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Cardiac Intercellular Communication: Are myocytes and fibroblasts fair-weather friends?

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Abstract

The cardiac fibroblast (CF) has historically been thought of as a quiescent cell of the heart, passively maintaining the extracellular environment for the cardiomyocytes, the functional cardiac cell type. The increasingly appreciated role of the CF, however, extends well beyond matrix production, governing many aspects of cardiac function including cardiac electrophysiology and contractility. Importantly, its contributions to cardiac pathophysiology and pathologic remodeling have created a shift in the field's focus from the CM to the CF as a therapeutic target in the treatment of cardiac diseases. In response to cardiac injury, the CF undergoes a pathologic phenotypic transition into a myofibroblast, characterized by contractile smooth muscle proteins and upregulation of collagens, matrix proteins, and adhesion molecules. Further, the myofibroblast upregulates expression and secretion of a variety of pro-inflammatory, pro-fibrotic mediators, including cytokines, chemokines, and growth factors. These mediators act in both an autocrine fashion to further activate CFs, as well as in a paracrine manner on both CMs and circulating inflammatory cells to induce myocyte dysfunction and chronic inflammation, respectively. Together, cell-specific cytokine-induced effects exacerbate pathologic remodeling and progression to HF. A better understanding of this dynamic intercellular communication will lead to novel targets for the attenuation of cardiac remodeling. Current strategies aimed at targeting cytokines have been largely unsuccessful in clinical trials, lending insights into ways that such intercellular cross-talk can be more effectively attenuated. This review will summarize the current knowledge regarding CF functions in the heart and will discuss the regulation and signaling behind CF-mediated cytokine production and function. We will then highlight clinical trials that have exploited cytokine-crosstalk in the treatment of heart failure and provide novel strategies currently under investigation that may more effectively target pathologic CF-CM communication for the treatment of cardiac disease.

The Societal Burden of Cardiovascular Disease

Cardiovascular diseases (CVD) are the leading cause of mortality in the United States¹ and account for over 15% of total health care expenditures (\$286 billion), exceeding any other major diagnostic group. Heart failure (HF) is the common final manifestation of most CVD, and is the leading hospital discharge diagnosis. With a 50% five-year survival rate, an aging population, and an alarming prevalence of CVD comorbidities such as obesity and diabetes, HF is predicted to be the leading cause of all morbidity by 2020². An increased understanding of disease pathophysiology led to limited clinical success with the now-standard therapeutic regimen of β -blockers, angiotensin-converting enzyme (ACE) inhibitors (or angiotensin receptor blockers, ARBs), aldosterone antagonists and/or

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diuretics^{3, 4}. However, despite improvements in symptom management and overall mortality rates, these approaches target secondary contributors to the disease^{5–8} (i.e. hypertension, neurohormonal compensation, etc) with limited and indirect effects on disease progression itself. Thus, current therapies can only delay HF progression and mortality.

Despite the varied etiologies and clinical manifestations of HF, impaired ventricular function is ultimately the result of pathologic cardiac remodeling. Upon cardiac injury, the heart undergoes a series of initially compensatory morphological and functional changes that aim to restore cardiac output. Over time, chronic cardiac stress exacerbates maladaptive responses, involving cardiac hypertrophy, interstitial fibrosis, ventricular dilation, chronic inflammation, and increased cellular apoptosis, producing a vicious cycle towards further cardiac dysfunction and decompensated HF^{9, 10}. Indeed, the extent of pathologic remodeling directly correlates with clinical outcome in HF patients¹¹.

The Cardiac Fibroblast in Physiology and Pathophysiology

Due to its important functional role in the heart, the cardiomyocyte (CM) has been the focus of most cardiac research aimed at developing novel therapeutic strategies for the attenuation of pathologic remodeling. However, CMs constitute only 30–40% of the total cardiac cell population¹². The majority of non-CM cells are cardiac fibroblasts (CF), the major supporting cells of the heart, responsible for governing many aspects of normal cardiac development, structure, and physiology. Historically, the best known function of the CF is to maintain structural integrity of the heart through regulation and turnover of the extracellular matrix (ECM). Tightly controlled production and secretion of matrix proteins such as collagens, fibronectin, matrix metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMPs) forms a highly organized three-dimensional network surrounding myocytes capable of tolerating mechanical stress and maintaining myocardial morphology. However, CF functions extend well beyond structural support, all of which are extensively reviewed elsewhere^{12–16}; CFs respond to and coordinate a variety of mechanical, chemical, and electrical inputs to maintain homeostasis, provide contractile coordination and electrical coupling between CMs¹⁷, contribute to angiogenesis¹⁸, and allow for mechanical force distribution throughout the myocardium. Diverse developmental origins and location (e.g. atria vs. ventricle) of the CF add further complexity to the roles of CF in myocardial physiology and homeostasis^{14, 19}.

In response to cardiac injury or stress, CFs undergo a phenotypic transition into a myofibroblast, characterized by expression of contractile proteins and smooth muscle cell markers (i.e. -smooth muscle actin; SMA)²⁰ and enhanced migratory and proliferative properties^{12, 21}. Increased deposition of collagens and other matrix proteins²² promotes scar formation critical for reparative healing of the myocardium. Conversely, sustained cardiac injury causes chronic myofibroblast activation and proliferation, leading to imbalanced collagen/MMP secretion and mislocalized (“reactive”) interstitial fibrosis, ventricular stiffening, myocyte ischemia, and arrhythmogenicity^{12, 21, 23}. Consequently, the CF has garnered increasing interest as a therapeutic target. Indeed, many currently marketed HF pharmaceuticals demonstrate “off-target” actions on the CF that may partially explain some of their salutary effects^{12, 24}. Further, direct modulation of CF function has proven beneficial in preclinical models of cardiac remodeling^{25–27}.

An additional role of the CF that has gained attention over the past decade, which is the major focus of this review and entire issue of the Journal, is its participation in a dynamic cross-talk with other myocardial cells (e.g. CMs) in the regulation of cardiac function and pathophysiology (Figure 1). Activated myofibroblasts are particularly responsive to mechanical stress and chemical stimuli induced upon cardiac injury, which enhances their

production and secretion of various pro-inflammatory and pro-fibrotic cytokines, growth factors, and chemokines^{12, 28}. The released mediators act in both autocrine and paracrine fashion to modulate local inflammatory cells, CMs, and CFs themselves to exacerbate pathologic remodeling. Recent strategies have therefore attempted to attenuate pathologic cardiac intercellular communication through cytokine targeting, although to date with limited clinical success. This review focuses on regulation and mechanisms of pathologic CF-CM paracrine cross-talk, (direct communication is discussed elsewhere in the current issue). Details of the major soluble mediators are provided, as well as discussion of past and current therapeutic strategies aimed at targeting this critical aspect of cardiac remodeling. Novel approaches beyond cytokine modulation that may more effectively attenuate pathologic CF activation and cardiac intercellular communication, including disruption of receptor cross-talk using PAR-1 inhibition, will be discussed. It is becoming clear that our traditional view of cardiac cells as isolated systems rather than as members of a dynamic functional network has limited our understanding of cardiac pathophysiology. A growing knowledge of complex cardiac intercellular communication will be crucial in developing new therapeutic approaches to directly attenuate cardiac disease and HF progression.

The Role of Intercellular Communication in Cardiac Disease

Initial studies in the early part of the decade demonstrated paracrine CF-CM interactions through classical conditioned media experiments and CF-CM co-culture studies²⁹. Fredj et al., for example, observed that adult murine CM displayed significantly enhanced cellular hypertrophy when in co-culture with CF or when treated with fibroblast-conditioned media concomitant with heightened IL-6 production, suggesting the potential involvement of IL-6 signaling in CM phenotypic modulation³⁰. Additional studies by Baudino's group demonstrated increased IL-6 and TNF α production by CF-CM co-cultures that was reversed upon antibody-mediated disruption of CF-CM interactions³¹. Later studies showed both CM contractile³² and electrophysiological³³ alterations upon treatment with CF-conditioned media, strongly supporting the idea of fibroblast-derived pathological mediators of CM dysfunction. These findings were substantiated by in vivo studies utilizing chimeric mice engineered to express myocytes lacking or expressing the AngII receptor³⁴. Upon AngII infusion, fibroblast proliferation and interstitial fibrosis were more prominent in mice expressing intact AngII receptors in the neighboring CMs.

Subsequent work has revealed that, upon cardiac injury, CFs undergo a phenotypic transition into a myofibroblast, a highly proliferative and secretory state that mediates most of the pathologic effects of this cell type, including elevated production and secretion of various pro-inflammatory mediators. Transition to a pro-inflammatory myofibroblast state results from a number of myocardial insults, including mechanical stress^{20, 35–37}, hypoxia³⁸, inflammatory cell-derived cytokine stimulation³⁹, and augmented neurohormonal input^{40, 41}. Of note, CFs harbor both AngII⁴² and β -adrenergic receptors (β ARs; predominantly β 2-ARs)^{43, 44} and accordingly directly contribute to the pathological responses of the enhanced renin-angiotensin-aldosterone system (RAAS) and adrenergic overdrive characteristic of the failing heart^{45–48}. We recently reported a novel mechanism of β -adrenergic-mediated CM hypertrophy via serotonin-AngII receptor coactivation on CFs^{49, 50}. Indeed, some of the salutary effects of β -blockers^{51–53} and ACE inhibitors⁵⁴ or angiotensin receptor blockers (ARBs)⁵⁵ may be explained, in part, by their “off-target” modulation of CF-CM cross-talk. Among others, mitogen-activated protein kinase (MAPK) pathways^{56–58}, NF- κ B^{59, 60}, PI3K⁶¹, and Smad signaling^{11, 62} have been implicated in CF activation in response to these upstream influences.

Elevated expression and release of various cytokines, chemokines, and growth factors (e.g. TGF β , TNF α , monocyte chemoattractant protein-1 (MCP-1), various interleukins,

endothelin-1, ANP, BNP, angiotensin II (Ang II), fibroblast growth factor-2 (FGF-2), and vascular endothelial growth factor (VEGF), etc), induces paracrine-mediated modulation of both CM and CF phenotypes, and also initiates an acute inflammatory response via recruitment of circulating immune cells to the heart (Figure 1)^{12, 28, 63}. Initially, the early inflammatory response is beneficial to an injured heart, necessary for mounting wound healing and tissue repair. Importantly, preventing early immune cell infiltration into cardiac tissue proves detrimental, and exacerbates cardiac remodeling post-myocardial infarction in mice^{64, 65}. However, sustained elevation of cytokines, as seen in both human^{66–69} and murine^{70–73} models of HF, correlates with NYHA functional class, severity, prognosis, and disease risk. In this context, the CF essentially becomes a persistently activated immune cell, mass-producing inflammatory cytokines and immune regulatory receptors (i.e. CD40)⁷⁴ that recruit and retain excessive numbers of circulating hematopoietic cells into the injured myocardial tissue. As a result, the immune response persists beyond its window of utility and transitions into a state of chronic deleterious inflammation⁷⁵. Further, increased levels of cytokines and growth factors cause persistent stimulation of myocardial cells (i.e. CF and CM), exacerbating each cell type's contribution to pathologic progressive cardiac remodeling and coordinating the post-injury response.

An emerging view of HF progression, aptly named the “cytokine hypothesis”⁷⁶, recognizes the strong inflammatory component of the disease and postulates the concerted contributions of a vast array of pro-inflammatory mediators, via a dynamic intercellular cross-talk, to its complex pathophysiology. The regulation and functional outcomes of individual cytokine mediators in the failing heart have been extensively reviewed elsewhere^{62, 77, 78}. Here we present an outline of the most predominately studied of these cytokines, highlight their roles in CF-CM cross-talk, and emphasize their pathologic relevance in disease progression. This is followed by a discussion of attempts to translate these basic science discoveries with cytokine inhibition into the clinic and speculation regarding their limited success to date, offering insight into development of improved therapeutics that target pathologic intercellular communication within the heart.

TGFβ

TGFβ is dramatically upregulated early in the failing heart, localized particularly to border zones of infarcted myocardium^{79, 80}. Its expression has been shown to be upregulated by adrenergic and AngII stimulation^{47, 48, 81} of CFs and subsequently mediates strong fibrotic and hypertrophic responses in post-infarction healing and remodeling through direct modulation of CF and CM function^{41, 82}. TGFβ stimulation of CF induces their transition to the myofibroblast state^{83–85}, and alters gene expression so as to promote a stable ECM. It does so through enhancement of matrix protein synthesis and deposition, such as collagen type I and III, as well as increased TIMP production with concomitant reductions in MMP expression, thus favoring a balance towards ECM synthesis rather than degradation^{85–87}. Effects on CM function by TGFβ stimulation include enhanced sensitivity to β-adrenergic-mediated CM hypertrophy⁸⁸. Interestingly, mice transgenically overexpressing TGFβ1 displayed a significantly increased myocardial density of β-adrenergic receptors and a corresponding decrease in GRK2 and G_i, classical mediators of β-AR desensitization, perhaps partly accounting for adrenergic toxicity in progressive HF⁸⁹. TGFβ-activated kinase (TAK1)-p38 MAPK⁹⁰ and Smad3^{91, 92} signaling axes have been implicated in mediating TGFβ-induced pathologic responses.

Importantly, preclinical models examining efficacy of TGFβ inhibition have proven successful in attenuating key aspects of pathologic post-infarct remodeling. A virally-injected soluble TGFβ type II receptor, which competitively sequesters circulating TGFβ, was observed to significantly improve survival, reduce remote and infarct zone fibrotic

lesions, and attenuate cardiac dysfunction (i.e. reduced ventricular dilation and end diastolic pressure, enhanced peak systolic pressure, and improved hemodynamics and contractility) four weeks post-infarction in mice. Further, MI thickness and fibroblast apoptosis were quantitatively reduced in treated mice⁹³. Similarly, mice undergoing TGF β neutralization with administration of an anti-TGF β antibody displayed reduced myofibroblast activity and attenuated fibrosis with improved diastolic function in response to pressure overload⁹⁴.

TNF α

TNF α is a member of a superfamily of ligands that have varied systemic physiologic roles. The TNF Receptor 1 (TNFR1), ubiquitously expressed (including the myocardium⁹⁵), mediates the effects of TNF α during pathologic conditions, mostly through activation of NF κ B or MAPK pathways⁵⁹. Elevated circulating levels of TNF α in human HF patients have been observed in a manner corresponding to functional class^{96, 97}, suggesting correlation with disease pathophysiology. Indeed, its plasma levels have served predictive roles for prognosis and disease risk in patients with myocardial infarction⁹⁸. Various mechanical and chemical stimuli provoke myocardial TNF α production^{35, 37}, upon which elevated concentrations contribute to HF progression through varied actions on CF and CM function. TNF α results in increased fibroblast proliferation, MMP secretion, and inflammatory cytokine secretion^{99–101}, translating to matrix breakdown and increased propensity for cardiac rupture in animal models¹⁰². Accordingly, murine myocardial infarction results in elevated TNF α myocardial expression, in conjunction with increased fibroblast proliferation and production of fibronectin in CFs isolated from both infarct and border zone regions, effects which were reversed with a neutralizing TNF α antibody¹⁰³. Additionally, TNF α results in altered Ca²⁺ homeostasis in the cardiomyocyte through multiple mechanisms that lead to impaired contractility. Through a sphingosine-dependent pathway, TNF α modulates both calcium release from the sarcoplasmic reticulum and Ca²⁺ responsiveness^{104, 105}. In addition, TNF α , through multiple mechanisms, reduces both SERCA2A protein expression and activity, increasing propensity for arrhythmia and contractile dysfunction^{106, 107}. Other studies have linked β -AR uncoupling¹⁰⁸ and increased nitric oxide production to TNF α -induced CM contractile dysfunction, even in the presence of adrenergic stimulation^{109, 110}. Further, TNF α has been demonstrated to induce CM hypertrophy¹¹¹ and may impact CM apoptosis through alteration of mitochondrial function and induction of mitochondrial cytochrome C release^{112, 113}.

Cell-specific effects of TNF α observed in vitro have translated to whole-animal cardiac dysfunction as well as successful attenuation of disease progression in preclinical models utilizing TNF α -targeted therapeutic strategies. Both cardiac-restricted overexpression or chronic infusion of TNF α were sufficient to induce pathologic ventricular remodeling in rodents, mimicking several hallmark characteristics of human HF after just 3 months, including hypertrophic growth and gene expression, apoptosis, ventricular dilation, increased fibrosis and inflammation, impaired function (i.e. reduced ejection fraction and fractional shortening) and greater mortality^{114–116}. Importantly, treatment with a soluble TNF α antagonist or cessation of TNF α infusion reversed these effects¹¹⁶. TNF α neutralization was also observed to reduce ex-vivo post-ischemic injury and improve calcium homeostasis in pressure-overloaded rabbit hearts¹¹⁷.

Interleukins

The interleukins are a family of pro-inflammatory cytokines with pleiotropic effects in the myocardium. Interleukin-6 (IL-6), in particular, is found to be upregulated post-myocardial infarction in rodent models^{72, 118} and in the ischemic human heart¹¹⁹. Fredj et. al. subsequently noted a correlation between increased levels of IL-6 in supernatants of CF-CM

co-cultures compared to isolated CF and CM alone, with concurrent increases in CF proliferation as well as CM size and hypertrophic gene expression³⁰, suggesting this cytokine's role in mediating pathologic CF-CM cross-talk. This same group showed that antibodies targeting IL-6 or its downstream target gp130 attenuated hypertrophic gene expression hypertrophy of CM either in culture with or treated with conditioned media from CF. IL-6 antagonism additionally attenuated CF proliferation when in coculture with CM. The authors postulated AngII to be the primary mediator of these effects¹²⁰, validating previous work implicating AngII-induced IL-6 production from CF in CM hypertrophy^{45, 121}. A key transducer of IL-6 effects in CF and CM is STAT3^{122, 123}; gp130 and STAT3 phosphorylation is accordingly dysregulated in hypertrophy¹²⁴. In vivo, mice transgenically expressing the chronically activated IL-6 receptor and its corresponding ligand develop significant cardiac hypertrophy¹²⁵.

Interleukin-1 β is another important member of the interleukin family; its expression is upregulated during cardiac injury and contributes directly to pathologic remodeling¹²⁶. CF-specific effects of IL-1 β stimulation include enhanced migration⁵⁶ and augmented MMP production¹²⁷, leading to ECM remodeling that can promote ventricular dilation and possible cardiac rupture. IL-1 β leads to CM contractile dysfunction through various mechanisms involving nitric oxide production¹⁰⁸, altered β -adrenergic signaling¹⁰⁹, and reduced expression of key calcium handling proteins such as phospholamban¹²⁸. In vivo work by Frangogiannis' group has demonstrated that mice lacking the IL-1 Receptor Type I are cardioprotected after ischemia-reperfusion injury, exhibiting an attenuated inflammatory response together with decreased collagen and MMP production¹²⁶, directly implicating this cytokine in pathologic cardiac remodeling.

Members of the Interleukin-17 (IL-17) family are a relatively new group of potent inflammatory cytokines released by activated T cells (primarily Th17) during immune responses^{23, 63}. While ligands, receptor specificities and signaling remain largely unknown, it is clear that their functions extend beyond immune regulation and into homeostasis of a wide range of tissues, including a role in heart (and vascular) pathology. Indeed, both gene and protein expression of IL-17A, the prototypical member of the family, is elevated in the infarct area of the left ventricle at four weeks post-myocardial infarction.¹²⁹ Mechanistic evidence came from Liao et al., who linked upregulated IL-17A levels after rodent ischemia/reperfusion injury with exacerbated remodeling¹³⁰. This is evidenced by amelioration of infarct expansion and cardiac dysfunction after genetic or antibody-mediated inhibition of IL-17A that was reversed with exogenous IL-17A administration. The authors showed that IL-17A promotes both Bax-mediated cardiomyocyte apoptosis and neutrophil vascular adherence and migration, which translate in vivo to attenuated apoptosis and cardiac neutrophil accumulation with IL-17A inhibition. Others have shown that IL-17 may indeed contribute to CF-CM crosstalk; IL-17 is expressed and secreted by CFs, which also harbor the IL-17 receptor¹³¹. IL-17 acts on the CF itself to enhance collagen deposition¹³¹ and MMP-1 expression¹³² and is upregulated in fibroblast-conditioned media shown to attenuate CM contractility³².

Finally, while many interleukins play a pro-inflammatory role in HF, proper regulation of any controlled immune response requires the actions of both pro- and anti-inflammatory mechanisms. Thus, anti-inflammatory cytokines may also govern post-cardiac injury responses. Interleukin-10 (IL-10) is one such anti-inflammatory cytokine, the levels of which are enhanced primarily in response to TNF α and are correlated with cardiac injury. While studies in animal¹³³ and human¹³⁴ HF show elevated levels of IL-10 after cardiac reperfusion or HF, there is a demonstrated increase in the ratio of TNF α :IL-10 after injury that is associated with depressed cardiac function^{135, 136}, suggesting that the anti-inflammatory response is an initial adaptive response that is later overwhelmed in the

chronic pro-inflammatory stages. Nevertheless, IL-10 has demonstrated cardioprotective and anti-inflammatory effects. For example, IL-10 reduced oxidative stress and apoptosis in TNF α -exposed adult rat cardiomyocytes¹³⁷. Importantly, recombinant IL-10 administration post-myocardial infarction suppressed inflammation via attenuation of inflammatory cell infiltrate and myocardial pro-inflammatory cytokine expression¹³⁸. Further, MMP activity and fibrosis were attenuated. Abrogated structural remodeling, as evidenced by a reduction in both infarct size and wall thinning, was seen with concomitant improvements in ejection fraction. These data suggest that a therapeutic approach may be to augment anti-inflammatory responses in parallel with pro-inflammatory cytokine targeting.

MCP-1

Monocyte chemoattractant protein-1 (MCP-1) is an inducible member of the pro-inflammatory chemokine superfamily that is rapidly upregulated in failing myocardium¹³⁹. As its name suggests, MCP-1 is critically involved in trafficking circulating monocytes and macrophages into the injured tissue from which it is released. Transgenically altered mice lacking the MCP-1 gene display impaired monocyte recruitment to inflamed tissue¹⁴⁰. Due to a large inflammatory component associated with vascular disease, MCP-1 inhibition has been extensively investigated in vascular pathologies including atherosclerosis^{7, 8, 141–143}. However, the increasingly recognized contribution of inflammation in cardiac disease has led to recent studies examining possible roles for MCP-1 in the failing heart. Serum MCP-1 levels have indeed been shown to be significantly upregulated in HF patients in a manner that correlates with the severity of cardiac dysfunction^{144, 145} and have been subsequently shown to contribute to the progression of cardiac disease. Mice exhibiting deletion of MCP-1's receptor¹⁴⁶, CCR2, knockdown of MCP-1 expression¹⁴⁷, or reduced MCP-1 activity through gene therapy¹⁴⁸ or neutralizing antibodies¹⁴⁷ demonstrated attenuated ventricular remodeling in response to ischemic injuries. Namely, both the fibrotic and inflammatory responses to cardiac injury were diminished upon MCP-1 inhibition, resulting in improved ventricular function. MCP-1-mediated effects in the myocardium include upregulated secretion of collagens and MMPs by CFs and their conversion into a myofibroblast phenotype^{149, 150} as well as increased apoptotic activity¹⁵¹. MCP-1's effects on extramyocardial inflammatory cells, however, are equally as important. MCP-1 induces upregulation of pro-inflammatory cytokine production, MMP secretion, and adhesion molecule expression on monocytes^{152–154}, increasing their recruitment into ischemic cardiac tissue¹⁵⁵, and enhancing their inflammatory potential. Additionally, MCP-1 appears crucial in the recruitment of bone marrow-derived CD34+/CD45+ fibroblast precursors into the heart that contribute to the endogenous CF population and exacerbate the fibrotic response^{156, 157}. Ongoing work in our laboratory and others seek to develop direct and/or indirect approaches to modulate MCP-1 expression and activity in HF.

Clinical Trial Outcomes of Cytokine Inhibition in Heart Failure

As outlined above, a number of pro-inflammatory cytokines are known to contribute to HF pathophysiology. Preclinical evidence strongly suggested that targeted cytokine inhibition, through gene therapy, knockdown strategies, or neutralizing antibody administration, can significantly attenuate pathologic cardiac remodeling. Thus, the clinical development of small molecule cytokine inhibitors could be a novel and promising therapeutic avenue. Indeed, cytokine antagonists, particularly those targeting TNF α , have proceeded to large-scale clinical trials. In particular, two TNF α antagonists were advanced; Etanercept, a recombinant human TNF α receptor used to competitively sequester circulating TNF α , and Infliximab, a monoclonal anti-TNF α antibody. Etanercept was tested in two separate trials differing only in the dose given; RECOVER patients received placebo or 25 mg of Etanercept once or twice weekly while RENAISSANCE patients were given 25 mg of

Etanercept twice or three times weekly, both for 24 weeks¹⁵⁸. Despite promising preclinical data with TNF α inhibition, as well as demonstrated safety and tolerability of Etanercept in Phase I studies, Etanercept provided no apparent clinical benefit towards event-free survival, hospitalization due to worsening HF, or patient assessment after six months in patients with NYHA class II to IV HF. Similar outcomes were observed in trials with Infliximab (i.e. ATTACH), in which patients with class III–IV HF received placebo or either 5 mg/kg or 10 mg/kg doses of the experimental drug for 28 weeks¹⁵⁹. While the lower dose yielded no clinical improvement, the higher dose surprisingly increased risk of death and hospitalization among patients.

Several explanations have been postulated to ratify the apparent discrepancy between preclinical and clinical data. Two main ideas include: inappropriate dosing that may not have sufficiently neutralized circulating TNF α , and existence of a “cytokine carrier” effect. The latter proposes that long-term administration of TNF α antagonists may ultimately stabilize active forms of the circulating cytokine, thus potentiating its actions¹⁵⁸.

A somewhat broader perspective may help to inform future drug development targeting the role of inflammatory cytokine signaling in HF. TNF α is one of several players in a complicated network of cytokines, growth factors, chemokines, and hormones that work in concert on multiple cell types, both systemically and in the myocardium, to affect cardiac function. Redundancy of function and complexity of their interactions may explain in part why inhibition of one signaling mediator alone may be insufficient to provide clinically translatable benefits. Moreover, individual cytokines have broad pleiotropic effects with varied local and systemic roles in maintaining physiologic homeostasis. Disruption or loss of homeostatic signaling, even with simultaneous attenuation of deleterious pathways, suggests the potential for off-target effects. An instructive example of deleterious consequences of cytokine manipulation are evident with TGF β signaling. TGF β signaling not only plays complex roles in the heart, but also in the tumor microenvironment, where it mediates biphasic pro- and anti- tumor growth promoting mechanisms^{160–164}. While our understanding of these dual functions is limited, it is clear that attenuating TGF β expression or signaling in the context of cancer is unfavorable, particularly in early stages of tumor development before it “evolves” mechanisms to resist, counteract, or even reverse TGF β ’s inhibitory effects¹⁶⁵. Alterations in the TGF β pathway that involve downregulation of signaling have been implicated in several forms of cancer, including brain¹⁶³, skin¹⁶⁶, and breast¹⁶⁷. Therefore, disruption of TGF β -mediated homeostatic signaling in healthy tissue in a non-targeted manner may promote transformation of pre-cancerous cells to a malignant state by relieving growth-promoting constraints imposed on them by this cytokine.

Perhaps most importantly, however, in the context of post-injury myocardial repair, is the consideration of timing¹⁶⁸. As mentioned previously, the early (i.e. 7-day) post-infarction stages of inflammation are protective, as demonstrated by exacerbated ventricular remodeling in response to disrupted monocyte recruitment⁶⁴. Thus, inflammation may be an adaptive response necessary for initiating early wound healing and reparative processes in the heart. Therefore, the time-dependency of the inflammatory response itself may implicate similar biphasic effects of pro-inflammatory cytokine mediators. This concept is illustrated by studies in which TGF β inhibition was initiated 7 days prior to or following left coronary artery ligation in mice¹⁶⁹. The authors observed exacerbated ventricular remodeling in mice pretreated with the inhibitor, including cardiac dysfunction and an enhanced inflammatory response. In mice treated 7 days post-MI, however, cardiac function and morphometry were preserved, suggesting early protective effects of TGF β post-myocardial injury that are lost with sustained expression. TNF α has been observed to have similar biphasic effects. One hour of infusion of a recombinant TNF α receptor into dogs produced a significant increase

in contractile function that ultimately became complete myocardial depression by seven hours of chronic infusion¹⁷⁰.

With some cytokines, it remains unclear whether they are predominately beneficial or detrimental in the setting of HF. With others, the cytokines may exert divergent effects in response to different types of cardiac injury. MCP-1¹⁷¹, for example, despite the wealth of data on its role in the exacerbation of remodeling, has been demonstrated to exert protective functions in both MI¹⁷² and ischemia/reperfusion (I/R)¹⁷³ murine models of HF, the latter through a reduction in oxidative damage. The protective effects may be mediated possibly in part through anti-apoptotic mechanisms¹⁷⁴. Likewise, although TNF α antagonism is protective after cardiac injury, it contributes to ischemic preconditioning prior to I/R injury in mice¹⁷⁵, further suggesting time-dependency of cytokine activity. Interestingly, TNF α -mediated cardioprotection is thought to be mediated primarily via PKC-p38 and JAK/STAT3-dependent pathways⁶⁰.

Further complexity arises from receptor subtype-specific effects of various cytokines, exemplified by the TNF α receptors, of which there are two types (Type I and Type II) expressed on cardiac cells¹⁷⁶. Four weeks post-myocardial infarction, TNFRI $^{-/-}$ and TNFRII $^{-/-}$ mice demonstrated opposing effects in respect to pathologic cardiac remodeling¹⁷⁷. Mice lacking the Type I receptor had preserved function and attenuated remodeling, while those without the Type II receptor endured exacerbated hypertrophy, fibrosis, apoptosis, inflammation and pathologic NF κ B and MAPK signaling. These data validated a prior study demonstrating similar results in addition to parallel divergent effects on post-MI mortality between the two receptor subtypes¹⁷⁸. A more recent study demonstrated that a possible mechanism by which TNFRII exerts its cardioprotective effects is via mitigation of β -adrenergic stimulation¹⁷⁹. Future therapeutic development targeting more specific or integrated signaling via the TNF-receptor subtypes or other downstream signaling pathways may provide therapeutic promise.

Novel Approaches to Attenuate Pathologic Intercellular Communication

The redundancy, complexity, and divergent functions of pro-inflammatory cytokines render these mediators a challenging target for the disruption of cardiac intercellular cross-talk. This highlights the need for a more integrative and targeted approach that can selectively attenuate deleterious signaling downstream of a wider range of pro-inflammatory modulators¹⁸⁰. Our lab is currently working towards understanding novel downstream mechanisms regulating cytokine expression, production, and signaling in hopes of identifying and validating new targets and small molecules for the attenuation of pathologic intercellular communication (Martin and Blaxall, unpublished data).

To explore additional mechanisms of pathologic intercellular communication in the pathogenesis of heart failure, we recently tested the hypothesis that G-protein coupled receptor (GPCR) transactivation may play a role. In particular, we have focused on the protease-activated receptor (PAR) family of GPCRs (Figure 2). Their unique mechanism of activation involves proteolytic cleavage of the extracellular N-terminal domain, classically by thrombin, that exposes a self-encoded ligand. Activation by thrombin elicits pathologic effects in cardiac cells, including neonatal cardiomyocyte hypertrophy and cardiac fibroblast DNA synthesis^{17, 18}. Indeed, we recently demonstrated that while cardiac-restricted PAR-1 (or PAR-2) overexpression led to heart failure, PAR-1 (or PAR-2)-deficient mice were less susceptible to myocardial injury^{36, 38}. Accordingly, inhibition of both PAR-1 by the synthetic antagonist SCH79797, and thrombin, using hirudin, also appears to be protective following myocardial injury^{29, 181}. Beyond thrombin, recent reports suggest that matrix metalloproteinase 1 (MMP-1) can cleave and thus activate PAR-1 in endothelial cells,

platelets and tumor cells, promoting disease processes including angiogenesis, thrombosis and tumorigenesis^{182–184}. Interestingly, expression and activity of MMPs correlate with cardiac disease and contribute to remodeling following injury, thus their role in PAR-1 cleavage may be involved in disease progression. The analogous major interstitial collagenase of MMP-1 in rodents, which do not express this homologue, is MMP-13, although its role in the heart beyond collagen degradation remains poorly understood^{153, 185}; recent work also suggests MMP1a may be a rodent homolog.¹⁸⁶ Prior work from Luttrell and others suggests a possible role for β 1-AR overstimulation in the elevation of extracellular proteases, such as MMPs, with signaling capabilities¹⁸⁷. Recent reports suggest that heart failure resulting from cardiac pressure overload, β 1-AR overstimulation or myocardial infarction is associated with elevated cardiac MMP-13 expression^{188–190}.

Our lab subsequently sought to determine whether MMP-13 can induce pathologic PAR-1 cleavage and activation in the heart in a non-ischemic (i.e. absence of thrombin) setting (Figure 2).¹⁹¹ Our data show that chronic β -AR stimulation of both cardiomyocytes and cardiac fibroblasts leads to PAR1 cleavage and transactivation and subsequent ERK1/2 activation via expression and secretion of active MMP-13. Importantly, this β -AR-elicited transactivation can be transmitted from fibroblasts to myocytes, elucidating another possible mechanism of pathologic cardiac intercellular communication. Further, pharmacologic MMP-13 inhibition attenuated cardiac dysfunction in an isoproterenol-induced (i.e. chronic β -AR stimulation) heart failure model.¹⁹¹

Conclusions

The past decade in cardiovascular research has seen a shift in our view of the cardiac fibroblast from the classically understood quiescent cells of the heart with little contribution to cardiac physiology to highly dynamic cells that take active roles in maintaining both cardiac function and pathophysiology. Importantly, their pathologic activation and release of a host of pro-inflammatory, pro-fibrotic, and pro-hypertrophic mediators creates a much more complex and integrated view of cardiac pathology. Our understanding of cardiac communication remains in its infancy, yet further investigation into this process is necessary to more effectively target HF disease progression. The limited success with targeted cytokine inhibition has provided fruitful lessons for current and future investigations that seek to develop novel strategies to ameliorate this key aspect of cardiac remodeling. Our lab is currently investigating novel targets of pathologic fibroblast activation and intercellular cross-talk that may more effectively and specifically ameliorate the action of multiple pro-inflammatory mediators in the heart. We believe that an increased understanding of the mechanisms underlying pathologic cardiac fibroblast activation and cardiac intercellular communication will yield novel therapeutic strategies that, unlike the current therapeutic paradigm, will directly target HF progression and will further contribute to the reduction in mortality and morbidity resulting from this devastating disease.

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Statement of Clinical Relevance

This review explores novel mechanisms to directly attenuate heart failure (HF) progression through inhibition of signaling downstream of pro-inflammatory cytokines that are elevated after cardiac injury.

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Figure 1. Pathologic Cardiac Intercellular Communication

In response to various chemical, mechanical, and ischemic stressors, the cardiac fibroblast (CF) undergoes a phenotypic transition to a myofibroblast, releasing a variety of growth factors, chemokines and cytokines that act both in an autocrine and paracrine fashion. Stimulation of CF results in a positive feedback loop to further enhance their activation, collagen deposition, and cytokine release, resulting in fibrosis and chronic inflammation. Cytokine effects on cardiomyocytes (CM) leads to pathologic effects including hypertrophy, apoptosis, and impaired contractile responses. Myocardial-generated cytokines also act on circulating inflammatory cells, such as monocytes, to enhance their activation and migration into cardiac tissue for wound healing and repair.

Figure 2. A Model for Pathologic Cardiac PAR-1 Receptor Transactivation

Adrenergic stimulation of the cardiac fibroblast (CF) via the β -AR leads to the production and release of MMP-13, which can, in a thrombin-independent manner, cleave PAR-1 receptors both on the CF and cardiomyocytes (CM) at a site distinct from thrombin cleavage. MMP-13-mediated PAR-1 activation results in ERK1/2-mediated CF and CM dysfunction. PAR-1-specific antagonism with SCH79797 attenuated the effects of MMP-13. Further, PAR-1 genetic ablation or MMP-13 inhibition with WAY170523 resulted in preserved cardiac function in response to chronic Isoproterenol infusion in wild-type mice, suggesting CF-CM receptor transactivation pathways may be novel therapeutic targets in the response to cardiac injury.