Targeting Interleukin-1 in Heart Disease

Benjamin W. Van Tassell, PharmD; Stefano Toldo, PhD; Eleonora Mezzaroma, PhD; Antonio Abbate, MD, PhD

Inflammation is a coordinated cellular-humoral response to injury. A close interaction between resident cells (ie, endothelial cells, fibroblasts, and dendritic cells) and leukocytes regulates the initiation and resolution of the acute inflammatory response. Constitutive membrane and cytoplasmic receptors function as guardians that “signal the alarm” when activated by products of cell destruction or microbial invasion. This first-line innate immune response initiates a process of leukocyte mobilization from the bone marrow, recruitment to the “activated” endothelium, and migration to the site of tissue injury to prevent infection and to facilitate tissue repair. Although critical for many forms of repair, the inflammatory response may also become a mechanism for progressive injury, impaired healing, and disease.

Central Role of Interleukin-1 in the Sterile Inflammatory Response

Interleukin-1 (IL-1) is an apical proinflammatory mediator in acute and chronic inflammation and a powerful inducer of the innate immune response. The production and activity of IL-1 are finely regulated at multiple levels, and very small concentrations of exogenous IL-1 can induce a sepsis-like syndrome and shock. IL-1 induces the synthesis and expression of several hundreds of secondary inflammatory mediators. IL-1 also induces its own production and processing, and this step is key in the pathogenesis of many autoinflammatory diseases. Two related genes code for 2 different proteins (IL-1α and IL-1β) that bind the same receptor (type I). IL-1α is synthesized as a fully active peptide that remains membrane bound or may be released from the cytoplasm during cell death. IL-1α thereby participates more prominently in local response to injury and less in the systemic inflammatory response. IL-1β, the main form of circulating IL-1, is initially synthesized as a precursor (proIL-1β) that becomes activated by caspase-1 cleavage in the setting of a macromolecular structure known as the inflammasome. Caspase-1 also participates in the secretion of active IL-1β that can then bind the membrane IL-1 receptor on the same cell (autocrine) or neighboring cells (paracrine) or enter the circulation targeting distant cells (endocrine). The constitutive expression and inducible expression of proIL-1β are highly cell-type specific. Both IL-1 isoforms induce proIL-1β synthesis, as do other proinflammatory stimuli such as the numerous Toll-like receptor agonists (ie, cell debris or bacterial products). In addition, many potential triggers of the inflammasome have been identified, including microbial antigens, cell debris, ATP, ischemia, cholesterol crystals, and other Toll-like receptor ligands such as danger-associated molecular patterns or pathogen-associated molecular patterns.

Activation of inflammasome after tissue injury induces a local surge of IL-1β that significantly amplifies the inflammatory response, recruiting more inflammatory cells, stimulating metalloproteinase activities, and ultimately inducing inflammatory cell death (pyroptosis) in leukocytes and resident cells. The sensitivity to stimuli and the intensity and duration of the ensuing inflammatory response are also highly dependent on genetic variability. Cryopyrin-associated periodic syndromes are rare, chronic autoinflammatory disorders in which the cryopyrin (NLRP3) “sensor” of the inflammasome is constitutively active, leading to a debilitating condition of IL-1β overproduction. Other polymorphisms in the cryopyrin gene have been linked to increased susceptibility to autoinflammatory diseases. Polymorphisms in the IL-1 gene cluster (coding for IL-1α, IL-1β, and IL-1 receptor antagonist [IL-1Ra]) that result in greater expression of the agonists or reduced expression of the antagonist have been associated with premature onset, increased severity, or worse outcomes in patients with various inflammatory conditions, ranging from rheumatoid arthritis, periodontitis, inflammatory bowel disease, Alzheimer disease, and others.

Heart Disease: An Inflammatory Disease

Cardiovascular disease is the leading cause of death in the United States and worldwide. Millions of patients succumb to the consequences of myocardial ischemia (triggered primarily by acute coronary syndromes), heart failure (impaired contractility/relaxation), and arrhythmias. The epidemics of cardiovascular risk factors such as diabetes mellitus, hyperlipidemia, obesity, arterial hypertension, and tobacco use have led to increased numbers of patients at risk for primary and secondary acute coronary events worldwide. Although the mortality for acute coronary events has dropped as a consequence of improved interventional treatments, this has resulted in an increased number of survivors at high risk of heart failure, recurrent events, and cardiovascular mortality. Cardiovascular disease has classically been considered a metabolic rather than inflammatory disease in which changes in lipid and glucose metabolism are linked to the
progression of coronary artery disease and impaired function. Nevertheless, reports of an inflammatory response during heart disease began to emerge several decades ago.\(^{18-20}\)

Patients with acute coronary events have increased inflammatory markers, including C-reactive protein (CRP), which serves a surrogate for IL-1 activity.\(^{21}\) For example, early studies observed that high CRP levels (>3 mg/L) in patients with unstable angina predicted a significantly worse prognosis.\(^{19}\) Subsequent studies validated that elevated CRP also predicted heart failure–related mortality in patients with impaired systolic function (ejection fraction <30%) and clinical recurrence after coronary revascularization procedures such as balloon angioplasty, coronary artery stenting, and coronary artery bypass graft surgery,\(^{22,23}\) as well as in apparently healthy subjects and even in patients with levels of CRP previously considered normal.\(^{24-27}\) Moreover, polymorphisms in the genes coding for IL-1\(β\), IL-1Ra, and other genes have been associated with premature onset of atherosclerosis and acute myocardial infarction (AMI).\(^{28}\)

The natural history of heart disease is not linear.\(^{29}\) Evidence shows that changes in the vessel wall and the heart start decades before the disease becomes clinically evident, and a chronic subclinical inflammation may promote or accelerate the disease (Figure 1).\(^{29}\) The atherothrombotic event leading to AMI is a rather stochastic event occurring on a predisposed substrate: a vulnerable plaque that experiences an intolerable stress, leading to rupture and acute thrombosis. An enhanced inflammatory response may predispose the plaques to rupture, and a heightened inflammatory response during myocardial injury may affect the degree of healing and progression to heart failure. Clinically stable patients with prior acute events and symptomatic heart failure display evidence of chronic inflammation, which may further aggravate the cardiac dysfunction or predispose the patient to further decompensation (Figure 1).

**Role of IL-1 in the Progression of Atherosclerosis**

Atherosclerosis and atherothrombosis are the underlying mechanisms for the great majority of first and recurrent coronary events.\(^{29}\) The degree to which plaques are formed, progress, and rupture is dependent, at least in part, on the degree of inflammation in the plaque. In human atherosclerotic plaques, the expression of IL-1\(α\) and IL-1\(β\) appears to correlate with the progression of atherosclerotic plaques, with minimal expression in healthy coronary arteries, increased expression in simple atherosclerotic plaques, and high expression in complicated plaques.\(^{30}\)

**Experimental Studies**

Experimental studies in atherosclerosis-prone animals have consistently shown that genetic deletion or pharmacological inhibition of IL-1 signaling reduces the formation and progression of atherosclerotic plaques, whereas increased IL-1 activity (by exogenous administration of IL-1\(β\) or downregulation of the naturally occurring IL-1Ra) favors its progression.\(^{31-33}\) Bone marrow chimeric studies identified IL-1 signaling in the vessel wall, rather than in leukocytes, as a major determinant of atheroma formation.\(^{34,35}\)

IL-1\(β\) mediates tissue response to endothelial injury, contributing to the proliferative intimal response.\(^{36}\) Treatment with IL-1 blockers inhibits the progression of atherosclerosis;\(^{31}\) however, the effects of IL-1 may be variable on the basis of the vascular district and may contribute to both plaque burden and outward vessel remodeling.\(^{37}\) IL-1\(β\) also exerts direct effects on thrombosis,\(^{38}\) insulin resistance,\(^{39}\) and obesity-related metabolic derangements.\(^{40}\) Figure 2 shows the central role of IL-1 in atherothrombosis.\(^{40}\)

**Clinical Studies**

As a result of the challenges of direct measurement of plasma IL-1\(β\) levels (often undetectable even in diseases with clear evidence of increased IL-1 activity), only a few studies have measured IL-1\(β\) levels and have shown that they were increased in patients with a greater atherosclerotic burden\(^{41-43}\) or that IL-1\(β\) levels were associated with less favorable prognosis after acute coronary syndromes.\(^{44-46}\) The IL-1 family of cytokines includes several different proteins with agonistic proinflammatory activity (such as IL-1\(β\), IL-18, and IL-33) and others with antagonist anti-inflammatory activity (IL-1Ra, IL-18 binding protein, and soluble ST2).\(^{1}\) Prospective and retrospective clinical studies have described that the plasma levels of IL-1Ra and ST2 (as surrogate markers for IL-1 activity) predict adverse outcome in patients with acute coronary syndromes.\(^{47-50}\) Increasing levels of IL-1Ra and of IL-6 (a secondary downstream mediator of IL-1\(β\)) predicted adverse outcome in patients with unstable angina.\(^{50}\) Similar results have been described more recently with the soluble ST2 biomarker.\(^{51}\)

---

**Figure 1.** Inflammation and interleukin-1 (IL-1) in the wide spectrum of ischemic heart disease. The natural history of heart disease is not linear. Evidence shows that atherogenesis is a slow process, and chronic subclinical inflammation may promote or accelerate the disease, whereas the atherothrombotic event leading to acute myocardial infarction is more of a sudden and rather stochastic event occurring on a predisposed substrate. An enhanced inflammatory response may predispose the plaques to rupture, and an even more intense response follows the myocardial injury, affecting the degree of healing and the progression to heart failure. Chronic inflammation also occurs in many clinically stable patients with prior acute events who are symptomatic for heart failure, potentially aggravating the cardiac dysfunction or predisposing to further decompensation.
Results from clinical trials testing the effects of IL-1 blockers on atherosclerosis or atherothrombosis are lacking.

Role of IL-1 in the Healing Process After AMI

Injury to the myocardium induces a sterile inflammatory response to promote healing, but unfortunately, restitutio ad integrum does not occur in the heart, and dead tissue is ultimately replaced by a nonfunctional scar. Healing is a dynamic process lasting for at least several weeks after myocardial injury. Cell death is initially necrotic in the infarct core as a result of an abrupt decrease in cellular ATP levels but progresses in the border zone and nonischemic myocardium through a more programmed form of cell death. This process is generally classified as apoptosis because of the characteristic DNA fragmentation and caspase-3 activation, but it also share features of inflammatory cell death or pyroptosis.

As in all forms of sterile inflammation, IL-1 is largely involved in the recruitment of the leukocytes and coordination of the inflammatory response to infarction. On cell death, local cardiac resident endothelial cells, cardiomyocytes, and fibroblasts release cytoplasmic IL-1α and proIL-1β into tissue that is susceptible to cleavage/activation of IL-1β by extracellular enzymes such as neutrophil elastase. Viable injured resident cells also release active IL-1β after activation of the inflammasome. Once leukocytes have been recruited to the infarct, the major source of IL-1β is likely to be the activated leukocyte. The formation of an active inflammasome in the heart follows and regulates the healing process. In preclinical models, genetic deletion of the scaffold component of the inflammasome (apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC)) leads to a significant improvement in cardiac healing after ischemia/reperfusion. Similarly, silencing or genetic deletion of cryopyrin (NLRP3), one of the sensor components of the inflammasome, prevented the formation of an active inflammasome (active caspase-1) and protected the heart from ischemic injury. These data are highly consistent with prior descriptions of a central role for caspase-1. The mechanisms leading to the formation of the inflammasome in the heart and the consequences of caspase-1 activation in the heart are not completely characterized. Silencing or pharmacological inhibition of the purinergic ATP/ADP receptor P2X7 prevented caspase-1 activation during AMI, suggesting that extracellular ATP is an important trigger for the formation of the inflammasome. Nevertheless, it cannot be excluded that other triggers may also be important or that P2X7 is important for other mechanisms such as release of mature IL-1β. Other purinergic receptors such as the adenosine A2B receptor may also be involved in the formation of the inflammasome. The expression of the components of the inflammasome also appears to be tissue and cell-type specific. One study reported the fibroblast (and not the cardiomyocyte) as the prevalent cell type forming the inflammasome on the basis of the finding of maturation of IL-1β in fibroblasts but not cardiomyocytes. A second study, however, demonstrated that the cardiomyocytes expressed all the components of the inflammasome (ASC, cryopyrin, and active caspase-1) but found no evidence of mature IL-1β in cardiomyocytes, suggesting that the processes of inflammasome formation, caspase-1 activation, and IL-1β maturation may be different and disconnected in different cell types. Moreover, the activation of caspase-1 in the cardiomyocyte, perhaps in other cells as well, may lead to IL-1β-independent effects such as inflammatory cell death by pyroptosis (Figure 3). In rare genetic diseases such as cryopyrin-associated periodic syndromes, the inflammasome is constitutively active, and patients suffer from the consequences of uncontrolled sterile inflammation. The finding that cryopyrin-associated periodic syndromes patients were highly responsive to IL-1β blockers has led to the theory that most inflammasome-mediated effects are mediated by increased IL-1β activity.

Experimental Studies

Genetic deletion of the IL-1 signaling receptor (IL-1R1) was protective in models of AMI as a result of ischemia/reperfusion and permanent ligation, as shown by smaller infarct size, reduced left ventricular enlargement, and reduced left

Figure 2. Interleukin-1 (IL-1) and atherosclerosis. The natural history of atherosclerosis is characterized by plaque formation, progression (growth), and complication. IL-1 plays a key role in the formation, progression, and complication of the atherosclerotic plaque. An increase in IL-1 activity causes a destabilization of the plaque, rupture, and superimposed thrombus formation. Data excerpted from Abbate et al.40

Figure 3. Interleukin-1 (IL-1) and atherosclerosis. The natural history of atherosclerosis is characterized by plaque formation, progression (growth), and complication. IL-1 plays a key role in the formation, progression, and complication of the atherosclerotic plaque. An increase in IL-1 activity causes a destabilization of the plaque, rupture, and superimposed thrombus formation. Data excerpted from Abbate et al.40
ventricular dysfunction. Conversely, mice lacking the naturally occurring IL-1Ra developed a severe form of cardiomyopathy characterized by increased cardiomyocyte apoptosis and ventricular dilatation after AMI. Moreover, overexpression of IL-1Ra appeared to consistently improve the cardiac response to AMI. Administration of the recombinant form of the IL-1Ra after the onset of ischemia led to a significant reduction in cardiomyocyte apoptosis and left ventricular dilatation independently of changes in infarct size, whereas pretreatment with anakinra induced a significant reduction in infarct size. These findings suggest that IL-1 signaling may be involved in myocardial infarction healing by multiple mechanisms. An initial surge of IL-1 activity early during AMI may be related to the release of intracellular IL-1α, which is active and is not processed by the inflammasome, or may be related to the release of proIL-1β, which can be cleaved to IL-1β extracellularly and independently of the inflammasome. A second wave of increased IL-1 activity occurs later in the course as a result of leukocyte recruitment to the injured site and activation of the inflammasome. Genetic deletion of IL-1R1 or administration of anakinra blocks signaling of both IL-1α and IL-1β, rendering it difficult to determine which mechanism is predominant. Studies assessing signaling downstream of the IL-1 receptor such as the myeloid differentiation factor-88 further strengthen the evidence of a critical role of IL-1 activity but do not allow discernment of the exact contribution of the isoforms. An additional antiapoptotic role of the intracellular IL-1Ra isoform in cardiomyocytes has been described. The contribution of IL-1 signaling in infiltrating leukocytes is strengthened by data obtained in experiments of bone marrow–chimeric mice with genetic deletion of the myeloid differentiation factor-88 or the IL-1 receptor–associated kinase-4. More recent data suggest that IL-1 signaling in the bone marrow also affects the regenerative potential in the heart. Several additional IL-1 blockers that have been developed recently have provided opportunities to validate the effect IL-1 blockade in preclinical models. The use of a recombinant chimeric protein composed of the IL-1 receptor, the IL-1 receptor–associated protein, and the Fc fragment of an immunoglobulin constitutes the “IL-1 trap.” The beneficial effects on cardiac remodeling seen with the use of an IL-1 trap in the mouse provided the initial evidence that IL-1 blockade in cardiac remodeling is a class effect. The IL-1 trap also blocks the effects of endogenous IL-1Ra without significant adverse effects. In none of these experiments was there evidence of impaired healing or increased risk of cardiac rupture, suggesting that IL-1 signaling may not be necessary for cardiac healing after AMI.

The evidence supporting the role of IL-1β versus IL-1α in cardiac remodeling after AMI is less straightforward. An initial report had suggested that blockade of IL-1β impaired healing in the heart and favored cardiac rupture in a model of severe nonreperfused myocardial infarction in the mouse. The investigators used a commercial hamster anti-mouse IL-1β antibody developed for immunohistochemistry. The characteristics of the antibody (IgG class), the presence or absence of stimulatory properties, and the ability to activate complement- or antibody-mediated cell death were not reported. The deleterious effects of IL-1β blockade in this study were based on a reduction of collagen deposition and increased incidence of cardiac rupture 5 to 7 days after surgery. This pattern of impaired healing and increased rupture was not seen in the studies using genetic models of IL-1 receptor deletion or in the

![Figure 3. Cryopyrin activation and inflammasome formation. Shown is a simplified scheme of cryopyrin activation and inflammasome formation in tissue resident cells and leukocytes recruited at the site of injury. Toll-like receptor (TLR)/interleukin-1 receptor type I (IL-1R1) agonists induce the inflammasome components (priming) through myeloid differentiation factor-88 (MyD88) signaling. ATP (through purinergic receptor 2X7 [P2X7]) or intracellular signals associated with lysosome destabilization activate cryopyrin, triggering the formation of the inflammasome. The inflammasome processes and releases active interleukin (IL)-1β, which further enhances the inflammatory response (autoinflammation). Data excerpted from Abbate et al. ASC indicates apoptosis-associated speck-like protein containing a C-terminal caspase-recruitment domain; DAMP, danger-associated molecular patterns; NF-κB, nuclear factor-κB; PAMP, pathogen-associated molecular pattern; and PKR, RNA-dependent protein kinase.](http://circ.ahajournals.org/issue/112/3/CIRC2004079707)
studies using pharmacological agents to nonselectively block IL-1α and IL-1β (ie, anakinra or IL-1 trap) and thus raised the question of whether selective IL-1β blockade would elicit adverse effects. More recent studies using well-described IL-1β antibodies specifically developed for in vivo use have shown a protective effect of IL-1β blockade. A mouse engineered IL-1β antibody of IgG1 isoclass that shows powerful modulation of IL-1 activity and was developed for in vivo use showed significant limitation of cardiac enlargement and dysfunction after experimental nonreperfused myocardial infarction in the mouse. Similar results were seen in the same model with another mouse monoclonal anti–IL-1β. Both of these antibodies had been shown to have powerful inhibitory effects on IL-1β signaling without cell toxicity. The discrepancies between these recent studies compared with the original antibody study are difficult to reconcile but likely are related to differences in the characteristics of the antibody used. The favorable data obtained with IL-1β blockers specifically developed for in vivo use are in line with the data from studies that inhibited caspase-1 or silenced components of the inflammasome.

In autoinflammatory diseases, IL-1β induces the synthesis, processing, and release of more IL-1β. When tested in the myocardial infarction model, IL-1β blockade with an IL-1β antibody ameliorated the cardiac remodeling process but did not affect the intensity of the inflammatory response or the caspase-1 activity in the tissue, suggesting that in AMI the mouse, IL-1–related inflammation may not follow an autoinflammatory pattern. Other triggers (ie, cell debris, ATP) must therefore be equally or more important in determining the intensity of the sterile inflammatory response. Figure 4 is a schematic representation of the mediators involved in the pathway of the sterile inflammation after AMI, the formation of the inflammasome, and the subsequent release of IL-1. Figure 4 also presents treatments that have been demonstrated to blunt the inflammatory response and to ameliorate the remodeling in preclinical models of AMI.

Clinical Studies
The MRC-ILA-HEART study randomized 186 patients with non–ST-segment–elevation AMI within 48 hours of symptom onset in 3 centers in the United Kingdom to either anakinra 100 mg daily for 14 days or placebo. The study was prompted by preclinical data from the same group showing that inappropriate arterial response to injury in animal models was mediated by IL-1β. The primary end point of the study was the area under the curve for CRP over the first 7 days. The results of the study are not available.

The Virginia Commonwealth University–Anakinra Remodeling Trial (VCU-ART1 and VCU-ART2 pilot studies included 10 and 30 clinically stable patients, respectively, with reperfused ST-segment–elevation AMI who were randomized to anakinra 100 mg daily for 14 days (as in the MRC-ILA-HEART study) or placebo. All 40 patients were followed up for 3 months with paired cardiac magnetic resonance analyses. Mortality and event rates were low in both groups, likely reflecting a selection bias toward lower-risk ST-segment–elevation AMI patients who were stable enough to undergo initial cardiac magnetic resonance imaging at 24 hours. Overall, anakinra showed an acceptable safety profile, with only 2 patients (10%) experiencing adverse events and requiring discontinuation of therapy (versus 2 patients [10%] in the placebo group). Injection-site reactions were the most commonly encountered side effect, occurring in 5 patients (25%) in the anakinra group and 2 patients (10%) in the placebo group. Anakinra was associated with a numerically greater incidence of infections (25% versus 15%) but not of serious infections (10% in each group). Nine adverse cardiac events (1 death, 2 recurrent myocardial infarctions, 6 instances of new-onset heart failure) occurred in 6 placebo patients, whereas 3 adverse cardiac events (2 recurrent myocardial infarctions and 1 instance of new-onset heart failure) occurred in 3 anakinra-treated patients (P=0.25). Anakinra-treated patients were significantly less likely to experience new-onset heart failure (1 patient, 5%) compared with placebo-treated patients (6 patients, 30%; P=0.035; Figure 5). Treatment with anakinra 100 mg daily significantly blunted,
but did not eliminate, the inflammatory response at 72 hours (CRP plasma levels of 8.4 mg/dL [anakinra] versus 18.9 mg/dL [placebo]; \( P < 0.001 \); Figure 5).\(^{31,82}\) Paired changes in left ventricular end-systolic volume index and left ventricular ejection fraction in the entire cohort were rather small over the 3-month follow-up, reflecting a group of ST-segment–elevation AMI patients at low risk for adverse cardiac remodeling yet at significant risk for heart failure. Anakinra-treated patients showed a trend toward more favorable changes in left ventricular end-systolic volume index and left ventricular ejection fraction, but placebo-corrected differences were not statistically significant (Figure 5).\(^{31,82}\) Anakinra had no effects on infarct size measured with cardiac magnetic resonance.\(^{81,82}\) A larger phase II study (VCU-ART3) will test 2 different anakinra regimens in patients with STEMI.\(^{82a}\)

**Role of IL-1 in Heart Failure**

Heart failure is a clinical condition of impaired cardiac contraction or relaxation at rest or with exercise, associated with symptoms of shortness of breath, fatigue, or exercise intolerance. Heart failure represents a final common pathway for many forms of cardiac injury (ischemia, pressure overload, toxicity) and leads to increased morbidity and mortality.

**Experimental Studies**

IL-1 was identified as one of the “soluble myocardial depressant factors” in the plasma of patients with sepsis.\(^{83,84}\) Without affecting the β-adrenergic receptor (β-AR) density or the affinity for its ligands, IL-1 impairs the coupling of the adenyl cyclase (responsible for the production of cAMP) with the β-AR, thus reducing the response to the endogenous and exogenous β-AR agonists (ie, isoproterenol).\(^{85,86}\) The mechanisms for this observation are not entirely clear.\(^{86,87}\) The \( I_{\text{Ca}} \) current \( (I_{\text{Ca}}) \) of the L-type calcium channel is also affected by IL-1β. This channel is responsible for the increase in \( I_{\text{Ca}} \) after β-AR stimulation, and IL-1 uncouples this channel from the receptor independently of cAMP concentration or β-AR density.\(^{88}\) Some reports show that nitric oxide produced in response to IL-1 affected the production of ATP (in the mitochondria) and the coupling of the calcium channel with the β-AR.\(^{89-92}\) IL-1 also regulates \( \text{Ca}^{2+} \) reuptake in the sarcoplasmic reticulum through downregulation of phosphorylamban and sarcoplasmic/endoplasmic reticulum calcium-ATPase mRNA and protein levels.\(^{90,93}\) More recently, a role for phosphoinositide-3-kinase-γ in IL-1 signaling has been identified as potentially linked to impaired contractility, phosphodiesterase-3 activity, and β-AR desensitization.\(^{94-98}\) A separate report describes the role of IL-1 in ischemia-induced systolic dysfunction.\(^{99}\) Figure 6 summarizes the proposed effects of IL-1 on cardiac contractility.

The impairment in contractility is experimentally reproducible in vivo by challenging the mouse with recombinant mouse IL-1β. A single injection of IL-1β (3 μg/kg) induces systolic dysfunction and impaired response to isoproterenol, and repeated injections of IL-1β give a reversible nonischemic cardiomyopathy.\(^{100,101}\) Interestingly, the injection of plasma from human patients with systolic heart failure (and elevated CRP) is sufficient to induce systolic dysfunction and impaired response to isoproterenol, whereas pretreating mice with IL-1 blockers preserves the systolic function and contractile reserve.\(^{102}\)

![Figure 5](http://irc.ahajournals.org/)

*Figure 5. Interleukin-1 blockade in acute myocardial infarction. The Virginia Commonwealth University–Anakinra Remodeling Trial (VCU-ART) and VCU-ART2 pilot studies showed significant blunting of the inflammatory response with anakinra 100 mg daily during ST-segment elevation acute myocardial infarction (STEMI) and a numerically lower incidence of heart failure at 3 months. Data excerpted from Abbate et al.\(^{82}\) LVEF indicates left ventricular ejection fraction; and LVESVi, left ventricular end-systolic volume index.*
IL-1 has also been shown to affect myocardial relaxation. In vitro studies describe impaired relaxation of cardiomyocytes. In our laboratory, we have observed that a single injection of recombinant murine IL-1β (3 μg/kg) in healthy adult mice leads to an increase in left ventricular end-diastolic pressure (4 mmHg in controls versus 8 mmHg in IL-1β-treated mice), accompanied by an overall reduction in contractility and relaxation, measured as dP/dt and −dP/dt, and an increase in isovolumetric relaxation, measured at Doppler echocardiography (personal communication by the authors, September 24, 2013).

Clinical Studies
The first study to show an improvement in cardiac function with IL-1 blockade enrolled a series of patients with rheumatoid arthritis in whom a single dose of anakinra 100 mg improved myocardial contractility and relaxation, coronary flow reserve, and brachial artery flow-mediated dilatation within 3 hours of administration. The Acute Inflammatory Response in Heart Failure (AIR-HF) pilot study showed tolerability of anakinra in 7 patients with symptomatic systolic heart failure and elevated CRP levels (>2 mg/L). Anakinra lead to a significant increase in peak VO2 (2.9 mL·kg−1·min−1), decrease in VE/VO2 (−3.2, reflecting greater ventilator efficiency), and increase in exercise time (2.9 minutes). CRP and IL-6 levels dropped by nearly 90%, suggesting that IL-1 activity may regulate the systemic inflammatory response in heart failure. Furthermore, the observation that IL-1 blockade also reduced the plasma concentration of IL-1β by nearly 90% suggests that IL-1 follows a positive feedback loop in heart failure wherein “IL-1 induces IL-1” and implicates heart failure as an autoinflammatory phenotype. A similar effect has been described by increasing amounts of nitric oxide (NO) secondary to augmented expression of the NO synthase (NOS), which not only contributes to the uncoupling of the Ca2+ regulation from the β-AR but also affects the production of ATP in the mitochondria. Altered levels of Ca2+, cAMP (through PKA), and ATP affect the contractility of the sarcocytes, the cardiomyocytes, and the heart as a whole. Class 3 PDE phosphodiesterases (PDE-3) oppose the effects of AC by hydrolyzing cAMP into AMP. Phosphoinositide-3 kinase-γ (PI3Kγ) regulates the activity of β-AR and PDE-3. PI3Kγ is also activated downstream of the IL-1R and may be involved in the impairment of contractility by activating PDE-3 and inducing β-AR desensitization.

Figure 6. Proposed mechanisms of interleukin-1 (IL-1)–induced contractile dysfunction. The diagram represents the intracellular pathways initiated by IL-1 and describes the proposed mechanisms by which IL-1 impairs the contractility of the cardiomyocyte. IL-1 (−α or −β) binds to the IL-1 receptor type I (IL-1R) and the IL-1R accessory protein (IL-1AcP) and recruits the intracellular adaptor protein myeloid differentiation factor 88 (MyD88). This interaction activates the IL-1R–associated kinases (IRAKs) and the tumor necrosis factor (TNF) receptor–associated factor (TRAF-6) and leads to the activation of p38 mitogen-activated protein kinase (p38 MAPK) and nuclear translocation of the nuclear factor-κB (NF-κB), causing transcriptional changes of several genes. IL-1 signaling induces uncoupling of the β-adrenergic receptor (β-AR) from the adenylyl cyclase (AC) and from the L-type calcium (Ca2+) channels, thus reducing the responsiveness of the cardiomyocytes to the β-AR agonist (such as isoproterenol [isopt]). The cAMP-dependent protein kinase A (PKA) is sensitive to increasing concentration of cAMP and regulates the function of several proteins, including sarcomere components and phospholamban, the calcium reuptake in the sarcoplasmic reticulum, and the transcription of several genes. The changes in gene transcription downstream of the IL-1R receptor affect the expression of phospholamban and the sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA) and increase the production of ceramide, which contributes to the uncoupling of the Ca2+ channel from the β-AR. A similar effect has been described by increasing amounts of nitric oxide (NO) secondary to augmented expression of the NO synthase (NOS), which not only contributes to the uncoupling of the Ca2+ regulation from the β-AR but also affects the production of ATP in the mitochondria. Altered levels of Ca2+, cAMP (through PKA), and ATP affect the contractility of the sarcocytes, the cardiomyocytes, and the heart as a whole.

Clinical Studies
The first study to show an improvement in cardiac function with IL-1 blockade enrolled a series of patients with rheumatoid arthritis in whom a single dose of anakinra 100 mg improved myocardial contractility and relaxation, coronary flow reserve, and brachial artery flow-mediated dilatation within 3 hours of administration. The Acute Inflammatory Response in Heart Failure (AIR-HF) pilot study showed tolerability of anakinra in 7 patients with symptomatic systolic heart failure and elevated CRP levels (>2 mg/L). Anakinra lead to a significant increase in peak VO2 (2.9 mL·kg−1·min−1), decrease in VE/VO2 (−3.2, reflecting greater ventilator efficiency), and increase in exercise time (2.9 minutes). CRP and IL-6 levels dropped by nearly 90%, suggesting that IL-1 activity may regulate the systemic inflammatory response in heart failure. Furthermore, the observation that IL-1 blockade also reduced the plasma concentration of IL-1β by nearly 90% suggests that IL-1 follows a positive feedback loop in heart failure wherein “IL-1 induces IL-1” and implicates heart failure as an autoinflammatory phenotype. A similar effect has been described by increasing amounts of nitric oxide (NO) secondary to augmented expression of the NO synthase (NOS), which not only contributes to the uncoupling of the Ca2+ regulation from the β-AR but also affects the production of ATP in the mitochondria. Altered levels of Ca2+, cAMP (through PKA), and ATP affect the contractility of the sarcocytes, the cardiomyocytes, and the heart as a whole. Class 3 PDE phosphodiesterases (PDE-3) oppose the effects of AC by hydrolyzing cAMP into AMP. Phosphoinositide-3 kinase-γ (PI3Kγ) regulates the activity of β-AR and PDE-3. PI3Kγ is also activated downstream of the IL-1R and may be involved in the impairment of contractility by activating PDE-3 and inducing β-AR desensitization.
Role of IL-1 in Myocardial and Pericardial Disease

Autoinflammatory diseases are often manifested with serositis (such as pericarditis) and occasionally with myocarditis. Isolated pericarditis without apparent cause is referred to as idiopathic recurrent pericarditis. Different studies have reported successful treatment of pericarditis with anakinra in patients with idiopathic recurrent pericarditis, parvovirus B19-associated myocardio-pericarditis, and myocarditis associated with Still disease. Preclinical clinical trials are lacking and much needed. In the meantime, considering its safety profile, anakinra may be considered for the treatment of refractory or recurrent pericarditis.

Preclinical studies also support a pathogenetic role of IL-1 in myocardial disease resulting from doxorubicin toxicity, radiation injury, or genetic mutations leading to dilated cardiomyopathy.

Overview of the Clinically Available IL-1 Blockers

Anakinra (Kineret)

Anakinra, recombinant human IL-1Ra, was originally approved for the treatment of rheumatoid arthritis in 2001. As a recombinant version of the naturally occurring IL-1Ra, anakinra binds to the IL-1 receptor to exert powerful IL-1 blocking activity without observable off-target effects. Anakinra is highly effective in the treatment of several inflammatory diseases, and preliminary studies show potential benefits in ischemic stroke, hemorrhagic stroke, and diabetes mellitus. The favorable safety profile of anakinra is demonstrated by the hundreds of thousands of patients who have been treated in the past decades. Anakinra is associated with an increased risk of infections (mainly upper respiratory infections). A review of clinical trials in patients with rheumatoid arthritis who were also receiving other immunomodulating agents and therefore were at greater risk of infection found that anakinra was associated with a 2- to 3-fold increase in serious infections (5%–6% versus 1%–2% in placebo) but not with increased infection-related mortality. Three randomized, controlled trials of anakinra in patients with severe sepsis showed a favorable trend toward reduced mortality. In contrast, tumor necrosis factor-α blockers are considered to be immunosuppressants and were associated with worse outcomes in sepsis trials. Moreover, anakinra prevents immunosuppression in a recent study of stroke patients and does not inhibit the production of interferon-γ. Nevertheless, given that IL-1 mediates fever and leukocytosis, it is expected that patients treated with IL-1 blockers (such as anakinra) may not develop fever or leukocytosis during infection. Close monitoring of treated patients is therefore warranted to identify more subtle symptoms of infection and to prevent potential delays in antimicrobial treatment.

The most common side effect of anakinra is injection-site reaction presenting as erythematous skin reactions. These reactions are generally mild and self-limiting, occur within a few days of initiation of therapy, and subside spontaneously within 1 or 2 weeks of continued treatment or within days on cessation of therapy. Rotation of the injection site may alleviate minor reactions, and topical corticosteroids may be helpful for severe reactions.

Anakinra is given as a fixed dose of 100 mg SC every 24 hours in adults or every 48 hours in patients with severely impaired kidney function. Nevertheless, the pharmacokinetic characteristics of anakinra after a 1-mg/kg subcutaneous injection (half-life, 4–6 hours) suggest that suboptimal IL-1 blockade may occur with this regimen. In many inflammatory diseases, physicians often use a higher dose of anakinra (up to 5 mg/kg) to achieve superior results. A much higher dose of anakinra (2-mg·kg⁻¹·h⁻¹ infusion for 72 hours) was used in the clinical trials of patients with sepsis while maintaining a favorable safety profile. Pilot clinical trials evaluating the use of anakinra in stroke patients have used the same high doses (2-mg·kg⁻¹·h⁻¹ infusion for 72 hours).

Before the approval of rilonacept (Arcalyst, 2008) and canakinumab (Ilaris, 2009), anakinra was the only available IL-1 blocker in clinical practice. Because of its safety record and short half-life, anakinra has been considered the ideal drug for pilot proof-of-concept studies. Compliance issues with daily injections and injection-site reactions may theoretically limit prolonged treatment courses; however, thousands of rheumatoid arthritis patients receive prolonged treatment of daily injections without significant problems. The pharmacokinetic characteristics of the newer agents (reviewed below) present the possibility of simplified dosing regimens. On the basis of current labeling, however, anakinra remains the only agent approved for a non orphan indication and therefore remains the mostly widely used IL-1 blocking agent in clinical practice.

Rilonacept (Arcalyst)

Rilonacept is a recombinant fusion protein based on elements of the IL-1RI and the IL-1 receptor accessory protein. Circulating rilonacept effectively functions as an IL-1 trap that binds with high affinity to circulating IL-1α and IL-1β and with less affinity to IL-1Ra. In 2008, rilonacept received Food and Drug Administration approval for the treatment of cryopyrin-associated periodic syndromes. Rilonacept offers the potential advantage over anakinra of weekly injections. A murine analog of rilonacept showed significant benefits compared with vehicle on cardiac remodeling after permanent coronary ligation in a mouse model of myocardial infarction. Notably, the benefits appeared to follow a U-shaped distribution in that moderate doses (5 mg/kg) appeared to be more efficacious than lower (1 mg/kg) or higher (30 mg/kg) doses. A small pilot study showed preliminary safety and efficacy in 10 patients with active gouty arthritis, but no follow-up studies have been published. As with anakinra, precautions are necessary to avoid infections, and the combination of rilonacept with other immunomodulating drugs has not been adequately tested. Minor infections are generally more common with rilonacept than placebo, and the risk of serious infections with rilonacept cannot be assessed because of limited data. Injection-site reactions are also common but unlikely to lead to drug discontinuation.
Canakinumab (Ilaris)
Canakinumab is a monoclonal IL-1β antibody (immunoglobulin G1/k isotype) that binds irreversibly to circulating human IL-1β and prevents activation of the IL-1 receptor. Unlike anakinra and rilonacept, canakinumab is specific for IL-1β blockade. Like rilonacept, canakinumab is approved by the Food and Drug Administration only for the treatment of cryopyrin-associated periodic syndromes; however, multiple reports already describe the use of canakinumab in other inflammatory disease states such as diabetes mellitus, gouty arthritis, rheumatoid arthritis, juvenile arthritis, uveitis, Behcet disease, and familial Mediterranean fever. Moreover, canakinumab is currently being investigated for the prevention of recurrent cardiovascular events in the Cardiovascular Risk Reduction Study (CANTOS), the largest anticytokine study ever conducted. CANTOS began enrollment in 2011 and will target a population of 17,200 patients with a history of AMI and elevated CRP. A murine analog of canakinumab showed significant benefits on cardiac remodeling and left ventricular function in the mouse after myocardial infarction caused by coronary artery ligation. Canakinumab offers the potential advantage over anakinra (and rilonacept) of a 3-month dosing interval (terminal elimination half-life, 26 days) that may be preferable for the treatment of chronic diseases. The prolonged duration of action, however, may be counterproductive in patients who experience significant adverse events.

In published clinical trials, canakinumab has shown potential safety and efficacy in the treatment of gouty arthritis, rheumatoid arthritis, and diabetes mellitus. In 2 studies of canakinumab in patients with acute gouty arthritis, canakinumab significantly improved pain and prevented new flares compared with triamcinolone. Patients randomized to canakinumab also experienced increased rates of infectious adverse events (20.4% versus 12.2%) and serious adverse events (7.6% versus 3.1%) compared with triamcinolone, accompanied by slight increases in serum urate and serum creatinine (decreased estimated glomerular filtration rate) and slight reductions in platelet, neutrophil, and leukocyte counts. Notably, however, no opportunistic infections were observed. As seen with other antibodies, canakinumab may induce an antibody reaction toward the drug, but this has not been shown to be clinically relevant. In patients with diabetes mellitus, canakinumab provided a highly significant reduction in CRP levels, thus proving that the elevation in CRP seen in such patients is highly dependent on IL-1β. Canakinumab had no significant effect on hemoglobin A1c, fasting glucose, insulin, insulin resistance, low-density lipoprotein cholesterol, or high-density lipoprotein cholesterol at 4 months; however, patients receiving canakinumab experienced a mild increase in triglyceride concentration. Neutropenia (absolute neutrophil count ≤1500 per 1 mm³) occurred in 10.7% of canakinumab patients compared with 4.6% of placebo patients; however, no serious adverse events were reported, and no patients experienced life-threatening consequences or need for intervention. The large effect of canakinumab on CRP as a surrogate for cardiovascular risk provided justification for the design of the large secondary prevention CANTOS study. Substudies of CANTOS study will also address the effect of canakinumab on carotid plaque burden, insulin secretion, and exercise capacity (among heart failure patients).

Gevokizumab
Gevokizumab (XOMA052) is a humanized monoclonal antibody (immunoglobulin G2 isotype) that binds to human IL-1β. Gevokizumab is not currently approved for any indications in the United States but has undergone preliminary testing in arthritis, uveitis, and diabetes mellitus. Compared with the Food and Drug Administration–approved IL-1 blockers, gevokizumab appears to be most similar to canakinumab because of IL-1β selectivity and a pharmacokinetic profile (terminal elimination half-life, 23 days) that allows extended dosing intervals up to 4 weeks. However, similar to rilonacept, gevokizumab displays a U-shaped dose response in which moderate doses may exert more favorable anti-inflammatory effects in vivo. In contrast to other agents, the IL-1β/gevokizumab complex appears to retain some degree of agonist activity at the IL-1 receptor. Whether the residual agonistic activity represents an advantage or a disadvantage from an efficacy and safety point of view is unclear at this time.

The Table summarizes the characteristics of the different IL-1 blockers.

**Current Indications of IL-1 Blockers**
As of May 2013, IL-1 blockers are approved only for the treatment of rheumatoid arthritis and cryopyrin-associated periodic syndromes. The favorable safety profile of anakinra in patients with rheumatoid arthritis who are at increased risk of infections, cancer, and cardiovascular disease provides some reassurance and justification for the many off-label uses of IL-1 blockers (as witnessed by the numerous

---

**Table. Comparison of IL-1 Blocking Agents**

<table>
<thead>
<tr>
<th>Name</th>
<th>Trade Name</th>
<th>Mechanism</th>
<th>Blockade</th>
<th>IL-1α</th>
<th>IL-1β</th>
<th>IL-1Ra</th>
<th>FDA approval</th>
<th>Dose</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anakinra</td>
<td>Kineret</td>
<td>Receptor antagonist</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>Rheumatoid arthritis</td>
<td>100 mg</td>
<td>Subcutaneously</td>
<td>Daily</td>
</tr>
<tr>
<td>Rilonacept</td>
<td>Arcalyst</td>
<td>IL-1 trap</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>CAPS</td>
<td>160 mg</td>
<td>Subcutaneously</td>
<td>Weekly</td>
</tr>
<tr>
<td>Canakinumab</td>
<td>Ilaris</td>
<td>IL-1β antibody</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>CAPS</td>
<td>150 mg</td>
<td>Subcutaneously</td>
<td>Weekly</td>
<td></td>
</tr>
<tr>
<td>Gevokizumab</td>
<td></td>
<td>IL-1β antibody</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>…</td>
<td>0.3 mg/kg</td>
<td>Intravenously</td>
<td>Monthly</td>
<td></td>
</tr>
</tbody>
</table>

Data excerpted from Abbate et al. CAPS indicates cryopyrin-associated periodic syndromes; FDA, Food and Drug Administration; IL, interleukin; and IL-1Ra, IL, interleukin receptor antagonist.
reports in the literature) and the many ongoing experimental studies in areas of medicine, including rheumatology, oncology, neurology, dermatology, gastroenterology and now cardiology. Perspectives

Although IL-1 blockade in cardiovascular disease is still in its infancy, it may represent a unique opportunity to quench the inflammatory response after tissue injury by selectively inhibiting a single apical mediator in the cascade. Observational data, pilot studies, and preclinical models suggest a beneficial role of IL-1 blockade in a variety of pathological processes, including atherosclerosis, atherothrombosis, AMI, heart failure, and pericarditis. Although pivotal clinical trial data are lacking, small pilot studies have shown promising safety and efficacy signals, and a large cytokine inhibition study is ongoing to test IL-1β blockade in the secondary prevention of patients with AMI.145

It remains unknown whether IL-1 blockade will significantly affect the morbidity and mortality of patients with cardiovascular disease and whether such potential benefit will be obtained with an acceptable risk and cost profile. The availability of multiple different IL-1 blockers offers potential benefits to patients but also additional challenges to determine whether any specific agent will provide superior benefits in individual disease states. It is also unclear how to best measure the pharmacodynamics or pharmacogenetic responses to IL-1 blockers and whether a “one size fits all” approach will suffice or tailored therapy will be necessary.

Sources of Funding

Dr Abbate is supported by an American Heart Association Scientist Development Grant and funds from the VCU Pauley Heart Center. Dr Van Tassell is supported by an institutional National Institute of Health K23TR000057 grant. Drs Mezzaroma and Toldo are supported by American Heart Association Postdoctoral Grants.

Disclosures

Dr Abbate has received research grants from Gilead, Novartis, and XOMA; has lectured for Glaxo-Smith-Kline, Novartis, and XOMA; and has consulted for Gilead, Janssen, Omni Biopharma, Swedish Orphan Biovitrum, and XOMA. Dr Van Tassell has received research grants from Gilead and Novartis and has consulted for Novartis. The other authors report no conflicts.

References

25. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, Gatto AM Jr; Air Force/Texas Coronary Atherosclerosis Prevention Study Investigators. Measurement of C-reactive protein for the targeting of statin


---

1922 Circulation October 22, 2013

---


**KEY WORDS:** heart diseases ■ inflammation ■ interleukins
Targeting Interleukin-1 in Heart Disease
Benjamin W. Van Tassell, Stefano Toldo, Eleonora Mezzaroma and Antonio Abbate

doi: 10.1161/CIRCULATIONAHA.113.003199
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/128/17/1910

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at: http://circ.ahajournals.org//subscriptions/