcomere structure, and phenotypically; LV dilatation, systolic dysfunction, and cardiomyocyte hypertrophy in naive animals. In response to pressure overload (TAC), autophagy was initially reduced during the hypertrophic response but was activated during the transition to chronic HF. Loss of Atg5 in this setting led to rapid HF and death. In chronic human HF, there is evidence of impaired autophagy and protein aggregates, indicating that this critical pathway can become dysregulated. In addition, impaired autophagy has been reported in a variety of clinical risk factors for HF, such as hypertension, ischemia, diabetes mellitus, aging, and genetic predisposition. During chronic ICM (4 weeks post MI), mice treated with resveratrol, which possess proautophagic functions, exhibited partially reversed LV dilatation and improved LV function in the chronic state, whereas the inhibition of autophagy worsened LV function.

Mitochondria are responsible for supplying the beating myocardium with ATP and are intimately involved in regulating apoptosis in stressed or damaged cells. During cardiac stress, such as ischemic injury or hypertrophy, mitochondria activate prosurvival and prodeath pathways regulating the balance between life and death of the cardiomyocyte. As a cardioprotective mechanism damaged/dysfunctional mitochondria (and their inflammatory contents) can be sequestered and recycled through a specialized autophagic process called mitophagy.

In the context of chronic HF, the impairment in mitophagy can lead to low-grade release of DAMPs over time that can trigger cardiac inflammation in a TLR-dependent manner. One such DAMP is mitochondrial DNA (mtDNA), mtDNA is derived from our symbiotic relationship with bacteria, and like bacterial DNA, it is not methylated. Our immune system is adept at identifying unmethylated DNA although recognition by TLR9, which leads to proinflammatory production (TNF-α, IL-1β, and IL-6) in a cell autonomous manner. Furthermore, unrestricted mtDNA activates the NLRP3 inflammasome. In the process of mitophagy, mtDNA is normally degraded by lysosomal DNase II and cardiac-specific loss of DNase II leads to an impaired ability to withstand pressure overload (via TAC), leading to rapid myocarditis and LV dysfunction. This was alleviated after TLR9 inhibition or ablation, which resulted in reduced systemic inflammation and increased cardiac function indicating mtDNA must be sensed to trigger inflammation, even if a component of mitophagy is impaired. mtDNA is rapidly induced after pressure overload; however, even when DNaseII is intact, loss of TLR9 improves cardiac function and suppresses inflammation, suggesting that low levels of mtDNA, or other TLR9 triggers, escape during pressure overload and can contribute to HF in the chronic setting (Figure 2).

Activation of TLR9 appears to be an important factor in the deterioration of established HF. As a model of chronic HF, inducible cardiomyocyte-specific deletion of Serca2a, a calcium ATPase, led to diastolic dysfunction >4 weeks. In this setting of established diastolic HF, TLR9 stimulation (via a TLR9 agonist) for an additional 4 weeks led to worsening diastolic function and a transition to systolic HF. This was accompanied by increased macrophage infiltration and proinflammatory cytokine production. Although this study assessed the role of TLR9 stimulation during chronic HF, it would have been interesting to determine whether macrophage infiltration and cytokine production was mechanistically involved in the transition to systolic HF. Nonetheless, these studies linking TLR9 activation to both initiation and progression of HF are intriguing, yet many questions remain in terms of the composition of the immune response and what role the individual cell types play after TLR9 is activated. More broadly, autophagy-related inflammation has been linked to the general process of aging, where the prevalence of many of the other risk factors for impaired autophagy area also seen. In this setting, low-grade chronic cardiac inflammation seen in aged humans and mice may be associated with impaired autophagy/mitophagy, leading to inflammation and a susceptibility to develop HF when stressed.

**Adaptive Immune Initiated Cardiomyopathies**

In contrast to HF that is initiated by one of the previously mentioned risk factors, immune-based myopathies develop after an antigen-specific T-cell response, resulting in ongoing inflammation. Infection and clearance of a virus in the heart is mediated by both the innate and the adaptive immune system. Although the majority of animal studies examining immune-based cardiomyopathies analyze viral infection, many other infectious (most commonly Chagas Disease in South America) and noninfectious (chemotherapeutic agents such as doxorubicin) agents have been shown to cause myocarditis.
Infectious Viral Myocarditis

Similar to humans, genetic susceptibility to viral-induced myocarditis has been well documented in mice, with some strains being susceptible to the development of dilated cardiomyopathy (A/J and BALB/c) and those that are resistant (C57BL/6). This led to the identification of several susceptibility genes including IFN-related genes (Fpgt, H28, and Tmfn3k). In acute viral coxsackievirus B–induced myocarditis, IFN-α and IFN-β (type I interferons) are important mediators of a Th1 response and reduced viral replication, whereas IFN-γ (type II interferon) regulates monocyte mobilization and fibrosis. A mouse model of limited IFN-β activity, induced by knocking out the TRIF protein, had severe chronic myocarditis and dilated cardiomyopathy. Similarly, IFN-γ knockout mice had an increase in chronic coxsackievirus B–induced myocarditis, associated with increased mast cell degranulation, circulating inflammatory cytokines (TGF-β, IL-1β, and IL-4) and cardiac fibrosis. Thus, it seems that a Th1 response is protective by reducing viral replication and inhibiting a Th2 response. Th2 responses, however, may be important in the acute setting of myocarditis by elevating regulatory T cells and anti-inflammatory cytokines. Failure to dampen this response late during infection leads to adverse cardiac remodeling and chronic myocarditis. This highlights the importance for cross talk between these 2 pathways in the resolution of inflammation after infection.

Similar to other types of cardiac injury, TLRs play an important role in mediating the immune response to viral infection. In the acute setting, loss of TLR4 leads to better viral clearance and reduced cardiac injury. TLR3, TLR9, and MDA5, on the contrary, are critical to viral recognition and antiviral responses, and loss of these receptors results in death during viral myocarditis. Depletion of monocytes, macrophages, and DCs also causes death during viral myocarditis; however, blunting monocyte activation and macrophage expansion have also been shown to be beneficial, suggesting that the exact role of monocytes, macrophages, and DCs remains to be defined. Because different cardiac macrophage lineages exist, some of which are monocyte derived, whereas others are embryonic derived and possess reparative functions, understanding the role of each macrophage lineage may yield additional clarity to this field.

Interpretation of studies focused on CD11c+ DCs must be done carefully, as CD11c is also expressed on a variety of other cell types, including recruited monocytes. Depletion of DCs using the CD11c^−/− mouse, for example, has the potential to induce loss of DCs, monocytes, macrophages, and natural killer cells. When compared with depletion of true classic DCs (Zbtb46^−/− mice), which specifically deplete classic DCs only, less pronounced results are seen in a variety of models. Based on our need to understand the acute effects of viral myocarditis, and the subsequent resolution of infection, mechanistic data on the contribution of viral induced inflammation to chronic HF are lacking. We suspect incomplete resolution of viral infection may play a role based on evidence in humans demonstrating viral persistence in myocarditis. It is also possible that additional risk factors, such as genetic susceptibility, may lead to an incomplete resolution of inflammation and thus altered homeostasis resulting in cardiac dysfunction. Our lack of data, as presented here, emphasizes a need to investigate this model further.

Experimental Autoimmune Myocarditis

After an initiating event, autoreactivity to self-antigen (typically myocardial proteins) can develop and has been linked with the initiation and persistence of chronic HF. There is a growing appreciation for the requirement of the innate immune system to maintain homeostasis, and a disruption in this process can lead to inflammation and cardiomyopathy. This has been modeled with DC loaded self-peptide (typically cardiac myosin) to induce experimental autoimmune myocarditis (EAM) via the activation of antigen-specific CD4 T cells. What was particularly interesting about this study is that after resolution, repeated inflammatory myocarditis could simply be reinduced in primed animals through activation of only TLR4 or injection of injured cardiomycocytes secondary to a recall T-cell memory response. EAM can progress during repeated stimulation over time, leading to fibrotic and dilated cardiomyopathy, and loss of MyD88 or IL-1 signaling is critical for transition from myocarditis alone to fibrosis and LV dysfunction.

Chronic release of DAMPs may also play an important role in EAM. HMGB1 is released from stressed and dying cells and binds the receptor for advanced glycosylation end products (RAGE). After induction of EAM, HMGB1 remained elevated >270 days post induction in animals (and in patients with myocarditis), and blockade of HMGB1 or deletion of RAGE prevented EAM development. These studies suggest that a critical component of disease pathogenesis in EAM is the release of DAMPs that drive inflammatory responses resulting in impaired LV function.

Monocytes may play an important role in EAM progression as inhibition of monocyte recruitment via CCR2 siRNA resulted in decreased immune cell infiltration, fibrosis, and increased cardiac function. This was observed when siRNA was injected either at the onset of disease or 21 days post-EAM induction, suggesting that monocytes play a role even at later stages after disease establishment. The authors also noted a decrease in CD4^+ T-cell numbers after CCR2 siRNA treatment; however, this may not have been because of the effect of siRNA on the T cells per se but likely represents a cross talk between the innate and the adaptive immune systems in the development of EAM.

Evidence has also implicated a role for regulatory T cells (Th17) in EAM, in particular, during the transition from acute myocarditis to fibrotic chronic HF. Baldeviano et al examined the role of IL-17A, a major cytokine expressed by Th17 cells, in EAM and reported an infiltration and persistence of IL-17A secreting Th17 T cells within the myocardium throughout the course of disease. Surprisingly, IL-17A knockout mice did not have any change in the acute/subacute autoimmune myocarditis response (out to 50 days), nor did loss of IL-17A change the antigen-specific CD4 T-cell response.
or the generation of autoantibodies. However, IL-17A was required to transition from myocarditis to dilated cardiomyopathy (from day 35 to day 62, with a peak myocarditis score at day 21). Il17a−/− mice were protected from LV dilation and LV dysfunction and were able to resolve acute autoimmune myocarditis without cardiac dysfunction. Moreover, neutralization of IL-17A during established myocarditis also preserved LV systolic function. IL-17A neutralization seemed to reduce the overall inflammatory response including a reduction in some proinflammatory cytokines, such as IL-6, IL-1β, and growth factors, such as G-CSF. Although not thoroughly investigated, there seemed to be a change in the composition of leukocytes, with decreased monocytes and increased regulatory T cells within the myocardium of Il17a−/− mice. Although additional mechanistic studies are required to define how IL-17A promotes the transition to dilated cardiomyopathy in the chronic stage of EAM, this study is important as it highlights that although IL-17A did not set the trajectory of the initially inflammatory response, it did play a pathological role in the chronic state.

IL-17A also plays a complex role in a variety of other cardiovascular disease states, coordinating intercellular processes between many cell types. IL-23 is an inducer of IL-17A, and loss of IL-23 (and presumably IL-17A) can impair tissue fibrosis—which can be pathological in some scenarios. As reviewed elsewhere, IL-17A can drive inflammation through several pathways, including being directly toxic to cardiomyocytes, and can also drive production of neutrophils in the bone marrow, which can later infiltrate the myocardium. In addition to being produced by Th17 cells, IL-17A can also be produced by innate γδ T cells and neutrophils themselves. In the setting of MI, loss of IL-23 or IL-17A in the acute setting improves infarct healing; however, it is unclear what role IL-17A has in chronic ICM or infectious models of myocarditis.

**Inherited Cardiomyopathies and HF: How Little We Know About Inflammation**

Genetics plays a major role in the development and progression of HF. There are >900 genetic mutations identified which either are themselves important initiating factors in disease or alter the way in which an individual copes with an initial insult leading to chronic HF. Familial dilated cardiomyopathy and hypertrophic cardiomyopathy account for the majority of sudden death in young people (≤35 years). There have now been upward of 80 genes recognized as genetic risk factors for familial dilated cardiomyopathy (including sarcomere proteins, cytoskeleton and nuclear envelope proteins, membrane ion channels, and desmosomes) with mutations in sarcomere genes being observed in ~60% of hypertrophic cardiomyopathy. Unfortunately, studies of transgenic mice that model human familial HF by over-expressing mutated cardiac proteins within cardiomyocytes do not often explore or examine the role of inflammatory cells and pathways. This represents a major gap in the field, and future studies should be focused on immune cell subsets that are altered/activated in response to these genetic predispositions. Given the close association between tissue fibrotic pathways and immune cells, it is likely that the immune system may at the least, modulate the fibrotic response to genetic abnormalities that alter cardiomyocyte structure and function leading to HF development. Moreover, macrophages promote angiogenesis, which is required for the hypertrophic response and the associated increase in nutrients required to maintain hypertrophied cardiomyocytes.

**Future Directions: The Promise of Immune-Based Therapeutic Strategies**

As we gain a better understanding of the immune cell subsets and their involvement in cardiac injury and repair, future work should focus on parsing these subsets as they may lead to disparate functional outcomes. Although there are many studies that target inflammation in general during chronic HF, these remain controversial, likely owing to the complex nature of the immune cells present. A greater understanding of the functional implication these immune cells have in the chronic setting will aid in our interpretation of these studies and help focus new therapies in the future. To achieve this, basic research studies should focus on administering interventions late after the establishment of HF, a task that is appreciatively laborious and expensive.

The most promising therapeutic strategies that are quickly emerging are stem cell–based therapies. As discussed in this review many stem/progenitor cell transplants have demonstrated beneficial effects caused by autocrine mechanisms. As we gain a better appreciation for the immunoregulatory roles these cells have, we can modulate these treatment strategies to optimize their potential. This has been exemplified in recent clinical trials combining stem cells and macrophages. Because macrophages are often thought of as conductors of the immune system, incorporating them into stem cell–based therapies may hold great potential.

**Conclusions**

Although the mechanisms of inflammation and subsequent initiation of tissue repair have been relatively well defined in the acute setting after cardiac tissue injury, the role of these critical pathways in the chronic setting remains largely unexplored. Extrapolating the role of immune subsets and autocrine/paracrine factors produced by those subsets into the chronic HF state is not a surrogate for true mechanistic studies that target individual inflammatory pathways after HF has been established. As a community, we have developed elegant Cre-based genetic systems that are both cell type specific and inducible, so that specific cells or genes can be deleted only during established chronic HF. This temporal approach to understand which components of the inflammatory response are physiologic (protective) or pathological (harmful) is required if we are to translate our findings in animals studies to the vast numbers of patients who have chronic HF worldwide. Although these long-term genetic studies are inherently more difficult, if we are to accurately model chronic human HF, we must specifically use animal models with established chronic disease to define the true role of immune system during this phase.
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References


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