Inflammation in Myocardial Diseases

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Abstract: Inflammatory processes underlie a broad spectrum of conditions that injure the heart muscle and cause both structural and functional deficits. In this article, we address current knowledge regarding 4 common forms of myocardial inflammation: myocardial ischemia and reperfusion, sepsis, viral myocarditis, and immune rejection. Each of these pathological states has its own unique features in pathogenesis and disease evolution, but all reflect inflammatory mechanisms that are partially shared. From the point of injury to the mobilization of innate and adaptive immune responses and inflammatory amplification, the cellular and soluble mediators and mechanisms examined in this review will be discussed with a view that both beneficial and adverse consequences arise in these human conditions. (Circ Res. 2012;110:126-144.)

Key Words: inflammation • ischemia • infection • rejection • myocardium

Inflammatory processes underlie many diseases and syndromes associated with injury of the heart muscle, and acutely, subacutely, and chronically cause structural, functional, and molecular deficits and defects. In this article, we address current knowledge and gaps in understanding regarding 4 common circumstances of myocardial inflammation: myocardial ischemia and reperfusion, sepsis, viral myocarditis, and immune rejection. Each of these pathological states has its own unique features in etiology, pathogenesis, and disease evolution, but they are also each characterized by inflammatory mechanisms that are partially shared (Table 1). From the point of injury, to the mobilization of innate and adaptive immune responses and inflammatory amplification, the orchestra of cellular and soluble mediators and mechanisms examined here will be addressed with a view that both beneficial and adverse consequences arise from inflammatory mobilization in these human conditions.

Ischemia and Reperfusion Injury and Inflammation

Prolonged Cardiac Ischemia Leads to Irreversible Cell Death

Thus, reperfusion is essential in order to salvage tissue from inevitable necrosis. However, although restoration of blood
flow is necessary, it also augments a localized oxidative burst and regional inflammatory response that can provoke further myocardial damage. A landmark study showing that reperfusion accelerates further myocardial injury was first documented in 1960 by Jennings et al. Since then, continuously intense research has been devoted to elucidating the mechanisms by which ischemia and reperfusion (IR) provoke myocardial injury and how such events can be targeted for the treatment of myocardial infarction, peri-transplant IR injury, and other conditions that are associated with IR. Cardiac IR injury is a multi-factorial process and many excellent reviews have been written on this process. The present section will focus exclusively on inflammation and its role in IR injury. Given the complexity, vast number of publications related to many of the individual immune components below, and space constraints, recent review articles are cited throughout this review for those seeking further detail.

Inflammation is an important contributor to the pathophysiology of cardiac IR injury and hypotheses pertaining to pathological mechanisms are an evolving area of intense research and debate. However, as further data accumulate it is becoming increasingly clear that acute and chronic inflammatory events that occur as a consequence of IR have profound effects on the functional deterioration of the heart.

**Initial Phase Following an Ischemic Insult**

**Triggers Oxidative Stress, Cellular Damage, and Cytokine Release Leading to Neutrophil Activation and Recruitment to the Injured Area**

Reactive oxygen species are produced by multiple sources during IR such as mitochondria, uncoupled eNOS, cytochrome p450, xanthine oxidase, and neutrophils (Figure 1). As a consequence of oxidative stress and ionic perturbations, microembolism formation, platelet activation, endothelial swelling, interstitial edema, and neutrophil plugging, normal blood flow may be disrupted in the capillaries and microcapillaries. This process is referred to as the “no-reflow” phenomenon. Oxidative stress also contributes to endothelial dysfunction leading to reduced nitric oxide, increased proinflammatory cytokine production and adhesion molecule expression, impaired endothelium-dependent vasodilation, and increased endothelial permeability. Neutrophil activation and leukocyte infiltration lead to further cytokine secretion, oxidative stress, and protease release thereby exacerbating myocardial damage and death.

**Animal Models Have Suggested That Many Types of Immune Cells May Be Involved in Postischemic Inflammation, Injury, and Repair**

Neutrophil depletion with antibodies and leukocyte depletion, as well as inhibition of neutrophil adhesion, attenuate IR-mediated myocardial injury (Table 1). Likewise, strategies targeting mediators of inflammation such as lipoxygenases complement or cytokines have also demonstrated efficacy in experimental models. T cells may also contribute to IR injury as RAG-/- mice exhibit reduced infarct size as compared to control mice. In the latter study, reconstitution of CD4+ T cells in RAG-/- mice resulted in an increase in infarct size. This effect appeared to be interferon (IFN)γ-dependent as T cells from IFNγ-deficient mice did not affect infarct size. Furthermore, T-cell depletion studies revealed that CD4+ but not CD8+ T cells contribute to myocardial injury. Mast cells may also contribute to myocardial injury through the release of cytokines and proteases as mast cell-deficient mice were found to exhibit reduced infarct size.

The innate immune system in IR has received much attention in recent years. Traditionally, the innate immune system has been regarded as the first line of defense against...
### Table 1. Selection of Key Inflammatory/Immune Effectors and Regulators Involved in Different Contexts

<table>
<thead>
<tr>
<th>Key Inflammatory/Immune Effectors and Regulators</th>
<th>Ischemia and Reperfusion Injury</th>
<th>Myocarditis</th>
<th>Cardiac Allograft Rejection</th>
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<tbody>
<tr>
<td><strong>T cells</strong></td>
<td></td>
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<td></td>
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<tr>
<td>CD8+cytotoxic T cells</td>
<td>Do not appear to play a significant role in I/R injury.</td>
<td>Role played by these cells has not been elucidated fully. Most likely act to clear viral infection, but contribute significantly to cardiac inflammation.</td>
<td>Cytoxic T cell-mediated direct lysis via perforin and granzyme-mediated pathways.</td>
</tr>
<tr>
<td>CD4+T helper cells</td>
<td>CD4+ reconstitution studies in Rag-/- mice suggest CD4+ cells contribute to I/R injury in an IFNγ-dependent manner</td>
<td>Role played by these cells has not been elucidated fully. Most likely act to clear viral infection, but contribute significantly to cardiac inflammation.</td>
<td>Thought to contribute to cell-mediated cardiac rejection via pathways dependent on donor allograft Fas expression and CD4+T perforin cell production.</td>
</tr>
<tr>
<td>Regulatory T cells</td>
<td>May suppress post-ischemic adaptive and innate immune responses. May play protective role in recovery phase.</td>
<td>IL-17 produced enhances TNF-α expression in cardiomyocytes and may contribute to autoantibody production and genesis of chronic heart failure.</td>
<td>Production of IL-17, as well as IL-21 and IL-22; IL-17 is considered to have proinflammatory properties.</td>
</tr>
<tr>
<td>B cells</td>
<td>May preserve ventricular function; B cell isotype IgM may be involved.</td>
<td>Antibody mediated immunity is crucial for clearance of enteroviruses like coxsackievirus, in particular, from the heart.</td>
<td>Considered homeostatic controllers of inflammation partly through production of antiinflammatory cytokines, e.g., IL-10 and TGF-β.</td>
</tr>
<tr>
<td>Neutrophils, Mast cells, NK cells and Macrophages</td>
<td>Neutrophils contribute to early microvascular changes, parenchymal damage and no-reflow phenomenon.</td>
<td>NK cells are among the first cells to infiltrate the myocardium during virus infection. These are accompanied by neutrophils and macrophages and all contribute to the immune infiltrate.</td>
<td>Early responders as part of the innate immunity; infiltration of recipient NK cells and macrophages has been observed as early as 3 hours post-transplant in animal cardiac transplant models.</td>
</tr>
<tr>
<td>Complement system</td>
<td>Complement system is activated in I/R injury and contributes to tissue destruction.</td>
<td>Complement receptors may play a role in clearing immune complexes from the extracellular milieu thereby reducing inflammation.</td>
<td>C4d is considered a key mediator shared between the classical and MBL-dependent pathways critical in the Ab-mediated complement activation process.</td>
</tr>
<tr>
<td>Inflammasome</td>
<td>Triggered in cardiac fibroblasts, but not myocytes in response to I/R. Infarct size, fibrosis and dysfunction are reduced in mice deficient for apoptosis-associated speck-like adaptor protein and caspase-1. I/R-mediated ROS and K+ in cardiac fibroblasts lead to inflammasome activation and recruitment of bone marrow-derived cells to the site of injury.</td>
<td>Complement receptors may play a role in clearing immune complexes from the extracellular milieu thereby reducing inflammation.</td>
<td>C3d, together with C4d, has been correlated positively and strongly to presence of donor-specific alloantibodies and allograft dysfunction.</td>
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(Continued)
Ischemia Reperfusion Results in Elevated Protease Activity That Can Contribute to ECM Cleavage

Such activity not only may contribute to a loss of myocardial integrity, but also may promote inflammation through the activation of protease-activated receptors (PARs) (reviewed in Ref. 29) or through the production of biologically active DAMPs that are recognized by a group of evolutionarily conserved transmembrane receptors referred to as toll-like receptors (TLRs). Although once thought to be strictly involved in recognition of so called pathogen-associated molecular patterns such as bacterial and viral DNA or proteins, these receptors are also capable of recognizing mammalian-derived molecules such as necrotic cells, oxidized lipoproteins, cDNAs, extracellular matrix (ECM) degradation products, heat shock proteins (HSP), fibronectin-extracellular matrix (ECM), and other factors. DAMPs function as indicators of cellular stress and, on recognition and binding to TLRs, invoke a proinflammatory or reparative response in cells. TLR recruitment results in the recruitment of the adaptor protein, myeloid differentiation primary response factor protein (MyD88), which leads to downstream phosphorylation and subsequent proteolytic degradation of the inhibitor of NF-κB (IκB) protein. Inhibitor of κB degradation results in the release and translocation of the transcription factor NF-κB (p52/p65) into the nucleus leading to transcription of proinflammatory genes such as cytokines, adhesion molecules and antiapoptotic proteins. For a detailed description of the role of TLR activation and signaling in cardiac ischemia, readers are encouraged to see the recent review by Aslan et al.26

Role for the Multi-Protein Inflammasome Complex in Cardiac Fibroblasts Has Recently Been Proposed for Cardiac IR Inflammation

The inflammasome comprises a high molecular weight, caspase-1 activating platform that is also activated by pattern recognition receptors known as NOD-like receptors (NLRs).27 Sterile inflammatory responses are believed to be triggered through the inflammasome pathway. In recent work by Kawaguchi et al.,39 inflammasome formation was triggered in cardiac fibroblasts, but not cardiac myocytes, resulting in interleukin-1β production and subsequent cardiac inflammation. In the latter study, infarct size, fibrosis, and dysfunction were reduced in mice that were deficient for apoptosis-associated speck-like adaptor protein and caspase-1. Further, the authors demonstrated a link between reactive oxygen species and the efflux of potassium ions in cardiac fibroblasts leading to inflammasome production and recruitment of bone marrow-derived cells to the site of injury. In summary, in addition to endothelial cells and myocytes, cardiac fibroblasts are also involved in the induction of post-IR inflammation.

Abundant Research Supports a Role for Tumor Necrosis Factor (Reviewed in Ref. 40) and NF-κB in Cardiac Myocardial Infarction MI (Reviewed in Refs. 41 and 42)

Although there is little doubt that these factors are activated in IR, there has been much controversy regarding their antiapoptotic versus proinflammatory roles in cardiac IR.
While it is beyond the scope of this review to enter into this debate, it is likely that tumor necrosis factor (TNF) and NF-κB activation and their consequences are cell type (eg, endothelial, cardiac myocyte, fibroblast, immune cell) and context-specific.

In addition to the aforementioned factors, IR and/or inflammation also promote phospholipase A2 (PLA2)-mediated liberation of arachidonic acid (AA), which may be further metabolized by cyclooxygenases (COX), cytochromes p450 (CYP), and lipooxygenases (LOX) into prostaglandins, eicosanoids, or leukotrienes, respectively. IR-mediated reactive oxygen species (ROS) generation can result in the release of proteases and danger-associated molecular patterns (DAMPs) that can promote inflammation through the activation of nuclear factor (NF)-κB. Alternatively, ROS also leads to inflammasome activation which leads to further inflammatory cytokine production. (Illustration Credit: Cosmocyte/Ben Smith). PAR indicates protease-activated receptors; TLR, toll-like receptor; IL, interleukin; MyD88, myeloid differentiation primary response factor protein; EET, epoxyeicosatrienoic acids; HETE, hydroxyeicosatetraenoic acids; IKK, I kappa B kinase; PG, prostaglandin (Illustration credit: Cosmocyte/Ben Smith).

Role of Inflammation in IR-Mediated Myocardial Injury Is a Complex, Multi-Factorial Process Involving Both Innate and Acquired Immunity

The innate immune response to IR appears to be the predominant cause of myocardial inflammation. Although beyond the scope of the present review, inflammation is a key player in the removal of dead cells and debris, hemostasis, provisional matrix deposition and granulation tissue formation, cytokine and growth factors, and scar formation. Understanding the role of inflammation in myocardial injury and postischemic scar formation is essential for guiding future therapeutic strategies. Although this response may be beneficial in the short term with respect to promoting wound repair, in chronic states it is likely to be maladaptive. Given the temporal and both beneficial and detrimental roles of the immune system in injury and repair, it is perhaps not surprising that therapies aimed at various immune targets have been disappointing. As such, further investigation is necessary to decipher how various inflammatory components can be targeted to minimize myocardial damage while evading any impairment of wound repair.

Sepsis and Myocardial Inflammation

Sepsis Induces Acute Cardiac Dysfunction

The advent of radionuclide cineangiography brought forth the realization that acute severe infection or severe sepsis can temporarily stun the heart and result in acute circulatory failure. Initially and erroneously attributed to inadequate cardiac filling, it has been demonstrated over the past 3 decades that there exists an inability to augment stroke volume in response to increases in preload. In other words, sepsis induces a primary depression of preload independent cardiac contractility or Emax. Depending on the timeframe and what is deemed acute reversible heart failure,
estimates of those suffering from acute cardiac dysfunction during sepsis range as high as 60% in the first 48 hours of admission for septic shock.54

Systemic Infection and/or Inflammation Results in Impaired Contractile Function Through a Conserved Innate Immune Response Within Cardiomyocytes
Cardiomyocytes are specialized cells whose primary function is contraction in order to provide the motive force that drives cardiac output and the generation of arterial pressure. There is mounting evidence that cardiomyocytes also respond to danger signals with a complex inflammatory and functional response.55–76 For example, in response to inflammatory stimuli, cardiomyocytes (1) express pro- and anti-inflammatory cytokines (IL-6, IL-10) that initiate and regulate local inflammatory response,67 (2) express chemokines, (MIP-2, MCP-1, KC)74,77 which recruit appropriate inflammatory cell subsets necessary for response and repair, and (3) increase expression of cell-surface-adhesion molecules (ICAM-1), allowing interaction and outside-in signaling from surface adhesion molecules63,66 leads to decreased cardiomyocyte contractility, thereby modulating peak systolic stress and strain and conceivably impairing the repair processes. It appears that some members of the TLR family of innate immune mediators recognize local danger signals and initiate an inflammatory response. We have recently shown that stimulation of TLR2, -4, and -5 with molecules derived from infectious pathogens activates the NF-kB signaling pathway to trigger a complex inflammatory response in the cardiomyocyte63 (Figure 2) and have also found that TLR stimulation results in rapid, intense signaling via IFN-regulatory factors (IRFs).

TLRs Recognize Exogenous Pathogen Associated Molecular Patterns and Are Also Stimulated by Endogenous Molecules Released During Tissue Damage
The TLRs known to induce cardiac dysfunction respond to a wide variety of endogenous and exogenous danger signals. Best characterized are the responses of TLR2 to Gram-positive bacterial products such as lipoteichoic acid, TLR4 to endotoxin from the cell wall of Gram-negative bacteria, and TLR5 to the flagellin of bacteria such as Pseudomonas species. TLR2 and TLR4 expressed by cardiomyocytes are also able to respond to endogenous molecules that are released as “danger signals” on tissue injury. These endogenous ligands include molecules that are upregulated and secreted as a result of cellular stress, including HSP60, HSP70,84–86 and HMGB1.87 Although it was initially thought that these proteins serve the unique function of protecting the cell against subsequent injury by binding misfolded peptides and proteins,88–90 recent data has linked increased expression of HSP60 by the cardiomyocyte with advanced heart failure, and correlated plasma membrane trafficking of this heat shock protein to apoptosis.91 HSP70 also appears to be highly upregulated in cardiac failure with easily detectable serum levels.92 These levels correlate with the degree of cardiac dysfunction and HSP70 acts as a functional biomarker in cardiac dysfunction and its substrate binding domain is able to decrease cardiomyocyte contractility via TLR signaling.93 Although it is unclear whether 1 or both TLR2 and TLR4 are required to induce cardiac inflammation as a result of recognition of these molecules,93,94 it is probable that high local concentrations of either exogenous pathogen-associated molecular patterns or endogenous damage molecules able to stimulate TLRs can result in a pathological inflammatory response. Therefore it seems that the TLR-induced inflammatory response might be of great importance not only in cardiac suppression because of severe infection, but for diseases involving cardiac injury such as ischemic heart disease.

Table 2. Selection of Key Inflammatory/Immune Effectors and Regulators Involved in the Context of Sepsis

<table>
<thead>
<tr>
<th>Key Inflammatory/Immune Effectors and Regulators</th>
<th>Sepsis</th>
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<tbody>
<tr>
<td>RAGE</td>
<td>Cell surface receptor whose ligands include highly glycosylated molecules and in acute inflammation the damage associated molecular patterns S100A8 and S100A9</td>
</tr>
<tr>
<td>S100A8/S100A9</td>
<td>Early response genes with distinct intra/extracellular roles. Extracellular interaction with RAGE results in an acute decline on cardiomyocyte contractility while intracellular calcium regulation mildly increases contractility. Extracellular (RAGE dependent) effects predominate</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Adhesion molecule upregulated in diverse inflammatory states. Its endogenous ligands include fibrinogen, following this interaction there is cytoskeletal changes leading to a decline in contractility</td>
</tr>
<tr>
<td>NFkB</td>
<td>A double edged sword responsible for both acute cardiac dysfunction in sepsis and for the cardioprotective aspects of pre-conditioning</td>
</tr>
<tr>
<td>TLR</td>
<td>See Supplemental Table 1; TLR2 and TLR4 activations are thought to contribute to myocardial inflammation via immune cell activation/regulation</td>
</tr>
</tbody>
</table>

RAGE indicates receptor for advanced glycation end products; ICAM-1, intercellular adhesion molecule; NFkB, nuclear factor-kB; TLR, toll-like receptor.

Systemic Infection and/or Inflammation Results in Impaired Contractile Function Through a Conserved Innate Immune Response Within Cardiomyocytes
Cardiomyocytes are specialized cells whose primary function is contraction in order to provide the motive force that drives cardiac output and the generation of arterial pressure. There is mounting evidence that cardiomyocytes also respond to danger signals with a complex inflammatory and functional response.55–76 For example, in response to inflammatory stimuli, cardiomyocytes (1) express pro- and anti-inflammatory cytokines (IL-6, IL-10) that initiate and regulate a local inflammatory response,67 (2) express chemokines, (MIP-2, MCP-1, KC)74,77 which recruit appropriate inflammatory cell subsets necessary for response and repair, and (3) increase expression of cell-surface-adhesion molecules (ICAM-1), allowing interaction and outside-in signaling from participating inflammatory cells and the extracellular matrix,55,66,71,76,78–80, and finally produce 2 small calcium regulated molecules (S100A8 and S100A9) that suppress calcium flux via the RAGE receptor (Table 2).81 This cardiomyocyte-inflammatory response involving cytokines,82 chemokines and the subsequently recruited leukocytes,57,63 and cell-surface-adhesion molecules63,66 leads to decreased cardiomyocyte contractility, thereby modulating peak systolic stress and strain and conceivably impairing the repair processes. It

In the 2 decades since their discovery, the known role of TLRs has expanded significantly: It was initially thought that TLR expression was restricted to leukocytes with a physiological repertoire limited to an exuberant proinflammatory response.95 It is now known that TLRs are expressed in nearly every tissue including the heart and lungs83,96 and are able to activate (1) early intense inflammatory activity via NFkB, (2)
late inflammatory responses via IRFs, (3) pro- or antiapoptotic pathways, and (4) the suppressive networks necessary to quench the induced inflammatory reaction. The effect of TLRs on the heart has been found to be a composite of these roles. The reported cardiac effects of TLRs include the MyD88 and TLR4 mediated induction of heart failure, protection against cell death, and the promotion of healing. For instance, pretreatment with low doses of the TLR4 ligand LPS activates cell survival pathways and thereby reduces infarct size and protects against ischemic cardiomyocyte apoptosis, whereas acute exposure to high levels of LPS results in intense cardiac suppression.

How is such a complex response regulated? Although TLR signaling does have unique molecular signatures dependent on the tissue, specific TLR, and dose and frequency of TLR ligand exposure, there is also a central or core inflammatory response. This core response to ligation regulates the pro- versus anti-inflammatory balance and occurs regardless of which TLR, tissue, or ligand is involved. The core proinflammatory response following stimulation with TLR ligands has been extensively studied and consists of molecules linked to inflammatory processes in all fields of medicine. These molecules include cytokines such as TNF, IL-1β, IL-6, IL-8, and GCSF, and the chemokines CCL3, CCL4, CCL20, CXCL1, CXCL2, and CXCL3. These molecules are not only regulated by NFκB but are capable of autocrine and paracrine induction of NFκB and other proinflammatory pathways. The core regulatory proinflammatory molecules include the NFκB family with the coregulator complex API and BCL3, mediators of interferon including IRF1, -4, and -7, STAT1, -4, and -5 and regulators of cAMP signaling including ATF3, ATF4, and cAMP responsive element modulator.

Core Inflammatory Response Includessuppressors of inflammation

Following stimulation of TLRs, in addition to the production of proinflammatory molecules there is also induction of suppressors of inflammation. These limit the inflammatory reaction and induce tolerance to repeated insults. Exposure to any TLR ligand induces tolerance to subsequent larger doses of any of these ligands. The molecular mechanisms are diverse and include the rapid production and secretion of anti-inflammatory cytokines such as IL-10 and decoy cytokine receptors such as the soluble decoy IL-6 receptor. Inhibition of NFκB signaling occurs at multiple sites and cellular locations and is of particular interest in the cardiomyocyte, given that activation of this pathway results in acute heart failure. Suppression occurs at multiple levels of the TLR-signaling cascade. The nonfunctional receptors ST2 and SIGIRR compete with the intracellular Toll/IL-1 receptor, and at the TLR-adaptor molecule level, a nonfunctional short MyD88 competes with the full-length adaptor molecule MyD88 and Tollip (Toll-interacting protein) inhibits interleukin-1 receptor-associated kinase (IRAK) phosphorylation. miR146 inhibits TNF receptor-associated factor (TRAF) production, and finally, IκBα pre-vents NFκB nuclear translocation. TNFAIP3 (A20) inhibits the ubiquitin dependent interaction of many NFκB signaling molecules and as such is a central molecule in inhibitory signaling. There are conflicting data regarding downregulation at the level of the TLRs, whereas the TLR-MyD88 complex is known to decrease following induction of tolerance. Following activation of TLRs evidence of inflammation (mainly early response genes) is discernable in beating cardiac myocytes within minutes of TLR activation, whereas the process through which the heart chooses to either augment or suppress inflammation evolves over the subsequent 2 to 3 hours. By 3 to 6 hours, dependent on the magnitude and duration of the inflammatory stimulus, cardiac dysfunction is evident via effector molecules such as ICAM-1 and S100A8/S100A9. (Illustration Credit: Cosmocyte/Ben Smith).
anti-inflammatory gene networks determine the transcriptional activity and targets of NFkB. In the heart, genetic targets of these transcriptional programs then produce both “core” and “noncore” molecules, some of which in turn regulate cardiac function.81,83,100

Myocarditis and Inflammation

The term myocarditis, meaning inflammation of the myocardium, was coined by Joseph Sobernheim in 1837.119 It was quickly appreciated that this inflammation was necessary to limit tissue damage, initiate the healing process, and eliminate dying cells and debris after injurious stimuli in the heart.120 On the other hand, persistent noxious stimuli, conditions including infection and adverse immune responses can partly explain the pathophysiology of inflammation and tissue injury in the myocardium.121 The inflammatory response promotes the recruitment of leukocytes and plasma proteins to the heart tissue.122 These constituents, in turn, contribute to a transient decline in function of the tissue, alter homeostasis, and may hasten the progression of disease to its sequelae, dilated cardiomyopathy (DCM) and congestive heart failure. We still do not understand the mechanisms that underlie prolonged inflammation and remodeling after a bout of acute myocarditis. However, recent discoveries in immunology and advances in experimental methods are bringing us closer to understanding the precise mechanisms involved in the transition from acute myocarditis to chronic manifestations, DCM, and heart failure.

Inflammation Is a Response to Microbial Damage in the Heart

Observations from several studies have provided compelling evidence that multiple stimuli, cardiotoxic viruses, and bacteria such as staphylococcus, streptococcus and diphtheria are among the most common causes of myocardial inflammation. Viruses, particularly coxsackievirus B3 (CVB3) can directly, through lysis of infected cardiomyocytes, and indirectly,123–127 by attraction of inflammatory cells through dead-cell debris and virus/microbial turnover, can cause myocarditis. Even protozoa and worms have been shown to cause myocarditis,128 perhaps through similar mechanisms that detect the cell debris and microbial turnover that are the hallmarks of infectious disease. In terms of enteroviral myocarditis, there is most frequently complete recovery from a transient inflammatory syndrome however some patients progress to DCM and congestive heart failure.129 This experience raises a question—why do only ~30% of patients diagnosed with myocarditis progress to develop DCM and heart failure? Hypothetically, unraveling the molecular mechanisms of the disease will allow us to understand the basis for susceptibility to chronic inflammation and propose an explanation for the different phenotypic manifestations of myocarditis,130 leading to heart failure.

Murine Model of Myocarditis Has Provided Significant Insight Into the Infectious and Inflammatory Queues Required to Induce Immune Infiltration and Acute Fulminant Myocarditis

In fact, even before intraorgan inflammation takes hold, significant morbidity and mortality are often seen as a direct result of virus infection alone, just 3 to 4 days postinfection.131–134 Significant cardiomyocyte death is observed at these early time points postinfection, concomitant with strong positivity for active virus replication; the immune-deficient C3H/HeSnJ SCID mouse model develops overwhelming myocardial damage and death tends to occur within 2 weeks of infection, in the absence of significant inflammation.132 Thus, virus infection and replication alone do significant damage in the absence of inflammation. Any process that results in tissue necrosis will release cellular debris, which in itself is very immunogenic.

Fulminant acute myocarditis precedes a chronic syndrome of inflammation and prolonged tissue remodeling. These events lead to weakening of the heart muscle that contributes to DCM. Here we discuss the immune cell types and potential mechanisms involved in acute and chronic myocarditis and the onset of DCM.

Immune Cells Infiltrate During Myocarditis

Tissue insults/injurious stimuli induce release of cytokines and chemokines and dead cell debris, attracting T cells and phagocytes due to pattern recognition receptor activation (Figure 3).135 Natural “killer-like” cells were demonstrated as the first wave of infiltrating immune cells within the first 5 days of experimental viral myocarditis in the murine model (Table 1).136 The proportion of these perforin-positive cells as a fraction of the total was reported as approximately 14% on day 5 of CVB3 infection, when massive cell infiltration begins to appear. This crescendo is followed by a gradual decrease in inflammation and a “second phase” of immune cell infiltration that has been proposed to consist of, primarily, T cells (Table 1).136 Infiltrating cell types in the heart are predominantly helper and cytotoxic T cells, in human cases of myocarditis137 and also by day 11 postinfection in the mouse model of CVB3 myocarditis.138
Autoimmunity May Play a Role in Chronic Myocarditis

If inflammation in the heart remains after a bout of acute myocarditis, the result is persistent remodeling and matrix turnover leading to pump dysfunction. Finer characterization of the major histocompatibility genes expressed during myocarditis in the murine model138 and human cases137,139 has suggested a dominant host epitope that is recognized during chronic myocarditis. The restriction of T-cell receptor V alpha as well as V beta genes suggests that a specific antigen in the heart is targeted in human myocarditis patients137 and in the murine model of CVB3-induced myocarditis.138 Some potential “specific antigens” have been implicated as dominant autoimmune targets during viral myocarditis. Immunization of susceptible strains of mice with cardiac myosin or cardiac protein C can induce “postinfection” myocarditis.128,140 Complementary studies have shown that streptococcus M protein with immunologic similarity to cardiac myosin can induce an autoimmune myocarditis in mice as well.141 Several autoantibodies against autoantigens have also been identified in DCM patients, supporting the “infection-immune” hypothesis derived from murine studies.128

Damage Done by Virus Replication Through Direct Killing of Infected Cardiomyocytes Has Been Established But the Relative Contributions of the Different CD4 and CD8 Immune-Cell Subsets to Myocardial Damage Remain Elusive

For example, experimental deletion of the different T-cell subsets has not provided clear answers as to the apportioned roles of immune cells during myocarditis. Whereas deletion of CD4+ T-cell subsets was effective at reducing early death in the murine model, it also enhanced the severity of myocarditis.133 The deletion of CD8 T-cells in this study decreased the severity of myocarditis and early death yet this model suffered from greatly enhanced viral loads. Thus, investigation of the classic T-cell subsets has clearly not provided answers to explain chronic myocarditis and continued matrix turnover.

T-Helper 17 Cells Play a Role in Chronic Inflammation

The discovery of new facets of the immune system has recently provided much needed insight into the pathobiology of inflammation during myocarditis. The recently identified Th17 subset142 has been implicated in the onset of chronic myocarditis.143 These cells secrete high levels of IL-17 and have been implicated in autoantibody production.142,144 Research conducted on the Th17 subset in the context of CVB3 myocarditis suggests that these cells may contribute to chronic myocarditis through persistent inflammatory signals provided by secretion of IL-17. Consistent with this hypothesis there is significant Th17 expansion approximately 2 weeks after CVB3 infection in the murine model. Enhanced IL-17 and Th17 specific transcription factor, RORyt, were also increased consistent with expansion of this cell type.143 Interleukin-17 and its various isotopes can induce expression of TNF-α and Th2 responses, which in turn induce a prolonged inflammatory environment that might foster the production of auto-antibodies. Though autoimmune mechanisms of damage to the heart during chronic myocarditis may seem to be exclusive of the initial infectious insult, there is reason to believe that virus infection and inflammation are more interdependent than previously thought. We will now discuss this intimate relationship between virus replication and inflammation.

T Regulatory Cells May Moderate Myocarditis

Not all T cells are proinflammatory. T regulatory (Treg) cells act to moderate the strength of a response, preventing autoimmunity through secretion of cytokines like IL-10 and tumor growth factor (TGF)-β.145 These cells express CD4 but they also express the α subunit of the IL-2 receptor, CD25. They are also high expresses of the transcription factor FoxP3. In a study reported by Li et al allografted M2 (anti-inflammatory) macrophages led to improvement of virus-induced myocarditis,146 which was associated with enhanced levels of Treg. Huber et al have also reported decreased viral load and immune infiltration after adoptive transfer of a CD4+/CD25+ regulatory-like T-cell population into a mouse model of CVB3 infection.147

In a study by Shi et al,148 Treg cells were adoptively transferred into CVB3 infected mice and the effect of the transferred cells on viral load and signaling within the heart were investigated. Decreased immune infiltration and enhanced Akt activation, as compared to the naïve CD4 T cell and PBS grafted controls, were notable in the Treg adopted mice. Perhaps the most surprising of the results was the decreased viral load, not only in the heart but also in the pancreas and spleen, associated with lower expression of the coxsackie-adenovirus receptor (CAR). The authors adoptively transferred, not only Treg cells, but CD25(−/−)CD4 T cells (naïve) as well, into a separate group of mice. In fact, the naïve CD4 T-cell treated control group fared worse than either the PBS or Treg-treated groups, related to reduced Akt activation and higher CAR receptor expression. As a result, the naïve T-cell population fostered a more severe myocarditis phenotype due to higher viral loads. Taken altogether, Shi et al described the protective role that Treg cells play in immune infiltration of the heart during viral myocarditis, suggesting that there is a balance to be struck between clearance of infection and immune-associated damage to the myocardium. In fact, in this study there was an observed decreased viral load concomitant with a decrease in immune infiltration due to reduced TNF-α release and lower CAR expression. The authors found that though TNF-α could enhance CAR expression in mouse cardiomyocytes the coaddition of TGF-β eliminated TNF-α-induced CAR expression. To conclude, these findings suggest a more intimate link between inflammation and virus replication than previously posited. Thus, moderating the immune response may be critical for prevention of chronic virus replication.

The alteration in signaling evoked by Treg is most likely also playing a significant role in modulating virus replication. It is entirely possible that the signaling environment altered by the adoptive transfer of Treg may have also contributed to a less favorable environment for virus replication. The authors10 reported activation of Akt in the
myocardium of the Treg-treated groups, which suggests that the activation of other signaling proteins may have been altered by Treg transfer. We have reported that Akt activation is required for successful CVB3 replication, a prosurvival protein required by the virus to optimize the longevity of the infected cell to promote optimal progeny virus production. On the other hand, the Akt activation reported by Shi et al may merely be a reflection of a healthier environment created by adopted Treg cells in the myocardium. It is quite clear that an unchecked, immunologically active environment supports enhanced cellular signaling driven by viruses for successful replication. For example, we have previously reported that the powerful immune-stimulating protein p38 is required for effective virus replication in a CVB3 myocarditis mouse model. The activation of p38 MAP kinase was not investigated by Shi et al, but we would predict less net activation of p38 in the presence of Treg, and thus an environment that is less conducive to virus replication. All of these examples point to a scenario where the inflammatory signaling environment can influence microbial replication.

**Virus Replication and Inflammation Are Caught in a Synergistic Loop**
Myocarditis is caused by viruses that rely on stimulatory signals influenced by inflammation. Activation of proinflammatory proteins such as p38 MAP kinase, Akt, IKK-α, NFκB, and IL-1β are required by CVB3, in particular, for successful replication in the host cell. Therefore a highly inflammatory and reactive environment will paradoxically foster provirus replication signals while attempting to clear the parasitic invasion. Support of this hypothesis is provided by a recent systems biology publication by Garmaroudi et al where they showed that IKK-α is a hub signaling molecule activated throughout the life-cycle of CVB3 in cardiomyocytes. Consistent with the intimate connection of TNF-α and IL-1β signaling with NFκB and IKK-α activation, the authors showed that replication of CVB3 could be abrogated by antagonizing IL-1β and TNF-α signaling in infected cells. These results suggest that proinflammatory signals provided by TNF-α and IL-1β will boost proviral signaling in infected tissue. Just as Shi et al showed that TNF signaling could enhance virus entry receptor levels the results by Garmaroudi et al show an intracellular role for TNF-α signaling in promoting CVB3 replication.

**It Is Becoming Clear That the Role of Inflammation During Acute Myocarditis Leads to Clearance of Cardiac Infection, Leading to Subsequent Resolution of the Myocarditis Itself**
However, prolonged, less-specific, and uncontrolled inflammation leads to chronic myocarditis and heart failure. Study of the virology of viral myocarditis has shown that the very signals that promote inflammation are the ones that also drive virus replication. Recent findings of the moderating role of regulatory T cells provide researchers with new therapeutic targets for encouraging the arm of the immune response that moderates inflammation and fosters beneficial immunity, meanwhile suppressing viral turnover. Similarly, therapeutic inhibition of Th17 cells may prove beneficial to the patient in preventing autoimmunity, and possibly as a diagnostic marker of prognosis during myocarditis. Many questions remain with regard to the complicated roles played by inflammatory cells, but recent advances in immunology continue to provide new information and new therapeutic targets.

**Cardiac Allograft Rejection and Inflammation**
Currently, cardiac allograft transplantation is the therapy of choice for patients with end-stage heart conditions. However, despite the advancements in immunosuppression, acute cardiac allograft rejection episodes are not infrequent during the early post-transplantation period (Figure 4).

**Acute Cellular Rejection Involves Multiple Immunologic Mechanisms**
The diagnosis of acute cardiac rejection relies largely on histological examination of the myocardium derived from endomyocardial biopsy (EMB) and is graded using the ISHLT standardized guidelines. Histologically, treatable acute cellular rejection, ie, ISHLT grading 2R or above, is typically characterized by the presence of inflammatory infiltrates with associated myocyte damage. The cellular infiltrates are generally comprised of lymphocytes, macrophages, and occasionally eosinophils.

Mechanistically, acute cardiac rejection is thought to involve both cellular and humoral processes. After transplantation, donor and recipient-derived antigen presenting cells (APCs; eg, dendritic cells) can trigger direct and indirect allorecognition, respectively. In direct allorecognition, the intact foreign donor major histocompatibility complex (MHC) and peptides presented on the surface of donor APCs are recognized by recipient T cells. Specifically, the donor organ-derived APCs can migrate from the allograft to the recipient’s lymphoid tissues, where they activate, through the direct pathway, CD4+ and CD8+ T cells. On the other hand, in indirect allorecognition, the recipient APCs first uptake and process the donor MHC antigens, before presenting the donor-derived allopeptides to recipient T cells. Although both direct and indirect pathways are activated as part of the alloimmune response post-transplant, it is thought that the direct pathway is primarily responsible for initiating the acute cellular rejection process, whereas the indirect pathway has been linked more so to the development of chronic rejection.

After the initiating allorecognition event, activated T cells can undergo clonal expansion, as well as produce cytokines, eg, IL-2, IL-4, IL-5, IFN-γ, and chemokines, thus creating an inflammatory milieu that further promotes the recruitment and activation of additional immune cells, eg, macrophages and eosinophils. Together, these events result in inflammation and immune cell infiltration in the myocardium, as observed in the EMBs taken during acute rejection. Insults to the local myocardial tissue, through mechanisms such as cytotoxic T-cell–mediated direct lysis and complement cascade activation and B-cell alloantibody production,
Immune Cells Play Important Roles in the Myocardial Inflammation of Acute Rejection

Allowing for the close interplay between (innate and adaptive) alloimmune responses and myocardial inflammation in the cardiac allograft, recent studies have continued to examine the role of specific immune cellular subtypes in acute cellular rejection (Table 1).

T Cells

The CD8+ cytotoxic T cell (CTL) population is considered a key effector in cardiac allograft rejection through CTL-mediated cytolysis, involving effector molecules such as perforin and granzyme. In minor histocompatibility antigen mismatched animal models, depletion of CD8+ T cells has been shown to prevent the onset of acute cardiac rejection. Although CD4+ T (helper) cells are also recognized as an important component of acute cellular rejection through inflammatory cytokine release and their interactions with CD8+ T cells and B cells, they are generally not associated with perforin- and granzyme-mediated pathways. However, recent in vivo work has indicated that CD4+ T cells alone are sufficient to trigger cell-mediated acute cardiac rejection, and that this process may be mediated through pathways dependent on donor alloantigen presentation, cytokine production, and regulation of T-cell-mediated inflammation. Th1 cells, which produce IFN-γ and can help activate the macrophages, and Th2 cells, which can produce IL-4, IL-5, IL-10, IL-13, and IL-25 and mediate B cell antibody production, Th17 cells are considered distinct from the Th1 and Th2 population in part due to their ability to produce IL-17, as well as IL-21 and IL-22. Although there are other cells (eg, some CD8+ cells) that can also produce IL-17, an interleukin with proinflammatory properties, increasing evidence of a relationship between IL-17 and acute cardiac rejection has implicated Th17 cells as a potential mediator in the process.

B Cells

The precise roles of B cells and the alloantibodies (alloAbs) that they produce in a cardiac transplant setting remain to be elucidated. However, it is thought that B cells may contribute to myocardial inflammation and acute cardiac rejection through both alloAb-mediated and alloAb-independent (eg, antigen presentation, cytokine production, and regulation of T-cell-mediated inflammation) mechanisms. Nevertheless, evidence regarding the effects of B-cell depletion on acute cardiac rejection has been controversial, with some in vivo studies reporting a significant decrease in cellular immunity and inflammation, and others finding minimal or no impact on the transplant outcome.
Natural Killer Cells and Macrophages
As described earlier, EMs demonstrating myocardial inflammation and acute rejection typically contain cellular infiltrates which are predominantly lymphocytes, along with macrophages. Involvement of both natural killer (NK) cells, a class of cytotoxic lymphocytes, and macrophages during early phases posttransplant, as part of the nonadaptive/innate immune response, has been demonstrated in in vivo studies. In a rat heart transplant model, infiltration of recipient NK cells and macrophages can be observed as early as 3 hours posttransplant in the myocardial interstitial regions and allograft vessel endothelium; at 3 days post-transplant, a maximum number of NK cells and macrophage were found in the endo-, epi-, and myocardial portions of the wall of the transplanted heart. This is typically followed by the infiltration of T- and B-cell populations in the endo- and subendomyocardial walls.

In CD28 mice heart transplant models (deficient of costimulatory signal; thought to be dependent on CD8+ T cell and NK cells-mediated effects to cause rejection), blockade of a NK cell-activating receptor (NKG2D) using anti-NKG2D monoclonal Ab prevented the occurrence of acute cardiac rejection. Based on this observation, it has been suggested that NK cells may mediate CD8+ T-cell proliferation, possibly via cytokine secretion or dendritic cell (DC) maturation promotion, and thereby contribute to the myocardial inflammation process, leading to acute cellular rejection.

Complement and (Allo)Antibodies Participate in Rejection-Related Inflammation
It is generally well accepted that brain death, as well as ischemia/reperfusion (IR) as part of the organ procurement/transplant process, favor a proinflammatory condition. In both settings, activation of the complement system cascade can occur (Figure 4). There are 3 major pathways of complement activation: classical, alternative, and mannose-binding lectin (MBL)-dependent pathways. The classical pathway is thought to be triggered by binding of immunoglobulin IgM and IgG (IgM; and certain classes of immunoglobulin G [IgG]) to the foreign antigens), whereas the alternative and MBL-dependent pathways involve recognition of foreign surfaces and binding of MBL to foreign microbes, respectively. Despite the difference in the initiating factors, all 3 pathways share the central process of complement component C3 activation, which can lead to the generation of (i) C3a and C5a, which can act as inflammatory mediators, (ii) C3b can mediate the opsonisation and engulfment of foreign material by phagocytes, and ultimately, (iii) terminal complement components that together form the C5b-9 membrane attack complex can trigger subsequent damage and destruction of the targeted (foreign) cells, eg, myocytes of the cardiac allograft.

Brain Death and IR Injury Activate Complement in Cardiac Allografts
In mouse models, brain death has been shown to induce significant elevation in serum complement factor C3a (a complement activation product), as well as increased cellular infiltration and complement component deposition in the myocardium of the donor cardiac allograft. This is in contrast to C3 mice after brain death, which demonstrated less injury of the myocardium, less myocardial infiltration of immune cells, eg, neutrophils, reduced adhesion molecule expression on the cardiac endothelial cells, and reduced levels of circulating proinflammatory cytokines, eg, TNF-α and IL-1β.

In mouse models of IR injury, both IgM and MBL have been shown as important mediators of myocardial IR-induced inflammation and complement activation (possibly via the classical and MBL-dependent pathways), ultimately leading to myocardial tissue damage. In another study, inhibition of the MBL via antibodies was shown to repress the severity of myocardial IR injury. It is important to note that although MBL is traditionally known for its ability to bind to specific carbohydrates on microbes, it has also been shown to bind to IgM and IgG antibodies, which further suggests its involvement in antibody (and complement)-mediated tissue injury and alloantibody-mediated cardiac allograft rejection.

Complement component C4 is often considered as a key mediator shared between the classical and MBL-dependent pathways critical in the antibody-mediated complement activation process. As such, C4 days, a complement fragment released during classical complement pathway activation, has gained increasing attention as a potential marker of antibody-mediated rejection (AMR). Examination of EMs from human cardiac transplant recipients has found that positive staining of C4 days in the myocardial capillaries was significantly and positively correlated with the presence of donor-specific, serum alloantibodies. A more recent study has also shown that immunofluorescence staining of C4 days, together with C4 days, can strongly and positively correlate with the presence of donor-specific alloantibodies, as well as allograft dysfunction in transplant recipients. Clinically, the presence and accumulation of C4 days in the allograft vasculature has been adopted as a key criteria to support the diagnosis of AMR.

Interactions Between Complement, Immune Cells, and Alloantibodies Are Complex
During the (direct and indirect) allore cognition processes posttransplant, activated immune cells, eg, T cells, B cells, macrophages, and dendritic cells (DC), can all interact dynamically with the complement system. APCs such as macrophages and DCs have receptors that can bind to specific components from all three pathways of complement activation (classical, alternative, and MBL-dependent), as well as Fc segments of antibodies. Furthermore, both macrophages and DCs are capable of producing complement components critical in the classical and alternative pathways, thus allowing them to activate and regulate complement at local sites of inflammation.

The presence of biologically active complement fragments can also modulate macrophage and DCs' abilities to clear apoptotic cells, as well as helping to facilitate T-cell and B-cell responses, effects that can have significant impact on the progression of myocardial inflammation. It is thought that...
the availability of complement may affect the alloreactivity of T cells. Complement component C3 deficient donor-derived macrophages and DCs are less capable of stimulating T cells in vitro; however, the exact pathway linking C3 receptor binding and T-cell alloreactivity remains unclear.\cite{182,189,190} Interestingly, T cells have also been shown to produce C3 in vitro after alloantigen stimulation.\cite{182}

Complement has also been shown to affect the antibody production and antigen presentation function of B cells. As described earlier, complement can be activated through the classical pathway, when secreted (allo)antibodies or membrane-bound immunoglobulins (IgM and certain class of IgG) on B cells bind to foreign antigens.\cite{182,191} C3 days, a complement activation product of C3, can bind to CR2 receptor present on B cell surfaces.\cite{182,192} This can lead to the crosslinking between membrane IgM, CR2 and CD19, which is thought to enhance the B cell-mediated activation of resting alloreactive T cells.\cite{182,192} Coincidentally, days are also been shown in vitro to regulate B-cell production of antibodies.\cite{182,193}

**It Has Been Estimated That AMR Occurs in up to 15% of Heart Transplant Recipients**\textsuperscript{159}

Once transplantation has taken place, the presence of recipient’s donor-specific alloantibodies can activate complements, interact with the aforementioned inflammatory infiltrates via Fc receptor and trigger additional complement production.\cite{181,182}

Currently, the precise role of alloantibodies and their contribution to acute cardiac rejection remains controversial. There have been reports of both pro- and anti-inflammatory effects of complement induced by antibodies, reviewed elsewhere.\cite{182} Moreover, the relative importance of complement-activating and noncomplement activating alloantibodies in cardiac allograft rejection has not been fully elucidated. Previous studies using a mouse model of heart transplant, with immunoglobulin knockout mice as the recipient, has shown that transfer of complement activating antibodies (IgG2b) can trigger significant cardiac allograft rejection and reduce allograft survival time in a dose-dependent manner, whereas transfer of noncomplement activating antibodies alone had minimal impact.\cite{181,194} Interestingly, transfer of a combination of both complement- and noncomplement-activating alloantibodies to the immunoglobulin knockout recipient mice increased the deposition of C4 days in the allograft (relative to either alloantibodies alone), and induced an accelerated severe cardiac allograft rejection, leading the authors to conclude that there may be a synergistic effect between the 2 types of alloantibodies in the context of AMR/graft rejection.\cite{194}

**In Addition to Complement- and Allo-Antibody–Mediated Pathways, Granzyme and Perforin Effector Pathways are also Considered Major Contributors to Myocardial Inflammation in the Context of Cardiac Rejection**\textsuperscript{165}

Cellular infiltrates observed in EMBs of cardiac recipients undergoing acute cardiac allograft rejection include lymphocytes (primarily T cells and some NK cells), as well as macrophages.\textsuperscript{160,178} Granzyme and perforin are considered key effector molecules of cytotoxic T cells and NK cells. Specifically, granzyme and perforin are critical components of cytotoxic granules, which are exocytosed by T cells and NK cells into the immune-target cell synapse on recognition of foreign cells.\textsuperscript{165} This process can cause inflammation and damage to the allograft through several mechanisms. Perforin released by the immune cells can form plasma membrane pores on cells recognized as foreign and help facilitate the entry of granzymes into cytoplasm of the targeted cells, which can in turn activate serine protease-mediated pathways and trigger necrosis and apoptosis of allograft cells, eg, endothelial and parenchymal cells.\textsuperscript{165,195} The formation of plasma membrane pores can also cause dysregulation of ion concentrations inside the cell, leading to effects such as contractility dysfunction of allograft cardiomyocytes.\textsuperscript{165} In the context of allograft transplantation, Granzyme B (and to some extent, Granzyme A) are considered major effector molecules involved in allograft cell deaths.\textsuperscript{165} Granzyme A is also thought to contribute to the myocardial inflammation process by inducing monocyte production of IL-1β, which may trigger increased cell surface adhesion molecule expression of endothelial cells and their release of additional proinflammatory cytokines and chemokines.\textsuperscript{165,196}

In vitro, both perforin and granzyme (ie, granzyme B) have been shown to induce death of cardiomyocytes and endothelial cells.\textsuperscript{165} However, activation of the perforin and granzyme pathways have not been shown as a required component for induction of acute cardiac allograft rejection in certain animal models in vivo.\textsuperscript{165} Specifically, in studies that used complete MHC-mismatched or minor histocompatibility antigen mismatched models of cardiac transplantation, in the absence of immunosuppression, perforin, or granzyme B knockout recipients showed no significant improvement in terms of allograft survival or the severity of cellular infiltrate in the allograft as compared to the wild-type recipients.\textsuperscript{165,197,198} This is attributed to the fact that in these antigen mismatch animal models, in the absence of immunosuppression, antibody-mediated rejection tends to occur at a much higher rate as compared to that seen clinically in human cardiac transplants.\textsuperscript{165} Thus, removal of important components of the cellular rejection process, eg, T-cell and NK-cell perforin/granzyme effector pathways, may not be sufficient to suppress the alloantibodies- and complement-mediated inflammation process seen in cardiac rejection to improve transplant outcome. Such further highlights the diversity of mechanisms involved in myocardial inflammation in the context of acute cardiac rejection.

**Beyond the Classical Components of Acute Cellular and Antibody-Mediated Rejections, Several Other Molecules Have Also Been Linked Closely to the Inflammation Process Post-Transplant**

For instance, the inflammasome, a multiprotein complex that contains the adaptor protein apoptosis-associated, speck-like protein containing a caspase recruitment domain, is thought to regulate the production of proinflammatory cytokines.
IL-1β and IL-18 in a caspase-1-mediated manner. A recent study using murine models has reported upregulation of apoptosis-associated, speck-like protein containing a caspase recruitment domain and IL-1β with associated myocardial inflammation in cardiac allografts posttransplantation, suggesting the potential importance of the inflammasome in cardiac allograft rejection. Various growth factors, eg, vascular endothelial growth factor, which has been traditionally recognized for angiogenic effects has also been linked to inflammation regulation in the context of cardiac allograft rejection. Increased vascular endothelial growth factor expression has been observed in cardiac allografts in relation to both acute and chronic rejection, as well as in regards to inflammatory cell recruitment and infiltration. Brain natriuretic peptide (BNP), a polypeptide released in the ventricles in response to increased pressure and stretching of cardiomyocytes, has been used as a marker to assess acute and chronic cardiac rejection. More recently, proteomic evidence generated using human samples has correlated proinflammatory cytokines with circulating BNP levels during acute cellular cardiac rejection. However, there remains a lack of evidence that directly implicates BNP upregulation as a contributor in the acute rejection process.

Given the significance of cellular infiltrates in the context of myocardial inflammation, numerous studies have examined pathways closely linked to immune cell functions in order to better define the relationship between specific signaling molecules/enzyme targets such as CCR, COX-2, CXCR, and TLR, and the effects of their inhibition in the context cardiac transplantation, ie, IR injury and cardiac rejection. A selection of these pertinent studies is summarized in Online Supplemental Table I, available at http://circres.ahajournals.org.

Cardiac Allograft Vasculopathy as an Expression of Chronic Cardiac Allograft Rejection Has Been Described as a Low-Grade, Chronic Inflammation Condition

The occurrence of acute cardiac rejection has been linked to increased incidence of cardiac allograft vasculopathy (CAV) development. Importantly, it is thought that the inflammatory events that occur during the acute rejection process, such as those described earlier (eg, immune cell infiltration, cytokine and chemokine production, complement and antibody deposition, endothelial cell and smooth muscle activation), can all contribute to the subsequent dysfunction of the vasculature. This is typically followed by an injury and repair mechanism that is central to the intimal thickening and vascular occlusion processes seen in CAV. The pathogenesis and pathophysiology of CAV have been reviewed elsewhere.

Summative Perspective on Roles and Mechanisms of Myocardial Inflammation

Beneficial and Detrimental Roles of Inflammation in the Heart

In this review we have discussed the arms of the immune system that mediate resolution or pathology during, ischemic-reperfusion injury, sepsis, viral myocarditis, and cardiac allograft responses. Although these disease settings may seem superficially disparate, there are common themes in health-supporting versus detrimental inflammation in the cardiac muscle. Inflammation in response to an allograft is a reaction to an unnatural process, but it has provided great insights into detrimental inflammation, in general. Such inflammation not only impairs the myocardium, but also contributes to a greatly accelerated form of atherosclerosis, termed allograft vasculopathy. The genesis of such vasculopathy is highly similar to that observed in atherosclerosis, the latter resulting in ischemia-reperfusion injury. Although cardiogenic shock during sepsis is on first-face seemingly very different from the other forms of inflammation addressed in this review, in that there is no direct damage to the heart muscle during the process, there are common innate immune pathways activated that result in reduced function of cardiomyocytes.

NF-κB: A Common Perpetrator?

A particularly dominant pathway that has emerged in all forms of myocardial inflammation covered in this review is NF-κB. The finding that IκB-α is a hub signaling molecule during viral myocarditis and the beneficial roles of Treg to moderate immune infiltration of the myocardium points to NFκB as a legitimate target in the myocarditis arena as well. Damage signaling and recognition of the presence of a pathogen is necessary for appropriate immune clearance, and NFκB activity is required to mediate these responses. Without completely inhibiting NFκB, there may be a therapeutic opportunity to at least moderate NFκB, and associated activity, through fine tuning of the NFκB response with a drug that dampens as opposed to obliterates NFκB activity. Perhaps important is the serendipitous observation that the Treg transcription factor FoxP3 acts to suppress NFκB activation, intrinsically suppressing the production of proinflammatory cytokines by Treg. As we noted earlier, Treg plays a moderating and beneficial role during both myocarditis and allograft rejection. Most likely, the largest obstacle will be in determining the tempo and dose of administration of a drug that could be used to modify the processes we have discussed, treating early to prevent cardiomyocyte damage or complete system failure due to cardiogenic shock. By comparing the immune and inflammatory responses during several different settings of cardiac threat or injury, we may identify common targets for common treatments of a disparate spectrum of immune related cardiac diseases. This said, the recognition of “prolife” roles for NF-κB in regards to the survival of ventricular myocytes reflects a complexity yet to be resolved.

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