Cardiac hypertrophy and remodeling are pathological features of many cardiac diseases, with underlying causes including hypertension, cardiomyopathy, valvular dysfunction, and myocardial infarction. In these diseases, ventricular hypertrophy occurs in response to pathological stimuli such as pressure and volume overload, sarcomere gene mutations, and neurohumoral activation, and a major consequence of prolonged and uncontrolled hypertrophic remodeling is cardiac dysfunction, which can lead to heart failure or cardiac arrest resulting from arrhythmia.

Despite the various pathological stimuli, there are many common features in the hypertrophic response in different cardiac diseases. In addition to increased cardiomyocyte mass, sarcomere rearrangement, and extracellular matrix deposition, other common features have recently been appreciated, including inflammatory signaling and immune cell activation. Numerous cell types are involved in orchestrating this complex pathological response. The heart consists of a heterogeneous population of cells, including cardiomyocytes and noncardiomyocytes, and it is now clear that intercellular signaling and communication between these cell types are critical in the pathophysiology of ventricular hypertrophy and remodeling (Figure 1).

Noncardiomyocytes display phenotypic changes during the development of cardiac hypertrophy. There is still much to be revealed about the specific roles of these cell types and their overall contribution to the hypertrophic response. Inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and transforming growth factor-β (TGF-β) and neurohumoral factors such as angiotensin II and aldosterone are involved in the pathophysiology and correlate with disease progression, but the cell type–specific targets and their effects on the cardiomyocyte in vivo are not well understood. The influence of both resident and infiltrating immune cells during myocardial infarction and postinfarction remodeling is well recognized. Recently, it has been shown that myeloid cell phenotypes play a critical role in ventricular hypertrophy and remodeling. In addition, there is a small body of literature examining specific immune cell interactions in other models of ventricular hypertrophy such as pressure overload. Although the early phases of myocardial infarction are dissimilar to the pathophysiology of progressive, chronic hypertrophy, studies focusing on the later phase of postinfarct hypertrophic remodeling may provide some insight into potential cellular mechanisms and therapeutic targets.

In this review, we summarize the current understanding of the role of noncardiomyocytes in the pathogenesis of cardiac hypertrophy, placing particular emphasis on relevant immune cell interactions and inflammatory signaling mechanisms. We highlight seminal findings demonstrating the importance of specific cell types in regulating the cardiomyocyte hypertrophic response, and we emphasize the relevant current and potential therapeutic targets. It is clear that this field is not fully developed and deserves increased attention.

### Renin-Angiotensin-Aldosterone System and TGF-β Signaling in the Hypertrophic Heart

Activation of the renin-angiotensin-alderosterone system (RAAS) has direct hypertensive effects that contribute to cardiac hypertrophy and remodeling, and these effects can be blocked by RAAS inhibition with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and mineralocorticoid receptor (MR) antagonists. RAAS inhibitors are widely used in the treatment of heart failure and significantly reduce morbidity and mortality; however, it is now well established that these protective effects extend beyond simply reducing blood pressure. Angiotensin II and aldosterone promote vascular and cardiac fibrosis and hypertrophy independently of blood pressure, and these signaling pathways have been shown to have pathogenic effects involving numerous cell types, including cardiomyocytes and immune cells.

The role of angiotensin II in both normal and pathological contexts is very complex. Cardiomyocytes express both angiotensin II type 1 and 2 receptors, and both appear to have an important but opposite role in maladaptive remodeling. In bone marrow–derived cells, angiotensin II type I receptors are involved in angiotensin II–induced hypertensive responses, and angiotensin II type 1 receptor has also been shown to regulate the mobilization of monocyte progenitor cells. Angiotensin II type 1 receptor responses may also be critical in regulating lymphocyte hypertensive responses. Our understanding of the many cell–specific effects is still undeveloped, but it is clear that localized, nonclassic RAAS activation is an important target for RAAS inhibitors and a potential mechanism for their beneficial effects.
In the past decade, there has been increasing interest in the direct role of MR activation during pathological remodeling. In severe heart failure, clinical trials have demonstrated that MR antagonism provides significant benefit independently of blood pressure lowering.6 MR is expressed in a wide range of cells, and the use of cell-specific knockout technology has now delineated some of the cell-specific effects. Both cardiomyocyte MR and myeloid MR have now been shown to directly influence cardiac remodeling.2,7,8

TGF-β is upregulated in the hypertrophied and fibrotic heart and is regarded as one of the major profibrotic cytokines and critical mediators of cardiac fibrosis. TGF-β has many pleiotropic effects in modulating cardiomyocyte and noncardiomyocyte function, and it induces cardiomyocyte hypertrophy and fibroblast proliferation and fibrosis. Inhibition of TGF-β signaling and genetic ablation of TGF-β have been shown to reduce fibrosis and to prevent cardiac dysfunction in several models of maladaptive cardiac remodeling,9,10 whereas TGF-β overexpression has been shown to induce cardiac hypertrophy.11

In pressure overload–induced cardiac remodeling, Koitabashi et al12 found that TGF-β–neutralizing antibody reduced myocardial fibrosis without affecting hypertrophy or cardiac function. They further revealed that cardiomyocyte-specific (Myh6-Cre) knockout of TGF-β receptor type II, but not type I, significantly reduced hypertrophy and fibrosis and prevented cardiac dysfunction through a TGF-β–activated kinase 1 signaling pathway. In fact, TGF-β–activated kinase 1 activation is known to induce cardiac hypertrophy; therefore, the TGF-β–TGF-β–activated kinase 1 signaling pathway may be useful for therapeutic targeting.13 However, different TGF-β signaling pathways are context dependent because knockout of both cardiomyocyte TGF-β receptors 1 and 2 significantly ameliorated postinfarct cardiac remodeling.14 Global blockade of TGF-β with neutralizing antibody, on the other hand, resulted in complete mortality within 5 days, suggesting that TGF-β has many diverse effects in specific cell types. In addition to highlighting the importance of specific TGF-β signaling mechanisms, this may indicate that TGF-β is necessary in other target cells for proper regulation of adaptive remodeling.

Inflammatory Signaling in Hypertrophic Remodeling

Inflammatory signaling molecules released during cardiac injury and hypertrophic remodeling can induce hypertrophic and fibrotic responses. Both cardiomyocyte and noncardiomyocyte cells secrete and respond to numerous cytokines, but the responses are complex, depend on the cell type, and are mostly characterized in vitro (Figure 2).

In fibroblasts, major proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 inhibit proliferation, decrease matrix synthesis, and increase MMP activity. In cardiomyocytes, they induce hypertrophy and can cause apoptosis, and in immune cells, they promote inflammation.15–18 However, the cell sources of the cytokines have usually not been identified, and even the target cells of the cytokines have not been fully defined in vivo. The complexity is further increased by the temporal changes that occur in the injury and immune responses, leading to an incomplete understanding of these cytokines during disease.

In patients with heart failure, the concentration of the inflammatory cytokines TNF-α, IL-6, and IL-1β correlates with disease severity.19 Experimental models have shown that infusion with TNF-α induces cardiac dysfunction and similarly that cardiomyocyte-restricted overexpression of TNF-α induces cardiac hypertrophy and fibrosis and leads to cardiac dysfunction and dilated cardiomyopathy.20 In contrast, global knockout of TNF-α ameliorates pressure overload–induced cardiac hypertrophy, fibrosis, and cardiac dysfunction.22 Anti-TNF-α therapies are also beneficial in animal models, but to a lesser extent. Neutralization of TNF-α significantly blocked
TNF-α–induced cardiac inflammation without affecting hypertrophy and producing minor decreases in cardiac dysfunction.23 Although TNF-α causes cardiac dysfunction and TNF-α inhibition ameliorates cardiac dysfunction in animal models, clinical studies have demonstrated that TNF-α inhibition with etanercept and infliximab had no benefit in chronic heart failure.24,25

IL-1β–deficient mice have reduced pressure overload–induced hypertrophy and cardiac dysfunction, suggesting that IL-1β has an exacerbating role in hypertrophic remodeling.26 In agreement with this, IL-1β injections were shown to induce cardiac dysfunction in mice.27 During heart failure, early clinical studies indicate that blocking IL-1β signaling provides a significant health benefit. Patients who received the IL-1 receptor antagonist anakinra had increased oxygen consumption and exercise performance.27 These results are promising, although larger trials that assess cardiac function and remodeling are needed. In other pathologies, anakinra has been shown to reduce adverse cardiac remodeling. In patients with rheumatoid arthritis, treatment with anakinra reduced IL-6, C-reactive protein, and endothelin and significantly improved left ventricular function.28 Similarly, patients with acute myocardial infarction with ST-segment elevation who received anakinra had a reduction in C-reactive protein, and treatment blocked the progression to heart failure.29

IL-6 has many pleiotropic effects on cardiomyocytes and noncardiomyocytes, and IL-6 infusion alone has been shown to induce cardiac hypertrophy, fibrosis, and diastolic dysfunction.30 In support of having a detrimental role during cardiac hypertrophy, genetic deletion of IL-6 has been shown to ameliorate cardiac damage and to suppress angiotensin II–induced cardiac hypertrophy, fibrosis, and inflammation, as well as hypertrophic and fibrotic signaling.31,32 IL-6 knockout has also been shown to prevent norepinephrine-induced hypertrophy and remodeling.33 However, our understanding of the IL-6 signaling cascade remains incomplete. Knockout of IL-6 in other hypertrophic disease models such as pressure overload has no effect on hypertrophy or fibrosis, and deletion of GP130, part of the IL-6 receptor complex, actually increases mortality after pressure overload and impairs cardiac function, leading to dilated cardiomyopathy.34 It is clear that our understanding of the IL-6 signaling pathways is inadequate within the context of these different disease models and requires further exploration.

**Anti-Inflammatory Signaling**

Inactivation of proinflammatory responses has been shown to mitigate hypertrophic cardiac dysfunction and remodeling; therefore, it is not surprising that many studies have targeted anti-inflammatory signaling pathways as a therapeutic strategy. IL-10 is a major anti-inflammatory cytokine, and IL-10–deficient mice have increased angiotensin II–induced vascular inflammation and impaired vascular relaxation, indicating that IL-10 has a protective role in maintaining vascular function.35 Another study demonstrated that IL-10–deficient mice had increased cardiac hypertrophy and fibrosis and increased cardiac dysfunction in response to isoproterenol.36 During pressure overload, IL-10–deficient mice had increased perivascular fibrosis but no differences in cardiac hypertrophy or cardiac function.37 However, administration of IL-10 significantly reduced cardiac hypertrophy and fibrosis and preserved cardiac function in both pressure-overload and isoproterenol models of cardiac hypertrophy.38

Glucocorticoids have major anti-inflammatory and immunosuppressive effects and are among the most widely used and
most effective anti-inflammatory therapeutic agents. Despite their highly potent anti-inflammatory effects, their utility in cardiovascular diseases is limited by a lack of efficacy in the treatment of hypertrophic remodeling. However, it appears that glucocorticoid receptors may have an important role in normal cardiac development and function because cardiomyocyte-specific (Myh6-Cre) glucocorticoid receptor knockout causes cardiac hypertrophy and impaired cardiac function, leading to heart failure and premature death.38

**Immune Cells**

It is now evident that resident and recruited immune cells respond much earlier to cardiac injury than previously thought. These changes precede hypertrophy and remodeling and persist throughout much of the major maladaptive hypertrophic response, resulting in cardiac dysfunction and failure. Immune cells coordinate cardiomyocyte and noncardiomyocyte responses during maladaptive remodeling, and the regulation of immune cell phenotypes represents an important pharmacological approach. These cells have critical roles in not only cardiomyocyte function but also injury responses involving scar formation and interstitial fibrosis, which affect cardiac function.

**Cardiac Macrophages**

The heart contains a heterogeneous population of macrophages that are present in both healthy and injured cardiac tissue in both humans and mice.39 Similar to tissue macrophages in brain and liver, most macrophages in the heart are established embryonically from yolk sac and fetal liver progenitors, and resident macrophage subsets are maintained through local proliferation and, to a lesser extent, monocyte recruitment.40,41 This is consistent with other recent findings demonstrating that in the absence of disease, most tissue macrophage populations are maintained locally though self-renewal.42 In the presence of tissue injury, monocyte-derived macrophages are much more prevalent.

During cardiac injury, the phagocytic function of the macrophage is critical for clearance of necrotic debris and matrix remodeling. However, the function of cardiac macrophages is much more extensive than previously thought, and they have a much more substantial role in regulating cardiac hypertrophy and remodeling. During myocardial infarction or angiotensin II infusion, expansion of macrophage populations occurs through both local proliferation and monocyte recruitment.40 Consistent with a potential causal role, expansion of cardiac macrophage populations occurred as early as 2 days after angiotensin II infusion and before significant hypertrophy and fibrosis occur.40 This suggests that during progressive cardiac hypertrophy and remodeling, inflammatory changes are occurring very early and precede hypertrophic remodeling.

Epelman and colleagues40 recently identified 4 distinct macrophage subsets in the mouse heart that have unique functional roles. All characterized subsets were capable of phagocytizing antigen and cardiomyocytes, and MHC-IIα subsets were capable of antigen presentation and T-cell activation. During angiotensin II infusion, the CCR2+ CD11c+ subset was derived predominantly from circulating monocytes and had robust inflammasome activation and inflammatory gene expression. Angiotensin II–induced inflammasome activation and IL-1β production were blocked by C-C chemokine receptor 2 (CCR2) deficiency, suggesting that the CCR2+ subset has a more predominant role in coordinating inflammation. In contrast, other cardiac tissue macrophage subsets probably function as sentinel immune cells and have roles in tissue surveillance, phagocytosis of dying cardiomyocytes, response to cardiomyocyte signaling, and T-cell activation.

**Macrophage Depletion**

Inhibition or depletion of specific immune cell types has provided some insight into their roles in hypertrophic remodeling (Table 1). Macrophage depletion studies using clodronate liposomes or CD11b-DTR transgenic mice have been performed in a wide range of pathologies to block inflammation. Depletion of macrophages in a hypertensive model with Ren-2 rats suggests that macrophages are necessary for cardiac repair. Although no differences in cardiac hypertrophy were observed, depletion with clodronate liposomes resulted in cardiac dysfunction with decreased ejection fraction and fractional shortening and increased end-diastolic volume.43 Greater cardiomyocyte loss and abundant increases in CD4+ T cells were present in macrophage-depleted rats, suggesting that macrophages are important in coordinating T-cell responses.

During myocardial infarction, macrophage depletion also impairs postsinfarction remodeling and repair.44–46 Macrophage depletion during the early inflammatory phase resulted in increased necrotic debris and neutrophil presence, whereas depletion during the later remodeling phase prevented collagen deposition and granulation tissue formation. Because different subsets of cardiac macrophages have different functional roles and because depletion of macrophages with clodronate liposomes is nonselective and depletes all macrophage subsets and peripheral monocytes, it is understandable that macrophage depletion would prevent important reparative functions. In addition, timing of macrophage depletion and targeting of specific macrophage subsets may be critical in achieving effective amelioration of maladaptive remodeling.

**Inhibition of Monocyte Trafficking**

The CCR2+ macrophage subset is monocyte derived and is thought to be involved mainly in promoting and regulating inflammation. Therefore, targeting inflammatory monocytes might be an effective means to limit this macrophage subset. Recruitment of monocytes occurs largely through monocyte chemoattractant protein 1 (MCP1)–CCR2 signaling, and CCR2 knockout in bone marrow cells markedly reduces angiotensin II–induced vascular inflammation and fibrosis without affecting hypertrophy.47 Similarly, inhibition of MCP1 with neutralizing antibodies significantly reduces macrophage infiltration and prevents myocardial fibrosis in response to pressure overload.47 Although there were no differences in cardiac hypertrophy, MCP1 neutralization restored diastolic function.

MCP1 knockout is also protective against angiotensin II–induced hypertrophic remodeling. MCP1 knockout mice exhibited suppressed inflammatory cytokine production and
Reduced fibrosis during early time points, but by 6 weeks, the inflammatory and profibrotic changes normalized, and no differences in hypertrophy or cardiac function were present.48 This suggests that blocking monocytes by targeting the MCP1-CCR2 signaling pathway may be a useful strategy to reduce CCR2+ inflammatory macrophage subsets while maintaining other resident populations carrying out sentinel, phagocytic, and remodeling functions. Blocking this chemotactic pathway appears to have a greater impact on fibrotic remodeling and might have a more direct role in regulating fibroblast function. Because many of the targeting strategies are time dependent, a more thorough characterization of the functional phenotypes at various pathophysiological stages will be necessary.

Table 1. Depletion or Inhibition of Immune Cells During Cardiac Hypertrophy and Remodeling

<table>
<thead>
<tr>
<th>Cell Type/macrophage/phagocytic cells</th>
<th>Treatment</th>
<th>Model</th>
<th>Function</th>
<th>Hypertrophy</th>
<th>Fibrosis</th>
<th>Inflammation</th>
<th>BP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clodronate liposomes</td>
<td>Ren2 rats</td>
<td>↓</td>
<td>NE</td>
<td>↓Mp, ↓Mo, ↑Tl</td>
<td>NE</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clodronate liposomes</td>
<td>Ang II</td>
<td>↓</td>
<td>NE</td>
<td>↓Mp, ↑TNF-α, ↓TGF-β</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM-CCR2−/−</td>
<td>Ang II</td>
<td>NE</td>
<td>NE</td>
<td>↓Mp</td>
<td>NE</td>
<td>45, 46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-MCP1</td>
<td>AAC</td>
<td>↑</td>
<td>NE</td>
<td>↓Mp, ↑TGF-β</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP1−/−</td>
<td>Ang II</td>
<td>NE</td>
<td>NE</td>
<td>↓Mp</td>
<td>NE</td>
<td>44, 48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LysM-iDTR</td>
<td>Ang II</td>
<td>↑</td>
<td>(PV)</td>
<td>↓Mp, ↑Tl</td>
<td>NE</td>
<td>49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T lymphocyte</td>
<td>Anti-CD3</td>
<td>CM–TNF-α Tg</td>
<td>↓</td>
<td>↓T1, ↑CD11b-</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rag2−/−</td>
<td>TAC</td>
<td>↑</td>
<td>NE</td>
<td>↓Mp</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rag1−/−</td>
<td>TAC</td>
<td>NE</td>
<td>NE</td>
<td>↑(PV)</td>
<td>37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rag1−/−</td>
<td>Ang II</td>
<td>↑</td>
<td>(PV)</td>
<td>↓Mp</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8−/−, anti-CD8</td>
<td>Ang II</td>
<td>NE</td>
<td>↓TGF-α, ↓TGF-β</td>
<td>NE</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APCs</td>
<td>MHCII−/−</td>
<td>TAC</td>
<td>↑</td>
<td>↓Mp</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cells</td>
<td>Deficient, cKit mutant</td>
<td>AAC</td>
<td>↑</td>
<td>↓Mp</td>
<td>53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient, cKit mutant</td>
<td>CM–TNF-α Tg</td>
<td>↑</td>
<td>↓Mp</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient, cKit mutant</td>
<td>TAC, ↓AF</td>
<td>NE</td>
<td>↓Mp</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cell stabilizer</td>
<td>AV fistula</td>
<td>↑</td>
<td>↓Mp</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mast cell stabilizer</td>
<td>SHR</td>
<td>NE</td>
<td>NE</td>
<td>↑IL-10, ↑IL-6</td>
<td>NE</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocytes</td>
<td>ICAM-I−/−</td>
<td>AAC</td>
<td>NE</td>
<td>↓Mp, ↑TGF-β</td>
<td>NE</td>
<td>58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AAC indicates abdominal aortic constriction; AF, atrial fibrillation; Ang II, angiotensin II; APC, antigen-presenting cell; AV, arteriovenous; BM, bone marrow; BP, blood pressure; CM, cardiomyocyte; ICAM-I, intracellular adhesion molecule-1; IL, interleukin; MCP1, monocyte chemoattractant protein 1; Mo, monocyte; Mp, macrophage; NE, no effect; Np, neutrophil; PV, perivascular; SHR, spontaneously hypertensive rat; TAC, transverse aortic constriction; Tg, transgenic; TGF-β, transforming growth factor-β; Tl, T lymphocyte; and TNF-α, tumor necrosis factor-α.

In contrast to depleting or blocking macrophage responses, manipulation of the macrophage phenotypes may provide a novel way to prevent specific deleterious inflammatory effects while still allowing other critical phagocytic and reparative responses.

Macrophages are capable of integrating a wide array of environmental signals and can respond through unique activation programs. Although initially designated M1 (classic) and M2 (alternative) on the basis of activation by T helper cell type 1 (Th1)– and type 2 (Th2)–mediated cytokines, macrophage activation falls within a spectrum of classically activated macrophage and alternatively activated macrophage (AAM) phenotypes.

AAMs are thought to have beneficial, wound-healing effects in many cardiovascular diseases and are one of the major macrophage subsets in the healthy heart.60,67 During myocardial infarction, the predominance of different macrophage phenotypes is phase dependent. During the initially inflammatory phase, there is an increase in classically activated macrophages, whereas during the later remodeling phase, AAMs predominate.68 Little is known about the functional macrophage phenotypes during the development of pressure overload and angiotensin II–induced hypertrophic remodeling.

Different Macrophage Phenotypes and Polarization

Macrophages display a range of functionally heterogeneous phenotypes, and a major focus of research has been an understanding of the roles of different macrophage phenotypes during disease. Modulating specific immune cell phenotypes to ameliorate disease is an enticing strategy and could be an important therapeutic approach (Table 2).
Regulation of Hypertrophic Remodeling by Macrophage and Myeloid Phenotype

Although our understanding of the roles for AAM phenotypes in cardiovascular diseases is limited, several studies have demonstrated that these phenotypes correlate with cardiovascular protection. Through the use of cell type–specific targeting and knowledge of specific signaling effectors that regulate macrophage activation, it is now possible to delineate the roles of specific macrophage phenotypes during cardiac hypertrophy. Although comprehensive data are lacking, numerous regulators of macrophage activation have been identified, and several studies have shown that modulation of the myeloid phenotype can regulate the hypertrophic and fibrotic response.

MR is a regulator of macrophage polarization, and activation by mineralocorticoids enhances proinflammatory classically activated macrophage phenotypes, whereas MR antagonists and MR knockout suppress the inflammatory response and skew macrophages toward an AAM phenotype. Importantly, myeloid-specific deletion of MR significantly reduced angiotensin II–induced cardiac remodeling. Knockout of miR155 reduced angiotensin II–induced cardiac hypertrophy and fibrosis similar to that of wild-type mice but had reduced cardiac dysfunction similar to that of mice with PI3K KD morph with PI3K KD, kinase-dead phosphatidylinositol 3-kinase (PI3K) is a downstream effector of many different signaling pathways, including certain Toll-like receptors and cytokine receptors, and studies have implicated PI3K signaling in cardiac pathophysiology. Insulin-like growth factor-1 has been shown to induce cardiac hypertrophy through a PI3K-dependent pathway. A study using a kinase-dead PI3K (PI3K KD) found that PI3K inactivation ameliorated pressure overload–induced inflammation, fibrosis, and cardiac dysfunction without affecting cardiomyocyte hypertrophy. Importantly, this protective phenotype was transferable to wild-type mice with PI3K KD marrow, suggesting that PI3K regulates bone marrow–derived cell phenotype, and this has a critical role in cardiac remodeling and dysfunction. PI3K KD inactivation in bone marrow was necessary to reduce pressure overload–induced cardiac inflammation and fibrosis. PI3K KD mice with wild-type marrow had inflammation and fibrosis similar to that of wild-type mice but had reduced cardiac dysfunction similar to that of mice with PI3K KD marrow. This implicates a role for PI3K in multiple cell types, likely through different mechanisms.

A wide array of noncoding miRNAs have been identified as modulators of cardiac hypertrophy and remodeling (reviewed by Kumarswamy and Thum), and many known miRNAs can be targeted to alter cellular function. Most targeting strategies have focused on understanding the role of miRNAs in regulating cardiomyocyte function, and cardiomyocyte-specific knockout or overexpression of many miRNAs can induce pathological hypertrophy. A recent study has expanded the field and revealed that miRNAs can regulate myeloid cell phenotype to modulate cardiac hypertrophy and remodeling. Knockout of miR155 reduced angiotensin II– and pressure overload–induced hypertrophy, inflammation, and cardiac dysfunction. miR155 knockout also suppressed angiotensin II–induced proinflammatory genes and mitigated Arg1 and IL-10 suppression, and bone marrow transplantation with miR155-deficient cells was sufficient to produce this phenotype.

### Table 2. Modulation or Enhancement of Immune Cells in Cardiac Hypertrophy and Remodeling

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment Model</th>
<th>Function</th>
<th>Hypertrophy</th>
<th>Fibrosis</th>
<th>Inflammation</th>
<th>BP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM/myeloid cells, modulators</td>
<td>MR KO</td>
<td>L-NAME/Ang II</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NE</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PHD2 KO</td>
<td>L-NAME/Ang II</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>BM-P13K-KD (inactive)</td>
<td>TAC</td>
<td>↑</td>
<td>NE</td>
<td>↓</td>
<td>↓</td>
<td>CD18</td>
</tr>
<tr>
<td></td>
<td>BM-mi155 KO</td>
<td>Ang II</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>CD45 leucocytes</td>
</tr>
<tr>
<td>T lymphocytes, enhancement</td>
<td>Treg adoptive transfer</td>
<td>Ang II</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>MP, IL, TNF-α</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td>Treg adoptive transfer</td>
<td>TAC</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>MP, IL, TNF-α</td>
</tr>
<tr>
<td></td>
<td>Treg adoptive transfer</td>
<td>Ang II</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>MP, IL, TNF-α</td>
</tr>
<tr>
<td></td>
<td>Treg-CVB3-H310A1</td>
<td>CM-TNF-α Tg</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>CD11b</td>
</tr>
</tbody>
</table>

Ang II indicates angiotensin II; BM, bone marrow–derived; BP, blood pressure; CM, cardiomyocyte; IFN-γ, interferon-γ; IL, interleukin; KO, knockout; L-NAME, L-N-nitroarginine methyl ester; MP, macrophage; MR, mineralocorticoid receptor; NE, no effect; PHD2, prolyl hydroxylase domain protein 2; PI3K-KD, kinase-dead phosphatidylinositol 3-kinase; TAC, transverse aortic constriction; Tg, transgenic; TGF-β, transforming growth factor-β; Tl, T lymphocyte; TNF-α, tumor necrosis factor-α; and Treg, regulatory T lymphocyte.
IL-4 is a potent inducer of AAMs and can be upregulated in cardiac tissue during injury. It also has direct effects on other cardiac cells and stimulates proliferation of fibroblasts. Inhibition of IL-4 with neutralizing antibodies attenuated cardiac fibrosis and hypertrophy during pressure overload, suggesting that IL-4 is profibrotic and may exacerbate the hypertrophic response. The effect of blocking IL-4 signaling in macrophages and its response in hypertrophic remodeling are unknown. The important effectors of this response are unclear because multiple cell types respond to IL-4, and further analysis using conditional targeting strategies will be necessary to understand how IL-4 signaling regulates specific cell types. It was suggested that mast cells might be important in initiating IL-4-induced hypertrophy and fibrosis because they secrete IL-4, although many other cells, including Th2 cells and cardiomyocytes, can also contribute to IL-4 production.

Manipulation of other regulators of macrophage polarization such as interferon regulatory factor-5 and class A scavenger receptor has been shown to be involved in cardiac remodeling after myocardial infarction, although whether these myeloid modulators are also involved in hypertensive cardiac remodeling is unknown. Even though AAMs are thought to be cardioprotective, the present data are inconclusive because the mechanisms by which macrophage phenotypes regulate cardiac remodeling are unclear.

Lymphocytes

T cells have the ability to regulate inflammation through interactions with various immune cells and can activate, modulate, or suppress other leukocyte inflammatory responses. T cells are recruited to the heart during cardiac inflammation, and there is evidence that they are involved in the pathophysiology of cardiac diseases.

Depletion studies have demonstrated that T cells are involved in cardiac remodeling and can significantly alter cardiac pathophysiology. In cardiac-TNF-α-overexpressing mice, which develop severe cardiomyopathy, neutralization of T cells with anti-CD3 antibody was shown to reduce inflammatory cell recruitment and to block hypertrophy. In pressure overload–induced hypertrophy, T-cell depletion using Rag2-deficient mice has reduced myocardial fibrosis with decreased macrophage infiltration. This is associated with significantly attenuated cardiac dysfunction. Major histocompatibility complex class II knockout mice exhibited a similar phenotype. This was suggested to be attributable to the lack of immature T cells but could also be attributable to impaired responses from antigen-presenting cells such as dendritic cells or macrophages. However, another study has also reported that Rag1-deficient mice had no differences in hypertrophy and actually had increased perivascular fibrosis. Rag1-deficient mice also have been reported to have reduced angiotensin II–induced hypertension and vascular dysfunction, indicating that blood pressure regulation may be an important mechanism by which T cells alter cardiac hypertrophy and remodeling.

Regulatory T cells (Tregs) have a major role in suppressing inflammatory responses and are increased in the heart after myocardial infarction and heart failure. Patients with heart failure have decreased circulating Tregs with reduced functional capacity to suppress T-cell activation, and plasma Treg concentration is correlated with cardiac function. In animal models, several studies have shown that FoxP3+ Tregs are beneficial and ameliorate cardiac damage. Adoptive transfer of Tregs reduced angiotensin II– and pressure overload–induced cardiac hypertrophy and remodeling and suppressed macrophage and T-cell populations in the heart. In cardiac-TNF-α-overexpressing mice, Treg induction by coxsackievirus B3 was also able to prevent cardiac hypertrophy and to suppress inflammation through an IL-10–dependent manner. A role for Tregs in regulating blood pressure responses is still unclear. One report found that adoptive transfer of Tregs reduced angiotensin II–induced hypertension and vascular dysfunction, whereas another report found no difference in blood pressure.

Tregs have been shown to attenuate hypertrophic remodeling and cardiac dysfunction after myocardial infarction. Adoptive transfer of Tregs, but not conventional T cells, reduced proinflammatory signaling while increasing anti-inflammatory IL-10 production, and this was associated with attenuated cardiac fibrosis and improved cardiac function. Although adoptive transfer of conventional T cells does not reduce cardiac damage, it appears that these cells are necessary during myocardial infarction because CD4 knockout mice have impaired scar formation and increased mortality. Depletion of Tregs with the FoxP3-DTR transgene also suggests a beneficial role for this T-cell subset because Treg depletion resulted in increased infarct size, exacerbated inflammation, and diminished clinical outcome.

Although the mechanisms by which Tregs ameliorate hypertension and hypertrophic remodeling are unknown, they likely involve suppressing or modulating other T-cell or immune cell responses. Tregs can suppress the Th17 T-cell subset and a role for the Th17 subset has been identified in the pathophysiology of hypertensive cardiac hypertrophy and remodeling. Th17 cells secrete IL-17, which induces a range of effects, and IL-17 concentrations are increased in hypertensive patients. In animal models, deletion of IL-17 significantly reduces angiotensin II–induced hypertension and attenuates vascular dysfunction. IL-17 blockade has also been shown to be beneficial in hypertensive cardiac hypertrophy and remodeling, dilated cardiomyopathy, and myocardial infarction disease models.

Th1 and Th2 cells can influence classic and alternative activation of macrophages and therefore could be potential targets for regulating macrophage phenotypes. Th1 responses are largely proinflammatory, whereas Th2 responses are in many situations profibrotic. Therefore, these cells may have critical roles and various stages during the pathogenesis of cardiac hypertrophy and remodeling. Macrophages and other antigen-presenting cells might also induce or modulate T-cell responses by presenting cardiomyocyte debris or through other signaling mechanisms. Collectively, these studies indicate that in addition to depleting T cells, enhancing or modulating specific T-cell responses may be an effective strategy to regulate cardiac remodeling and to suppress unresolved inflammatory responses. In addition, how the modulation of T cells alters other immune cell phenotypes is still mostly unknown.
Mast Cells

Mast cell numbers in the heart increase significantly in response to cardiac injury, and numerous studies have shown that mast cells have a role in maladaptive cardiac remodeling. Mast cell–deficient WBB6F1-W/Wv mice have a marked reduction in pressure overload–induced cardiac hypertrophy and fibrosis with preserved cardiac function, and they are protected from decompensated cardiac hypertrophy and failure during pressure overload. Similarly, mast cell deficiency also reduced cardiac hypertrophy, fibrosis, and dysfunction in cardiac-TNF-α–overexpressing mice.

Mast cell function can also be inhibited pharmacologically with mast cell stabilizers. In spontaneously hypertensive rats, the mast cell stabilizer nedocromil significantly ameliorated cardiac fibrosis, although it had no effect on hypertrophy or cardiac function. Nedocromil is also protective in a volume-overload model and significantly suppressed ventricular hypertrophy while preventing cardiac dysfunction and reducing mortality.

The mechanisms by which mast cells contribute to cardiac remodeling and dysfunction are not fully elucidated. Mast cells secrete molecules such as histamine, growth factors, cytokines, and proteases, which may influence other cell types and adversely affect adaptive remodeling. Mast cells induce cardiomyocyte apoptosis, fibroblast and myofibroblast proliferation, and matrix deposition through the secretion chymase and tryptase. Mast cells secrete IL-4, which may have profibrotic and immunomodulatory effects. Therefore, it will be important to fully delineate the role that mast cells have in regulating cardiomyocyte and noncardiomyocyte function during cardiac remodeling.

Other Immune Cells

Inhibition of intercellular adhesion molecule-1 with neutralizing antibodies was shown to reduce infiltrating macrophages and to suppress cardiac fibrosis during pressure overload. However, intercellular adhesion molecule-1 inhibition prevents transmigration of many different leukocytes. Therefore, it is difficult to delineate the cell type–specific contributions. Many other immune cell populations that likely contribute to pathological cardiac remodeling are not thoroughly discussed because of a lack of data.

Neutrophils are the most abundant circulating leukocyte in humans, and they respond quickly to acute injury. Depletion of neutrophils is protective in models of myocardial infarction, but until recently, there have been limited data examining the role of neutrophils during the development of cardiac hypertrophy and remodeling. Neutrophils have now been recognized as having a more significant and direct role in promoting cardiac inflammation and regulating hypertrophic remodeling. A recent finding has shown that neutralization of the neutrophil-secreted molecule S100A9 reduces angiotensin II–induced cardiac hypertrophy and fibrosis, which implicates neutrophils in the pathophysiology of hypertrophic remodeling. Although the presence of neutrophils is commonly noted, few studies have focused on defining neutrophil function. Therefore, the role of neutrophils during pathological hypertrophy and remodeling requires further exploration.

Eosinophils are thought to have a major pathological role during eosinophilic myocarditis but may also have a potential role during other more common cardiac diseases. Recent reports using eosinophil-deficient (ΔdblGATA) and hyporesinophilic (IL-5TG) mice have shown that eosinophils secrete IL-4 and regulate AAM phenotypes in adipose tissue. Importantly, eosinophils were found to affect insulin sensitivity, thermogenesis, and beige fat formation. It is currently unknown how the macrophage phenotype is controlled during cardiac injury, and the possibility of eosinophils regulating macrophage phenotype in this context remains explored.

Experiments examining the roles of dendritic cells in cardiac hypertrophy and remodeling are also absent. Dendritic cells are present in injured cardiac tissue and have been shown to be important in cardiac remodeling after myocardial infarction. Although this may suggest a possible role in other forms of chronic hypertensive remodeling, no studies have confirmed this.

Fibroblasts

Fibroblasts are one of the most prevalent cell types in the heart and have a major role in matrix deposition during cardiac remodeling. During cardiac development and injury, the origin of cardiac fibroblasts and myofibroblasts has remained somewhat controversial, and it has been speculated that proliferating cardiac fibroblasts are derived from a variety of sources such as resident fibroblasts, bone marrow–derived progenitors, fibrocytes, epithelial cells, and endothelial cells. Recently, a lineage tracing study using multiple Cre lines and reporter systems to identify fibroblasts found that cardiac fibroblasts originate from epicardium- and endocardium-derived resident fibroblast populations during development.

Interestingly, this was also found to be true during cardiac injury. During pressure overload, rapidly expanding populations of fibroblasts were also derived from these 2 populations, not from hematopoietic precursors.

The important fibroblast signaling mechanisms that influence cardiomyocyte function and hypertrophy in vivo are still poorly understood, partly because of a lack of fibroblast-specific genes. Several Cre lines (Postn-Cre, Fsp1-Cre, Tcf21-Cre, Col1a1-Cre, Col1a2-Cre) have been created for fibroblast-specific gene deletion; however, some of these lines have been shown to be nonspecific or the specificity is unknown under specific pathological conditions. Therefore, studies using these Cre lines need to be interpreted carefully. Krüppel-like factor 5 has been shown to be an important regulator of pressure overload–induced cardiac remodeling through regulation of fibroblast function. Fibroblast-specific (Postn-Cre), but not cardiomyocyte-specific (Myh6-Cre), deletion of Krüppel-like factor 5 significantly attenuated cardiac hypertrophy and fibrosis. After “high-intensity” pressure overload with greater aortic constriction, mice displayed similar reductions in hypertrophy and fibrosis but developed heart failure and had increased mortality. This suggests a role for Krüppel-like factor 5 in adaptive remodeling during pressure overload and provides evidence that fibroblasts have direct roles in regulating cardiac hypertrophy and remodeling. Furthermore, this highlights the need to analyze cardiac function and mortality rates because the measurement and analysis of select pathological...
findings such as hypertrophy and fibrosis do not always predict the functional consequences.

Coculture studies have shown that fibroblast and cardiomyocyte interactions can influence hypertrophic and fibrotic responses and modulate contractile function, but these influences are difficult to verify in vivo because of the multitude of different cell types with overlapping signaling mechanisms. In a mouse model of hypertrophic cardiomyopathy, Kim et al found a marked induction of profibrotic genes that occurred very early, before cardiomyocyte hypertrophy and functional abnormalities had developed. Transcriptional sequence analysis subsequently found a large number of profibrotic genes upregulated in nonmyocytes in both prehypertrophic and overtly hypertrophic mice. Thus, fibrotic signaling is occurring much earlier than originally thought, and it is not just a secondary manifestation resulting from hypertrophy. It also suggests that specific mutations in sarcomere proteins may induce early intercellular communication that initiates profibrotic signaling mechanisms and in turn influences hypertrophy and remodeling.

In these studies, TGF-β inhibition was able to reduce nonmyocyte proliferation and to attenuate cardiac hypertrophy and remodeling. Similarly, treatment of mice with an angiotensin II type 1 receptor blocker also significantly ameliorated hypertrophy and fibrosis, and this beneficial effect was dependent on early administration before established hypertrophic cardiomyopathy. This further suggests that inhibiting early profibrotic signaling is essential for pharmacological inhibition of hypertrophic remodeling. Although these results are intriguing, TGF-β and angiotensin II have effects in numerous noncardiomyocyte populations. Therefore, these pharmacological interventions could reflect the functional roles of many cellular interactions.

Transcriptome profiling with microarrays and deep RNA sequencing has identified hundreds of noncoding RNAs with altered expression during pathological hypertrophic remodeling. Numerous studies have shown that overexpression, deletion, or silencing of these noncoding RNAs can regulate the pathophysiological response to cardiac injury, although most of these studies have focused on manipulating and deleting or silencing of these noncoding RNAs can regulate cardiac hypertrophy and demonstrate an important mechanism of fibroblast-cardiomyocyte cross-talk.

Summary

Although cardiac hypertrophy was once thought of as a simple response to increased workload, it is now clear that it is a complex process involving all cell types present in the heart and interactions with circulating cells. Identifying the cell types involved, particularly the many immune cells, is critical to a full understanding of the physiological and pathological responses. We rely largely on specific markers to identify different cell populations and phenotypes while having little knowledge about functional importance. In addition to the identification of specific immune cell populations, it will be necessary to understand how specific functional phenotypes contribute. This understanding may lead to potential clinical interventions.

In the future, we can bring a number of approaches and technologies to delineating the mechanisms of physiological and pathological hypertrophy. Novel technologies to test the cell type–specific roles such as conditional knockouts and cell-type depletion are available, but most of these studies have focused on myocardial infarction models. Although many of these results might suggest a role in other hypertrophic responses in other cardiac pathologies, further studies will be necessary to determine that role. There are also limitations on which cell types can currently be addressed with these technologies. Furthermore, cardiac diseases are multifacase, and most studies focus on only single time points. To fully understand the hypertrophic response, it will be necessary to identify critical, early time points and to understand the functional role of specific immune cells during the early phases of hypertrophic remodeling. Although this may prove to be difficult because of time and cost, the benefits of understanding the early immune responses could be critical to understanding the pathogenesis and for identifying specific therapeutic targets.

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