Effects of interleukin-1 on cardiac fibroblast function: Relevance to post-myocardial infarction remodelling

Neil A. Turner

Division of Cardiovascular and Diabetes Research, and Multidisciplinary Cardiovascular Research Centre (MCRC), University of Leeds, Leeds, UK.

Address: Dr Neil A. Turner, Division of Cardiovascular and Diabetes Research, School of Medicine, Worsley Building, Clarendon Way, University of Leeds, Leeds LS2 9JT, UK. Tel: +44(0)113-3435890. Fax: +44(0)113-3434803. E-mail: n.a.turner@leeds.ac.uk

Keywords:

Cardiac fibroblast; Myocardial infarction; Interleukin-1; Signal transduction pathways; Therapies

Abstract

The cardiac fibroblast (CF) is a multifunctional and heterogeneous cell type that plays an essential role in regulating cardiac development, structure and function. Following myocardial infarction (MI), the myocardium undergoes complex structural remodelling in an attempt to repair the damaged tissue and overcome the loss of function induced by ischemia/reperfusion injury. Evidence is emerging that CF play critical roles in all stages of post-MI remodelling, including the initial inflammatory phase that is triggered in response to myocardial damage. CF are particularly responsive to the proinflammatory cytokine interleukin-1 (IL-1) whose levels are rapidly induced in the myocardium after MI. Studies from our laboratory in recent years have sought to evaluate the functional effects of IL-1 on human CF function and to determine the underlying molecular mechanisms. This review summarises these data and sets it in the context of post-MI cardiac remodelling, identifying the fibroblast as a potential therapeutic target for reducing adverse cardiac remodelling and its devastating consequences.

1. Introduction

Cardiac fibroblasts (CF) are the major cellular components of the heart, outnumbering cardiomyocytes by as many as 2:1 (Jugdutt, 2003; Banerjee et al., 2007). These multifunctional and heterogeneous cells play important roles in many aspects of cardiac structure and function including its embryonic development, normal physiology and pathophysiology (Brown et al., 2005; Camelliti et al., 2006; Porter and Turner, 2009; Krenning et al., 2010; van den Borne et al., 2010; Turner and Porter, 2013). CF are particularly important regulators of the myocardial extracellular matrix (ECM). As well as controlling synthesis of structural ECM components (e.g. collagens, laminins, fibronectin), CF are also a source of ECM-regulatory molecules including matricellular proteins (e.g. thrombospondins, CCNs, tenascins), matrix metalloproteinases (MMPs) and MMP inhibitors (TIMPs). The close proximity of fibroblasts to cardiomyocytes and other myocardial cell types facilitates inter-cellular communication via both physical interactions (e.g. gap junctions) and via local paracrine signalling networks (e.g. synthesis and secretion of growth factors and cytokines), and hence CF are able to influence multiple aspects of cardiac function.

CF are intrinsically involved in the complex structural remodelling of the heart that occurs following myocardial infarction (MI), as well as in other cardiac pathologies that can lead to heart failure including hypertension, cardiomyopathy and myocarditis (Brown et al., 2005; Porter and Turner, 2009; Krenning et al., 2010; van den Borne et al., 2010; Turner and Porter, 2013). Aspects of CF function that are particularly pertinent to myocardial remodelling include their ability to proliferate, migrate in response to chemotactic stimuli, differentiate into myofibroblasts, regulate ECM turnover and synthesise and secrete numerous autocrine/paracrine signalling molecules, including growth factors, angiogenic factors and inflammatory cytokines and chemokines (Porter and Turner, 2009).

Following MI, the myocardium undergoes complex structural remodelling in an attempt to repair the damaged tissue and to overcome the loss of function induced by ischemia/reperfusion injury (Jugdutt, 2003). Post-MI remodelling occurs through a highly organised series of events involving both resident myocardial cells and infiltrating extra-cardiac cells such as neutrophils, monocyte/macrophages and myofibroblast precursors. The post-MI healing process can be divided into three overlapping stages referred to as the inflammatory, granulation (proliferative) and maturation phases (Frangogiannis, 2008; van Nieuwenhoven and Turner, 2013). It is becoming increasingly apparent that CF are critically involved in all these stages of myocardial repair and remodelling.

2. Cardiac Fibroblasts and Post-MI Remodelling

2.1. Inflammatory phase

Recent evidence suggests that CF can act as early triggers of the myocardial inflammatory response, preceding the infiltration of inflammatory cells (Kawaguchi et al., 2011). Although cardiomyocytes undergo rapid necrotic cell death in response to ischaemia, CF appear to be less sensitive to oxygen and nutrient starvation (Zhang et al., 2001) and could therefore be important sensors of early cardiomyocyte damage. The close positioning of myocytes and fibroblasts in the heart, coupled with recent evidence that CF express components of the innate immune system including Toll-like receptors and NOD-like receptors (Strand et al., 2013; Fernandez-Velasco et al., 2012), suggests that fibroblasts are able to rapidly sense endogenous danger signals known as damage-associated molecular patterns (DAMPs) that occur following myocyte damage and necrosis (Arslan et al., 2011; Zheng et al., 2011). Importantly, CF express a functional inflammasome that facilitates activation and secretion of the proinflammatory cytokine IL-1β (Kawaguchi et al., 2011); one of the main triggers for the myocardial inflammatory response. In addition to IL-1β, CF are capable of secreting a host of

other proinflammatory cytokines and chemokines including tumor necrosis factor (TNF)α, IL-6, IL-33, CC chemokines (e.g. CCL2/MCP-1) and CXC chemokines (e.g. CXCL1/GROα/KC and CXCL8/IL-8,) in a feed-forward loop driven by the inflammatory milieu, thereby exacerbating the initial inflammatory response (Turner et al., 2007; Turner et al., 2009; Turner et al., 2011; Demyanets et al., 2013; LaFramboise et al., 2007). Although further studies are required to determine the precise relative contribution of CF in this early inflammatory stage of remodelling compared with other cell types (mast cells, neutrophils, monocyte/macrophages etc), the available evidence to date suggests that CF play an important role (Chen and Frangogiannis, 2012).

2.2. Granulation phase

CF are also integrally involved in the granulation phase of remodelling, the transitional stage between inflammation and fibrosis (Chen and Frangogiannis, 2012; van Nieuwenhoven and Turner, 2013). Granulation tissue comprises mostly macrophages and myofibroblasts, the latter being derived from a variety of sources including resident CF, endothelial cells, epithelial cells, bone marrow-derived fibrocytes, pericytes and smooth muscle cells (Krenning et al., 2010; Zeisberg and Kalluri, 2010). Myofibroblasts are a differentiated form of fibroblasts, characterised by increased alpha-smooth muscle actin (αSMA) expression, that are not present in the normal healthy myocardium (Tomasek et al., 2002; van den Borne et al., 2010). In response to proinflammatory stimuli such as IL-1 and TNFα, myofibroblasts produce high levels of ECM-degrading proteases (especially MMPs) to degrade the damaged tissue prior to clearance by phagocytic leukocytes. This ECM degradation also facilitates (myo)fibroblast migration into the infarct area where they undergo increased proliferation in response to mitogenic stimuli, thus rapidly increasing local myofibroblast numbers. Neovascularisation is an important component of the granulation phase, and (myo)fibroblasts

likely contribute to this through their ability to modulate endothelial cell function, for example through secretion of VEGF (Zhao and Eghbali-Webb, 2001). In addition to their effects on MMPs, proinflammatory cytokines such as IL-1 may also be important stimulators of VEGF secretion and activity at the level of the fibroblast (Turner et al., 2010).

2.3. Maturation phase

As the granulation phase progresses, increased levels of pro-fibrotic molecules (e.g. TGFβ, CTGF/CCN2), combined with a reduction in inflammatory signals (e.g. IL-1), drive a switch in myofibroblast function away from ECM degradation towards synthesis of structural ECM components (particularly collagen I and III) and scar formation (Chen and Frangogiannis, 2012; van Nieuwenhoven and Turner, 2013). Myofibroblasts are the most prevalent cell type in scar tissue and are the main effectors of fibrogenesis (Peterson et al., 1999). Their expression of contractile cytoskeletal proteins (e.g. αSMA) and focal adhesion proteins (e.g. paxillin, integrin αVβ3) enables mechanical contraction of the scar edges to facilitate wound healing and scar maturation (Tomasek et al., 2002).

2.4. Reactive fibrosis

Although myofibroblast numbers decrease rapidly after scar formation, they can persist in the healed scar for many years after MI (Willems et al., 1994) and may be important for maintaining the strength and flexibility of the scar (van den Borne et al., 2010; Turner and Porter, 2013). However, persistent myofibroblast activation and continual local production of inflammatory cytokines, particularly in non-damaged areas of the myocardium (reactive fibrosis), can promote sustained inflammation, neurohormonal activation, ventricular wall stiffening, cardiac dysfunction and eventually heart failure (Brown et al., 2005).

3. Interleukin-1 and Post-MI Remodelling

3.1. IL-1 in the heart

The proinflammatory cytokine IL-1 comprises two distinct gene products (IL-1α and IL-1β) that have indistinguishable biological activities mediated via activation of the cell surface receptor IL-1R1 (Dinarello, 2011; Arend et al., 2008). A third receptor ligand, IL-1RA (IL-1 receptor antagonist), is structurally related to IL-1α and IL-1β but acts as an inhibitor of IL-1 signalling as it binds to, but does not activate, the IL-1R1 receptor complex. Increased myocardial IL-1α/β levels are associated with many cardiovascular pathologies including MI, cardiomyopathy, hypertension and myocarditis (Long, 2001; Bujak and Frangogiannis, 2009). As neither IL-1α nor IL-1β molecules possess leader sequences they are unable to be secreted from cells via the normal Golgi-mediated vesicular transport pathway.

IL-1α is an intracellular cytokine localised to the cytosol and also the nucleus where it appears to activate transcription of inflammatory genes (Werman et al., 2004). IL-1α is only released from cells when they are damaged or undergo necrotic cell death, and is one of the DAMPs that trigger the innate immune response. A similar role has been ascribed to other IL-1 family members such as IL-33 (Demyanets et al., 2013). IL-1α is a key trigger for sterile inflammation in the liver (Chen et al., 2007), and it likely plays a similar role in the heart following myocyte necrosis given that cardiac myocytes (Westphal et al., 2007) and fibroblasts (Turner et al., 2007) express IL-1α, and IL-1α levels are increased in the infarcted myocardium (Timmers et al., 2008).

In contrast to IL-1α, IL-1β is actively secreted from cells via non-classical pathways in response to specific stimuli (Arend et al., 2008) and is one of the earliest cytokines detected in patient plasma following MI (Guillen et al., 1995). Innate immune responses triggered by tissue damage often require assembly of inflammasomes, cytoplasmic multiprotein complexes incorporating caspase-1, that are required for proteolytic activation of

IL-1β (Lamkanfi, 2011). Formation of active inflammasomes is observed in fibroblasts, neutrophils, macrophages and endothelial cells within granulation tissue following experimental MI, and in cardiomyocytes bordering the infarct zone (Mezzaroma et al., 2011; Kawaguchi et al., 2011).

3.2. IL-1 signalling

The biological effects of IL-1α and IL-1β are mediated through binding to their cognate receptor, IL-1R1. Ligand binding stimulates recruitment of the IL-1 receptor accessory protein (IL-1RacP) which in turn recruits the MyD88 (myeloid differentiation factor 88) and IRAK4 (IL-1 receptor-activated protein kinase 4) signalling proteins **(Figure 1)**. This tetrameric complex is formed within seconds of IL-1 binding and acts as a stable first signalling module (Brikos et al., 2007; Weber et al., 2010). A complex assortment of additional adaptor proteins and kinases (e.g. TRAF6, TOLLIP, IRAK-1, IRAK-2) are then recruited to the core IL-1R1 signalling module, facilitating activation of downstream signalling pathways including the MAP kinase (ERK, p38, JNK) and NFκB cascades (Weber et al., 2010). Whilst the vast majority of IL-1R1 signalling has been shown to have an absolute requirement for the MyD88 adaptor protein (Adachi et al., 1998), IL-1R1 can activate the PI3K/Akt pathway via both MyD88-dependent and -independent mechanisms in some cell types (Davis et al., 2006; Ko et al., 2011) **(Figure 1)**.

Several negative regulators of the signalling pathways downstream of IL-1R1 have also been described (O'Neill, 2008; Flannery and Bowie, 2010). These include the tyrosine phosphatases SHP-1 and SHP-2 and the suppressors of cytokine signalling SOCS-1 and SOCS-3. IRAK-M is a novel member of the IRAK family that is particularly highly expressed in monocytes and macrophages, but has also been shown to be expressed by CF

(Chen et al., 2012). IRAK-M lacks kinase activity and acts as a negative regulator of IL-1R1 signalling.

Negative regulation of IL-1 signalling at the receptor level occurs in several ways (Dinarello, 2005; Dinarello, 2011). Firstly, IL-1α/β can bind to a second cell-surface IL-1 receptor, IL-1R2, which lacks the cytosolic domain and therefore acts as a decoy receptor that is unable to activate intracellular signalling pathways. Secondly, IL-1RA acts as a natural receptor antagonist by binding to IL-1R1, but it does not elicit downstream signalling as IL-1RAcP is not recruited. Finally, IL-1α/β can be sequestered in the plasma by soluble forms of the IL-1R2 and IL-1RAcP proteins (sIL-1R2 and sIL-1RAcP) that are formed following inducible receptor cleavage.

3.3. Role of IL-1R1 signalling in post-MI cardiac remodelling

IL-1 levels are generally elevated in the first few hours after MI and return to basal levels within 3 days, although this varies depending on the species in question (Dewald et al., 2004). For example, IL-1β mRNA levels in rat myocardium are markedly elevated over a 3-24 h period following experimental MI (without reperfusion) and return to basal levels within 3 days (Deten et al., 2002). In a reperfused mouse MI model, IL-1β mRNA was elevated 3-6 h after MI and returned to basal levels within 3 days (Dewald et al., 2004). In MI patients, plasma levels of IL-1β are transiently elevated 1-5 h after infarction before returning to control levels (Guillen et al., 1995).

Both IL-1α and IL-1β are able to drive cardiac dysfunction in animal models. For example, transgenic mice with cardiomyocyte-specific overexpression of IL-1α develop LV hypertrophy (Nishikawa et al., 2006), while those ubiquitously over-expressing IL-1α die of heart failure within 2 weeks of birth (Isoda et al., 2001). Moreover, intraperitoneal injection of IL-1β induces proinflammatory cytokine expression, cardiac fibrosis and heart failure in

mice within 3 weeks (Blyszczuk et al., 2009; Van Tassell et al., 2010).

Knockout mouse studies have revealed a critical role for the IL-1 receptor in mediating cardiac myofibroblast accumulation, MMP expression and fibrotic remodelling of the infarcted heart (Bujak et al., 2008). The beneficial effects on cardiac remodelling observed in IL-1R1 knockout mice were not only the result of suppressed inflammation, but also attributed to the loss of direct IL-1-mediated effects on CF (Bujak et al., 2008). MyD88 and IL-1R1 signalling were also shown to be critical for development of cardiac fibrosis during progression to heart failure in a mouse model of inflammatory heart disease; effects shown to be mediated predominantly at the level of CF progenitor cells (Blyszczuk et al., 2009). A recent study demonstrated that IRAK-M, a negative regulator of IL-1R1 signalling, was upregulated in both macrophages and fibroblasts infiltrating the infarcted heart (Chen et al., 2012). As well as being important for preventing excessive inflammatory activation of macrophages, IRAK-M also appeared to restrain fibroblast-derived matrix-degrading activity (Chen et al., 2012).

The above findings, together with a wealth of other pre-clinical data (reviewed by (Bujak and Frangogiannis, 2009; Dinarello et al., 2012; Abbate et al., 2012), suggest that inhibition of IL-1 signalling may be an effective therapeutic strategy for improving cardiac function and outcome following MI (Abbate et al., 2010b; Van Tassell et al., 2010). Subsequent clinical trials have evaluated one such approach in MI patients, namely subcutaneous injection of a recombinant form of the human IL-1RA, Anakinra. In these randomised studies, patients were injected with a single daily dose of 100 mg Anakinra or placebo over a 2-week period. In the VCU-ART small pilot study of 10 patients with acute myocardial infarction with ST-segment elevation (STEMI), Anakinra administered within a few hours of the onset of symptoms appeared to have beneficial effects on cardiac remodelling (Abbate et al., 2010a). However, in the recently reported VCU-ART2 follow-up

trial with 30 clinically stable STEMI patients, early Anakinra administration did not show significant improvement in cardiac function although it did blunt the acute inflammatory response (Abbate et al., 2013). The outcome of the larger MRC-ILA-HEART study in 182 patients with smaller infarcts (non-STEMI) treated with or without Anakinra (Crossman et al., 2008) is expected soon, however this also appears likely to produce disappointing results (Morton et al., 2011). Thus, despite encouraging pre-clinical data, these preliminary findings in MI patients suggest that blanket inhibition of IL-1 signalling does not offer the anticipated beneficial effects on cardiac remodelling.

The discrepancy between these early clinical results and the strong preclinical animal data obtained with recombinant IL-1RA may relate in part to the larger severity of cardiac remodelling induced in experimental MI models compared with the relatively low level of LV dysfunction observed in the STEMI (Abbate et al., 2010a; Abbate et al., 2013) or non-STEMI (Crossman et al., 2008; Morton et al., 2011) patient cohorts studied. Whether more pronounced beneficial effects of Anakinra would be observed with a group of patients at greater risk of severe cardiac remodelling remains to be determined. It is also a possibility that more targeted approaches aimed at IL-1 responses in individual cardiac cell types may offer superior outcomes, and pre-clinical studies are needed to evaluate this hypothesis.

4. Effects of Interleukin-1 on Cardiac Fibroblast Function

Studying the effect of IL-1 on CF function *in vivo* is complicated because of the interplay between different cell types through intercellular autocrine/paracrine signalling. Thus, much of our knowledge on the direct effects of IL-1 on CF has come from *in vitro* cell culture studies. Whilst these systems are some way from the physiological situation they are useful in that they allow direct transcriptional and functional responses to be measured, as well as the underlying molecular mechanisms. Many studies have used neonatal rat CF as a convenient

model system, although these cells may exhibit significant differences compared with adult cells, and compared with CF derived from other species (Porter and Turner, 2009). In our laboratory we have studied adult human CF cultured from different patients to gain a more accurate picture of the mechanisms underlying CF function in man.

4.1. Cell proliferation, migration and differentiation

For several years, the general consensus has been that IL-1 inhibits CF proliferation, although most of the evidence for this notion comes from studies using cultured neonatal rat CF (Palmer et al., 1995; Koudssi et al., 1998; Piacentini et al., 2000; Xiao et al., 2008). In contrast, some recent reports have described increased proliferation of neonatal and adult rat CF in response to IL-1 (He et al., 2011; Szardien et al., 2012), although the reasons for these opposing results are not immediately apparent. We are not aware of any reports describing the effects of IL-1 on human CF proliferation.

IL-1 is a chemoattractant for several cell types and has been shown to be a potent inducer of *in vitro* cell migration of both neonatal and adult rat CF (Mitchell et al., 2007; Brown et al., 2007), as well as adult human CF (Mughal et al., 2009).

IL-1β was shown to reduce myofibroblast differentiation (i.e. the proportion of αSMA-positive cells) in a recent study on neonatal rat CF (Szardien et al., 2012). Moreover, we recently demonstrated that IL-1α, even at relatively low concentrations (0.1 ng/ml), could oppose the ability of TGFβ to induce fibroblast-to-myofibroblast differentiation (van Nieuwenhoven et al., 2013). IL-1α caused a marked reduction in TGFβ-induced αSMA mRNA and protein expression in human CF and also reduced their ability to contract collagen gels, a functional assay for myofibroblast activity (van Nieuwenhoven et al., 2013).

4.2. Inflammatory gene expression in CF

In order to create an overview of the many effects of IL-1 on CF function we have undertaken several studies in recent years to investigate IL-1α-induced changes in gene expression in human CF. Cells were cultured from atrial tissue biopsies from multiple patients undergoing coronary artery bypass surgery and were found to consistently co-express vimentin and αSMA throughout culture over several passages (Mughal et al., 2009), characteristic of the myofibroblast phenotype. We studied the effects of IL-1α on expression of proinflammatory cytokines, chemokines, adhesion molecules, angiogenic factors and molecules involved in ECM synthesis and degradation **(Figure 2)**. IL-1α was found to be a potent stimulator of several proinflammatory cytokines including IL-1α, IL-1β, TNFα, IL-6 and leukaemia inhibitory factor (LIF), but not the other IL-6 family members cardiotrophin-1 or oncostatin M (Turner et al., 2009). IL-1α also stimulated gene expression and secretion of the neutrophil-attracting CXC chemokines CXCL-1 (KC/GROα), CXCL-2 (MIP-2a/GROβ), CXCL-5 (ENA-78) and CXCL-8 (IL-8) (Turner et al., 2011). Using a focused RT-PCR microarray we identified a number of inflammatory adhesion molecules that were also upregulated following IL-1α stimulation, including ICAM-1, VCAM-1, E-selectin and to a lesser extent the integrin β3 subunit and CD44 (Turner et al., 2011). Another important myocardial proinflammatory cytokine, TNFα, was able to stimulate a similar profile of cytokines, chemokines and adhesion molecules in human CF; however it was consistently less potent than IL-1α (Turner et al., 2007; Turner et al., 2009; Turner et al., 2011). This relative sensitivity of CF to IL-1 is in agreement with previous reports that fibroblasts in the heart are particularly responsive to IL-1 compared with other cytokines, and compared with other sources of fibroblasts (Brown et al., 2005).

4.3. Synthesis and degradation of ECM by CF

We used a focused RT-PCR microarray to evaluate mRNA levels of structural ECM proteins, MMPs and TIMPs in cultured human CF and their modulation by IL-1α (Turner et al., 2010). Human CF expressed relatively high basal levels of multiple ECM proteins under basal levels including collagens I, IV, V, VI, VIII, XII and XIV, laminins α2, β1 and γ1 and fibronectin, but none of these were markedly affected by IL-1 stimulation over a 6 h period (Turner et al., 2010). It is possible that IL-1α may modulate expression of some ECM proteins at later time points, particularly as we have since observed a 20% reduction in COL1A1 mRNA expression 24 h after stimulation with 10 ng/ml IL-1α; an inhibition that was not evident after 6 h (N.A. Turner, unpublished observation). This finding is in agreement with earlier studies in neonatal and adult rat CF (Siwik et al., 2000; Xiao et al., 2008), and appears to be specific for fibrillar collagens. Interestingly, we also reported recently that IL-1α can rapidly (within 4 h) and potently (by up to 80%) reduce protein levels of the profibrotic matricellular protein CTGF/CCN2 in human CF (Maqbool et al., 2013). It is therefore possible that the delayed inhibitory effect of IL-1 on COL1A1 gene expression occurs indirectly via an autocrine/paracrine feedback loop involving down-regulation of profibrotic factors such as CTGF/CCN2.

CF are capable of expressing several, but not all, members of the MMP family in a regulated manner (Turner and Porter, 2012). In our own studies, human CF were found to express particularly high basal mRNA levels of MMP-1 (interstitial collagenase), MMP-2 (gelatinase A), MMP-14 (MT1-MMP) and MMP-16 (MT3-MMP), and even higher levels of TIMP-1 and TIMP-2, but not TIMP-3 (Turner et al., 2010). Exposure of cells to IL-1α (10 ng/ml, 6 h) elicited 80-200-fold increases in mRNA levels of MMP-3 (stromelysin 1), MMP-9 (gelatinase B) and MMP-10 (stromelysin 2), and an 8-fold increase in MMP-1, while TIMP-2 mRNA levels were increased by a modest 2-fold (Turner et al., 2010). IL-1 was also previously reported to stimulate MMP-3 and MMP-9 expression in neonatal and adult rat CF

(Siwik et al., 2000; Brown et al., 2007). Studies using human and rat CF have reported additional stimulatory effects of IL-1 on MMP-2 expression and activity (Siwik et al., 2000; Brown et al., 2007; Guo et al., 2008), although we did not observe this in our own shorter-term studies (Turner et al., 2010).

Protein and mRNA expression levels of ADAMTS1, a protease that is a potent inhibitor of angiogenesis, were rapidly reduced following IL-1α treatment (Turner et al., 2010), and this finding coupled with the up-regulation of VEGF mRNA by IL-1α (Turner et al., 2010) would suggest a pro-angiogenic effect of IL-1α at the level of the fibroblast.

Taking all these data together, we propose that by modulating expression of a specific subset of genes, IL-1α can stimulate human CF to adopt a pro-inflammatory, pro-angiogenic, pro-migratory, ECM degrading phenotype, whilst suppressing myofibroblast differentiation **(Figure 2)**. These findings are of particular relevance to the early inflammatory phase of post-MI infarct healing when IL-1 levels are elevated, prior to myofibroblast activation, ECM synthesis and scar maturation (van Nieuwenhoven and Turner, 2013; Chen and Frangogiannis, 2012).

4.4. IL-1 signalling in CF

IL-1α/β stimulates all the major canonical kinase signalling cascades in CF **(Figure 1)**, including the ERK, JNK, p38, PI3K/Akt and NFκB pathways (Xie et al., 2004; Mitchell et al., 2007; Turner et al., 2009). All of these pathways have also been shown to be activated in the myocardium early after experimental MI; see recent comprehensive reviews on NF-κB (Gordon et al., 2011), p38 (Marber et al., 2011; Turner, 2011), JNK (Turner, 2011), ERK and Akt; the latter being components of the reperfusion injury salvage kinase (RISK) pathway that is considered cardioprotective (Hausenloy et al., 2005). However, these studies do not shed particular light on IL-1 signalling in CF due to the heterogeneity of cell types in the

heart and the multitude of potential stimuli for these pathways after MI (in addition to IL-1). Only a very few *in vivo* studies have attempted to study signalling pathways at a cellular level post-MI. One particularly good example of this is a recent study by Yeh and colleagues that used a permanent LAD occlusion model to reveal differential temporal changes in ERK and p38 (and to a lesser extent JNK and Akt) activation in distinct cell types in infarcted and remote areas of the mouse myocardium over a 1-12 week period (Yeh et al., 2010). Clearly the specific role of IL-1-induced signalling pathways in CF *in vivo* is extremely difficult to evaluate and hence most of our current understanding has been obtained from *in vitro* CF cultures treated with IL-1.

We used pharmacological inhibitors to delineate the pathways responsible for mediating increases in gene expression in cultured human CF (Turner et al., 2009; Turner et al., 2010; Turner et al., 2011). These studies (summarised in **Table 1**) revealed major roles for the NFκB and p38 MAPK pathways in mediating IL-1α-induced expression of multiple proinflammatory genes. NFκB-regulated genes included IL-1β, TNFα, IL-8, ICAM-1 and E-selectin. Although we did not evaluate the role of NFκB signalling in mediating expression of MMPs in human CF, previous studies suggest that several of the major MMPs expressed by CF (including MMPs 1, 3 and 9) are regulated via this pathway due to NFκB binding sites in their gene promoter regions (Turner and Porter, 2012).

The p38 MAPK pathway plays an important role in regulating various aspects of the myocardial remodelling process, including CF function (Marber et al., 2011; Turner, 2011). The IL-1α-induced, p38-regulated genes that we identified using the p38-α/β-selective inhibitor SB203580 included IL-6, IL-8, TNFα and MMP-3 **(Table 1)**. We therefore investigated the role of individual p38 MAPK subtypes in mediating IL-1α-induced IL-6 and MMP-3 gene expression in human CF (Sinfield et al., 2013). Quantitative RT-PCR and Western blotting of cells from multiple patients revealed a consistent pattern of p38 subtype

expression in human CF, with p38-α being most abundant, followed by p38-γ and p38-δ, and little or no expression of p38-β (Sinfield et al., 2013). This pattern of subtype expression is similar to that of whole heart tissue from adult humans (Lemke et al., 2001). Treatment of human CF with IL-1α led to selective activation of p38-α and p38-γ (Sinfield et al., 2013). Moreover, the p38-α/β-selective inhibitor SB203580 ablated IL-1α-induced IL-6 and MMP-3 gene expression (Turner et al., 2009; Turner et al., 2010). We subsequently confirmed a specific role for p38-α in mediating the effects of IL-1α on IL-6 and MMP-3 expression and secretion in these cells by using an siRNA gene silencing strategy to selectively knock down the p38-α subtype (Sinfield et al., 2013).

4.5. Important considerations

In the course of these studies we have noted some potentially important differences in the signalling pathways regulating expression of specific genes in human CF compared with CF from other species. For example in our studies using human CF, IL-1-induced MMP-3 expression was found to be p38-dependent whereas IL-1-induced MMP-9 expression was p38-independent (Turner et al., 2010; Sinfield et al., 2013). However, the exact opposite was previously reported in adult rat CF in which IL-1-induced MMP-3 expression was p38-independent and IL-1-induced MMP-9 expression was p38-dependent (Brown et al., 2007). Whether such discrepancies relate to genuine differences in regulatory mechanisms between different species is an important issue that requires clarification as this could be an important consideration when modelling human disease.

It is worth noting that our studies on human CF used atrial fibroblasts from multiple patients with coronary artery disease. It is feasible that CF from different regions of the heart may exhibit differences in their functional responses, as has been reported for canine cells (Burstein et al., 2008). It is also possible that CF derived from our cohort of donors may have

modified function compared with those from "healthy" subjects, although we have no evidence to support this notion.

Another consideration that should be highlighted is that under physiological conditions, CF are exposed to a plethora of bioactive molecules and the net effect on cell function depends on relative amounts of these stimulatory and inhibitory signals that vary in a spatiotemporal manner. Therefore whilst we can gain mechanistic insights from studying responses to a single stimulus such as IL-1 in isolation, we should keep in mind that this is a vast over-simplification of the *in vivo* scenario. Indeed, we have recently reported that even when studying just two cytokines (IL-1α and TGFβ), a complex interplay between factors becomes evident at the level of gene expression and human CF function (van Nieuwenhoven et al., 2013).

5. Conclusions

Fibroblasts play a critical role in many aspects of post-MI remodelling and as such represent a viable therapeutic target for reducing adverse cardiac remodelling and its devastating consequences. Understanding the origin, role and regulation of CF in the remodelling heart is therefore imperative to guide such strategies. IL-1 is a proinflammatory cytokine that is an early trigger of post-MI remodelling and has multiple effects on CF function. Emerging clinical studies suggest that blanket inhibition of IL-1 signalling does not offer beneficial effects on post-MI remodelling in man. However, more targeted approaches aimed at the IL-1/CF axis may offer superior outcomes, and pre-clinical studies are needed to evaluate this hypothesis.

References

Abbate, A., Kontos, M.C., Grizzard, J.D., Biondi-Zoccai, G.G., Van Tassell, B.W., Robati, R., Roach, L.M., Arena, R.A., Roberts, C.S., Varma, A., Gelwix, C.C., Salloum, F.N., Hastillo, A., Dinarello, C.A., Vetrovec, G.W., 2010a. Interleukin-1 blockade with anakinra to prevent adverse cardiac remodeling after acute myocardial infarction (Virginia Commonwealth University Anakinra Remodeling Trial [VCU-ART] Pilot study). Am. J. Cardiol. 105, 1371-1377.

Abbate, A., Van Tassell, B.W., Biondi-Zoccai, G., Kontos, M.C., Grizzard, J.D., Spillman, D.W., Oddi, C., Roberts, C.S., Melchior, R.D., Mueller, G.H., Abouzaki, N.A., Rengel, L.R., Varma, A., Gambill, M.L., Falcao, R.A., Voelkel, N.F., Dinarello, C.A., Vetrovec, G.W., 2013. Effects of interleukin-1 blockade with Anakinra on adverse cardiac remodeling and heart failure after acute myocardial infarction [from the Virginia Commonwealth University-Anakinra Remodeling Trial 2 (VCU-ART2) Pilot Study]. Am. J. Cardiol.

Abbate, A., Van Tassell, B.W., Biondi-Zoccai, G.G., 2012. Blocking interleukin-1 as a novel therapeutic strategy for secondary prevention of cardiovascular events. BioDrugs. 26, 217-233.

Abbate, A., Van Tassell, B.W., Seropian, I.M., Toldo, S., Robati, R., Varma, A., Salloum, F.N., Smithson, L., Dinarello, C.A., 2010b. Interleukin-1beta modulation using a genetically engineered antibody prevents adverse cardiac remodelling following acute myocardial infarction in the mouse. Eur. J. Heart Fail. 12, 319-322.

Adachi, O., Kawai, T., Takeda, K., Matsumoto, M., Tsutsui, H., Sakagami, M., Nakanishi, K., Akira, S., 1998. Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity. 9, 143-150.

Arend, W.P., Palmer, G., Gabay, C., 2008. IL-1, IL-18, and IL-33 families of cytokines. Immunol. Rev. 223, 20-38.

Arslan, F., de Kleijn, D.P., Pasterkamp, G., 2011. Innate immune signaling in cardiac ischemia. Nat. Rev. Cardiol. 8, 292-300.

Banerjee, I., Fuseler, J.W., Price, R.L., Borg, T.K., Baudino, T.A., 2007. Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse. Am. J. Physiol Heart Circ. Physiol 293, H1883-H1891.

Blyszczuk, P., Kania, G., Dieterle, T., Marty, R.R., Valaperti, A., Berthonneche, C., Pedrazzini, T., Berger, C.T., Dirnhofer, S., Matter, C.M., Penninger, J.M., Luscher, T.F., Eriksson, U., 2009. Myeloid differentiation factor-88/interleukin-1 signaling controls cardiac fibrosis and heart failure progression in inflammatory dilated cardiomyopathy. Circ. Res. 105, 912-920.

Brikos, C., Wait, R., Begum, S., O'Neill, L.A., Saklatvala, J., 2007. Mass spectrometric analysis of the endogenous type I interleukin-1 (IL-1) receptor signaling complex formed after IL-1 binding identifies IL-1RAcP, MyD88, and IRAK-4 as the stable components. Mol. Cell Proteomics. 6, 1551-1559.

Brown, R.D., Ambler, S.K., Mitchell, M.D., Long, C.S., 2005. The cardiac fibroblast: therapeutic target in myocardial remodeling and failure. Annu. Rev. Pharmacol. Toxicol. 45, 657-687.

Brown, R.D., Jones, G.M., Laird, R.E., Hudson, P., Long, C.S., 2007. Cytokines regulate matrix metalloproteinases and migration in cardiac fibroblasts. Biochem. Biophys. Res. Commun. 362, 200-205.

Bujak, M., Dobaczewski, M., Chatila, K., Mendoza, L.H., Li, N., Reddy, A., Frangogiannis, N.G., 2008. Interleukin-1 receptor type I signaling critically regulates infarct healing and cardiac remodeling. Am. J. Pathol. 173, 57-67.

Bujak, M., Frangogiannis, N.G., 2009. The role of IL-1 in the pathogenesis of heart disease. Arch. Immunol. Ther. Exp. (Warsz.) 57, 165-176.

Burstein, B., Libby, E., Calderone, A., Nattel, S., 2008. Differential behaviors of atrial versus ventricular fibroblasts: a potential role for platelet-derived growth factor in atrial-ventricular remodeling differences. Circulation 117, 1630-1641.

Camelliti, P., Green, C.R., Kohl, P., 2006. Structural and functional coupling of cardiac myocytes and fibroblasts. Adv. Cardiol. 42, 132-149.

Chen, C.J., Kono, H., Golenbock, D., Reed, G., Akira, S., Rock, K.L., 2007. Identification of a key pathway required for the sterile inflammatory response triggered by dying cells. Nat. Med. 13, 851-856.

Chen, W., Frangogiannis, N.G., 2012. Fibroblasts in post-infarction inflammation and cardiac repair. Biochim. Biophys. Acta.

Chen, W., Saxena, A., Li, N., Sun, J., Gupta, A., Lee, D.W., Tian, Q., Dobaczewski, M., Frangogiannis, N.G., 2012. Endogenous IRAK-M attenuates postinfarction remodeling through effects on macrophages and fibroblasts. Arterioscler. Thromb. Vasc. Biol. 32, 2598-2608.

Crossman, D.C., Morton, A.C., Gunn, J.P., Greenwood, J.P., Hall, A.S., Fox, K.A., Lucking, A.J., Flather, M.D., Lees, B., Foley, C.E., 2008. Investigation of the effect of Interleukin-1 receptor antagonist (IL-1ra) on markers of inflammation in non-ST elevation acute coronary syndromes (The MRC-ILA-HEART Study). Trials 9, 8.

Davis, C.N., Mann, E., Behrens, M.M., Gaidarova, S., Rebek, M., Rebek, J., Jr., Bartfai, T., 2006. MyD88-dependent and -independent signaling by IL-1 in neurons probed by bifunctional Toll/IL-1 receptor domain/BB-loop mimetics. Proc. Natl. Acad. Sci. U. S. A 103, 2953-2958.

Demyanets, S., Kaun, C., Pentz, R., Krychtiuk, K.A., Rauscher, S., Pfaffenberger, S., Zuckermann, A., Aliabadi, A., Groger, M., Maurer, G., Huber, K., Wojta, J., 2013. Components of the interleukin-33/ST2 system are differentially expressed and regulated in human cardiac cells and in cells of the cardiac vasculature. J. Mol. Cell Cardiol. In press.

Deten, A., Volz, H.C., Briest, W., Zimmer, H.G., 2002. Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats. Cardiovasc. Res. 55, 329-340.

Dewald, O., Ren, G., Duerr, G.D., Zoerlein, M., Klemm, C., Gersch, C., Tincey, S., Michael, L.H., Entman, M.L., Frangogiannis, N.G., 2004. Of mice and dogs: species-specific differences in the inflammatory response following myocardial infarction. Am. J. Pathol. 164, 665-677.

Dinarello, C.A., 2005. The many worlds of reducing interleukin-1. Arthritis Rheum. 52, 1960-1967.

Dinarello, C.A., 2011. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. Blood 117, 3720-3732.

Dinarello, C.A., Simon, A., van der Meer, J.W., 2012. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. Nat. Rev. Drug Discov. 11, 633-652.

Fernandez-Velasco, M., Prieto, P., Terron, V., Benito, G., Flores, J.M., Delgado, C., Zaragoza, C., Lavin, B., Gomez-Parrizas, M., Lopez-Collazo, E., Martin-Sanz, P., Bosca, L., 2012. NOD1 activation induces cardiac dysfunction and modulates cardiac fibrosis and cardiomyocyte apoptosis. PLoS. One. 7, e45260.

Flannery, S., Bowie, A.G., 2010. The interleukin-1 receptor-associated kinases: critical regulators of innate immune signalling. Biochem. Pharmacol. 80, 1981-1991.

Frangogiannis, N.G., 2008. The immune system and cardiac repair. Pharmacol. Res. 58, 88-111.

Gordon, J.W., Shaw, J.A., Kirshenbaum, L.A., 2011. Multiple facets of NF-κB in the heart: to be or not to NF-κB. Circ. Res. 108, 1122-1132.

Guillen, I., Blanes, M., Gomez-Lechon, M.J., Castell, J.V., 1995. Cytokine signaling during myocardial infarction: sequential appearance of IL-1 beta and IL-6. Am. J. Physiol 269, R229-R235.

Guo, X.G., Uzui, H., Mizuguchi, T., Ueda, T., Chen, J.Z., Lee, J.D., 2008. Imidaprilat inhibits matrix metalloproteinase-2 activity in human cardiac fibroblasts induced by interleukin-1β via NO-dependent pathway. Int. J. Cardiol. 126, 414-420.

Hausenloy, D.J., Tsang, A., Yellon, D.M., 2005. The reperfusion injury salvage kinase pathway: a common target for both ischemic preconditioning and postconditioning. Trends Cardiovasc. Med. 15, 69-75.

He, Y.H., Zhang, H.N., Zhang, G.P., Hou, N., Xiao, Q., Huang, Y., Wu, J.H., Luo, M.S., Zhang, G.S., Yi, Q., Chen, M.S., Luo, J.D., 2011. A physiological concentration of glucocorticoid inhibits the pro-inflammatory cytokine-induced proliferation of adult rat cardiac fibroblasts: roles of extracellular signal-regulated kinase 1/2 and nuclear factor-kappaB. Clin. Exp. Pharmacol. Physiol 38, 739-746.

Isoda, K., Kamezawa, Y., Tada, N., Sato, M., Ohsuzu, F., 2001. Myocardial hypertrophy in transgenic mice overexpressing human interleukin 1α. J. Card Fail. 7, 355-364.

Jugdutt, B.I., 2003. Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? Circulation. 108, 1395-1403.

Kawaguchi, M., Takahashi, M., Hata, T., Kashima, Y., Usui, F., Morimoto, H., Izawa, A., Takahashi, Y., Masumoto, J., Koyama, J., Hongo, M., Noda, T., Nakayama, J., Sagara, J., Taniguchi, S., Ikeda, U., 2011. Inflammasome activation of cardiac fibroblasts is essential for myocardial ischemia/reperfusion injury. Circulation 123, 594-604.

Ko, N.Y., Mun, S.H., Lee, S.H., Kim, J.W., Kim, d.K., Kim, H.S., Her, E., Kim, S.H., Won, H.S., Shin, H.S., Kim, H.S., Kim, Y.M., Choi, W.S., 2011. Interleukin-32α production is regulated by MyD88-dependent and independent pathways in IL-1β-stimulated human alveolar epithelial cells. Immunobiology 216, 32-40.

Koudssi, F., Lopez, J.E., Villegas, S., Long, C.S., 1998. Cardiac fibroblasts arrest at the G1/S restriction point in response to interleukin (IL)-1β. Evidence for IL-1β-induced hypophosphorylation of the retinoblastoma protein. J. Biol. Chem. 273, 25796-25803.

Krenning, G., Zeisberg, E.M., Kalluri, R., 2010. The origin of fibroblasts and mechanism of cardiac fibrosis. J. Cell Physiol 225, 631-637.

LaFramboise, W.A., Scalise, D., Stoodley, P., Graner, S.R., Guthrie, R.D., Magovern, J.A., Becich, M.J., 2007. Cardiac fibroblasts influence cardiomyocyte phenotype in vitro. Am. J. Physiol Cell Physiol 292, C1799-C1808.

Lamkanfi, M., 2011. Emerging inflammasome effector mechanisms. Nat. Rev. Immunol. 11, 213-220.

Lemke, L.E., Bloem, L.J., Fouts, R., Esterman, M., Sandusky, G., Vlahos, C.J., 2001. Decreased p38 MAPK activity in end-stage failing human myocardium: p38 MAPK α is the predominant isoform expressed in human heart. J. Mol. Cell Cardiol. 33, 1527-1540.

Long, C.S., 2001. The role of interleukin-1 in the failing heart. Heart Fail. Rev. 6, 81-94.

Maqbool, A., Hemmings, K.E., O'Regan, D.J., Ball, S.G., Porter, K.E., Turner, N.A., 2013. Interleukin-1 has opposing effects on connective tissue growth factor and tenascin-C expression in human cardiac fibroblasts. Matrix Biol. 32, 208-214.

Marber, M.S., Rose, B., Wang, Y., 2011. The p38 mitogen-activated protein kinase pathway-A potential target for intervention in infarction, hypertrophy, and heart failure. J. Mol. Cell Cardiol. 51, 485-490.

Mezzaroma, E., Toldo, S., Farkas, D., Seropian, I.M., Van Tassell, B.W., Salloum, F.N., Kannan, H.R., Menna, A.C., Voelkel, N.F., Abbate, A., 2011. The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse. Proc. Natl. Acad. Sci. U. S. A 108, 19725-19730.

Mitchell, M.D., Laird, R.E., Brown, R.D., Long, C.S., 2007. IL-1β stimulates rat cardiac fibroblast migration via MAP kinase pathways. Am. J. Physiol Heart Circ. Physiol 292, H1139-H1147.

Morton, A.C., Foley, C., Rothman, A., Gunn, J., Greenwood, J.P., Hall, A., Fox, K., Lees, B., Flather, M., Crossman, D., 2011. Investigation of IL-1 inhibition in patients presenting with non-ST elevation myocardial infarction acute coronary syndromes (the MRC ILA Heart Study). Heart 97, A13 (abstract) doi:10.1136/heartjnl-2011-300198.15.

Mughal, R.S., Warburton, P., O'Regan, D.J., Ball, S.G., Turner, N.A., Porter, K.E., 2009. Peroxisome proliferator-activated receptor γ-independent effects of thiazolidinediones on human cardiac myofibroblast function. Clin. Exp. Pharmacol. Physiol. 36, 478-486.

Nishikawa, K., Yoshida, M., Kusuhara, M., Ishigami, N., Isoda, K., Miyazaki, K., Ohsuzu, F., 2006. Left ventricular hypertrophy in mice with a cardiac-specific overexpression of interleukin-1. Am. J. Physiol Heart Circ. Physiol 291, H176-H183.

O'Neill, L.A., 2008. When signaling pathways collide: positive and negative regulation of toll-like receptor signal transduction. Immunity. 29, 12-20.

Palmer, J.N., Hartogensis, W.E., Patten, M., Fortuin, F.D., Long, C.S., 1995. Interleukin-1β induces cardiac myocyte growth but inhibits cardiac fibroblast proliferation in culture. J. Clin. Invest 95, 2555-2564.

Peterson, D.J., Ju, H., Hao, J., Panagia, M., Chapman, D.C., Dixon, I.M., 1999. Expression of Gi-2 alpha and Gs alpha in myofibroblasts localized to the infarct scar in heart failure due to myocardial infarction. Cardiovasc. Res. 41, 575-585.

Piacentini, L., Gray, M., Honbo, N.Y., Chentoufi, J., Bergman, M., Karliner, J.S., 2000. Endothelin-1 stimulates cardiac fibroblast proliferation through activation of protein kinase C. J. Mol. Cell Cardiol. 32, 565-576.

Porter, K.E., Turner, N.A., 2009. Cardiac fibroblasts: at the heart of myocardial remodeling. Pharmacol. Ther. 123, 255-278.

Sinfield, J.K., Das, A., O'Regan, D.J., Ball, S.G., Porter, K.E., Turner, N.A., 2013. p38 MAPK alpha mediates cytokine-induced IL-6 and MMP-3 expression in human cardiac fibroblasts. Biochem. Biophys. Res. Commun. 430, 419-424.

Siwik, D.A., Chang, D.L., Colucci, W.S., 2000. Interleukin-1β and tumor necrosis factor-α decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. Circ. Res. 86, 1259-1265.

Strand, M.E., Herum, K.M., Rana, Z.A., Skrbic, B., Askevold, E.T., Dahl, C.P., Vistnes, M., Hasic, A., Kvaloy, H., Sjaastad, I., Carlson, C.R., Tonnessen, T., Gullestad, L., Christensen, G., Lunde, I.G., 2013. Innate immune signaling induces expression and shedding of the heparan sulfate proteoglycan syndecan-4 in cardiac fibroblasts and myocytes, affecting inflammation in the pressure-overloaded heart. FEBS J.

Szardien, S., Nef, H.M., Voss, S., Troidl, C., Liebetrau, C., Hoffmann, J., Rauch, M., Mayer, K., Kimmich, K., Rolf, A., Rixe, J., Troidl, K., Kojonazarov, B., Schermuly, R.T., Kostin, S., Elsasser, A., Hamm, C.W., Mollmann, H., 2012. Regression of cardiac hypertrophy by granulocyte colony-stimulating factor-stimulated interleukin-1β synthesis. Eur. Heart J. 33, 595-605.

Timmers, L., Sluijter, J.P., van Keulen, J.K., Hoefer, I.E., Nederhoff, M.G., Goumans, M.J., Doevendans, P.A., van Echteld, C.J., Joles, J.A., Quax, P.H., Piek, J.J., Pasterkamp, G., de Kleijn, D.P., 2008. Toll-like receptor 4 mediates maladaptive left ventricular remodeling and impairs cardiac function after myocardial infarction. Circ. Res. 102, 257-264.

Tomasek, J.J., Gabbiani, G., Hinz, B., Chaponnier, C., Brown, R.A., 2002. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat. Rev. Mol. Cell Biol. 3, 349-363.

Turner, N.A., 2011. Therapeutic regulation of cardiac fibroblast function: targeting stress-activated protein kinase pathways. Fut. Cardiol. 7, 673-691.

Turner, N.A., Das, A., O'Regan, D.J., Ball, S.G., Porter, K.E., 2011. Human cardiac fibroblasts express ICAM-1, E-selectin and CXC chemokines in response to proinflammatory cytokine stimulation. Int. J. Biochem. Cell Biol. 43, 1450-1458.

Turner, N.A., Das, A., Warburton, P., O'Regan, D.J., Ball, S.G., Porter, K.E., 2009. Interleukin-1α stimulates pro-inflammatory cytokine expression in human cardiac myofibroblasts. Am. J. Physiol Heart Circ. Physiol 297, H1117-H1127.

Turner, N.A., Mughal, R.S., Warburton, P., O'Regan, D.J., Ball, S.G., Porter, K.E., 2007. Mechanism of TNFα-induced IL-1α, IL-1β and IL-6 expression in human cardiac fibroblasts: Effects of statins and thiazolidinediones. Cardiovasc. Res. 76, 81-90.

Turner, N.A., Porter, K.E., 2012. Regulation of myocardial matrix metalloproteinase expression and activity by cardiac fibroblasts. IUBMB. Life 64, 143-150.

Turner, N.A., Porter, K.E., 2013. Function and fate of myofibroblasts after myocardial infarction. Fibrogenesis. Tissue Repair 6, 5.

Turner, N.A., Warburton, P., O'Regan, D.J., Ball, S.G., Porter, K.E., 2010. Modulatory effect of interleukin-1α on expression of structural matrix proteins, MMPs and TIMPs in human cardiac myofibroblasts: role of p38 MAP kinase. Matrix Biol. 29, 613-620.

van den Borne, S.W., Diez, J., Blankesteijn, W.M., Verjans, J., Hofstra, L., Narula, J., 2010. Myocardial remodeling after infarction: the role of myofibroblasts. Nat. Rev. Cardiol. 7, 30-37.

van Nieuwenhoven, F.A., Hemmings, K.E., Porter, K.E., Turner, N.A., 2013. Combined effects of interleukin-1α and transforming growth factor-β1 on modulation of human cardiac fibroblast function. Matrix Biol. In press doi: 10.1016/j.matbio.2013.03.008.

van Nieuwenhoven, F.A., Turner, N.A., 2013. The role of cardiac fibroblasts in the transition from inflammation to fibrosis following myocardial infarction. Vascul. Pharmacol. 58, 182-188.

Van Tassell, B.W., Varma, A., Salloum, F.N., Das, A., Seropian, I.M., Toldo, S., Smithson, L., Hoke, N.N., Chau, V.Q., Robati, R., Abbate, A., 2010. Interleukin-1 trap attenuates cardiac remodeling after experimental acute myocardial infarction in mice. J. Cardiovasc. Pharmacol. 55, 117-122.

Weber, A., Wasiliew, P., Kracht, M., 2010. Interleukin-1 (IL-1) pathway. Sci. Signal. 3, cm1.

Werman, A., Werman-Venkert, R., White, R., Lee, J.K., Werman, B., Krelin, Y., Voronov, E., Dinarello, C.A., Apte, R.N., 2004. The precursor form of IL-1α is an intracrine proinflammatory activator of transcription. Proc. Natl. Acad. Sci. U. S. A 101, 2434-2439.

Westphal, E., Li, C., Pilowski, C., Koch, S., Ebelt, H., Muller-Werdan, U., Werdan, K., Loppnow, H., 2007. Endotoxin-activated cultured neonatal rat cardiomyocytes express functional surface-associated interleukin-1α. J. Endotoxin. Res. 13, 25-34.

Willems, I.E., Havenith, M.G., De Mey, J.G., Daemen, M.J., 1994. The alpha-smooth muscle actin-positive cells in healing human myocardial scars. Am. J. Pathol. 145, 868-875.

Xiao, H., Ji, A.M., Li, Z.L., Song, X.D., Su, D., Chen, A.H., 2008. Interleukin-1β inhibits collagen synthesis and promotes its decomposition in cultured cardiac fibroblasts. Sheng Li Xue. Bao. 60, 355-361.

Xie, Z., Singh, M., Singh, K., 2004. Differential regulation of matrix metalloproteinase-2 and -9 expression and activity in adult rat cardiac fibroblasts in response to interleukin-1β. J. Biol. Chem. 279, 39513-39519.

Yeh, C.C., Li, H., Malhotra, D., Turcato, S., Nicholas, S., Tu, R., Zhu, B.Q., Cha, J., Swigart, P.M., Myagmar, B.E., Baker, A.J., Simpson, P.C., Mann, M.J., 2010. Distinctive ERK and p38 signaling in remote and infarcted myocardium during post-MI remodeling in the mouse. J. Cell Biochem. 109, 1185-1191.

Zeisberg, E.M., Kalluri, R., 2010. Origins of cardiac fibroblasts. Circ. Res. 107, 1304-1312.

Zhang, X., Azhar, G., Nagano, K., Wei, J.Y., 2001. Differential vulnerability to oxidative stress in rat cardiac myocytes versus fibroblasts. J. Am. Coll. Cardiol. 38, 2055-2062.

Zhao, L., Eghbali-Webb, M., 2001. Release of pro- and anti-angiogenic factors by human cardiac fibroblasts: effects on DNA synthesis and protection under hypoxia in human endothelial cells. Biochim. Biophys. Acta 1538, 273-282.

Zheng, Y., Gardner, S.E., Clarke, M.C., 2011. Cell death, damage-associated molecular patterns, and sterile inflammation in cardiovascular disease. Arterioscler. Thromb. Vasc. Biol. 31, 2781-2786.

Figure Legends

Figure 1. IL-1 receptor signalling. IL-1α or IL-β binding to the IL-1 receptor (IL-1R1) stimulates recruitment of the IL-1 receptor accessory protein (IL-1RAcP). This in turn facilitates association with MyD88 (myeloid differentiation factor 88) and IRAK4 (IL-1 receptor-activated protein kinase 4) signalling proteins. Activation of MAP kinase (ERK, p38, JNK), NFκB and PI-3-kinase/Akt pathways occur in a MyD88-dependent manner after recruitment of additional adaptor proteins and kinases. Activation of the Akt pathway can also be MyD88-independent in some cell types.

Figure 2. Effects of IL-1 on human cardiac fibroblast function. IL-1 modulates expression of specific genes in human CF to promote a pro-inflammatory, pro-angiogenic, pro-migratory, ECM degrading, non-differentiated fibroblast phenotype. Figure summarises results previously published by our group using human CF from multiple patients (Mughal et al., 2009; Turner et al., 2009; Turner et al., 2010; Turner et al., 2011; Maqbool et al., 2013; van Nieuwenhoven et al., 2013). See main text for details.

Table legends

Table 1. Role of specific signalling pathways in mediating the effects of IL-1 on human CF gene and protein expression. Pharmacological inhibitors were used to delineate the signalling pathways underlying IL-1α-induced increases in gene and protein expression in human CF. Specific inhibitors were PD98059 (ERK pathway inhibitor), SB203580 (p38 MAPK pathway inhibitor), SP600125 (JNK pathway inhibitor), LY294002 (PI-3-kinase/Akt pathway inhibitor) and IMD-0354 (NFκB pathway inhibitor). "+" and "-"symbols represent positive and negative roles of the pathways respectively, with a greater number of symbols indicating a larger influence. Empty grey boxes represent lack of effect of inhibitor, while

cross-hatch boxes indicate pathway not studied. Summary is based on previously published RT-PCR, Western blotting and ELISA data from our laboratory (Turner et al., 2009; Turner et al., 2011; Turner et al., 2010).

Table 1

Gene	mRNA/protein	ERK	p38	JNK	PI3K	NFκB	Reference
IL-1β	mRNA				+	++	Turner et al., 2009
IL-6	mRNA	-	++	-			
TNFα	mRNA		++			++	
IL-8	mRNA						Turner et al., 2011
	prot		++			+++	
ICAM-1	mRNA					++	
	prot					+++	
E-Selectin	mRNA	-		- -		+	
	prot			- -		+++	
MMP-1	mRNA						Turner et al., 2010
MMP-2	mRNA						
MMP-3	mRNA		+++				
MMP-9	mRNA						
MMP-10	mRNA						

Figure 1

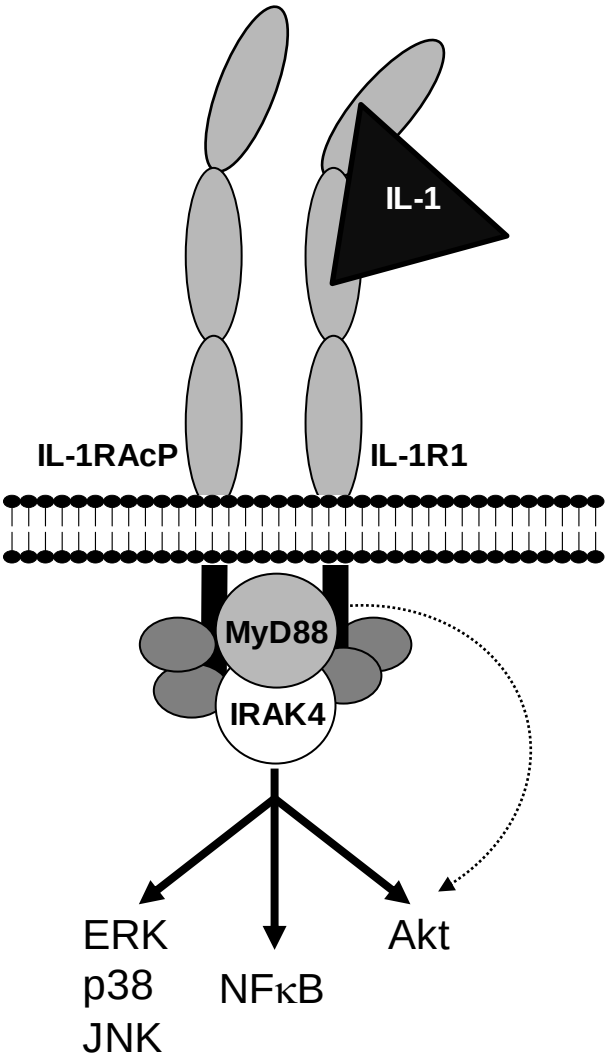
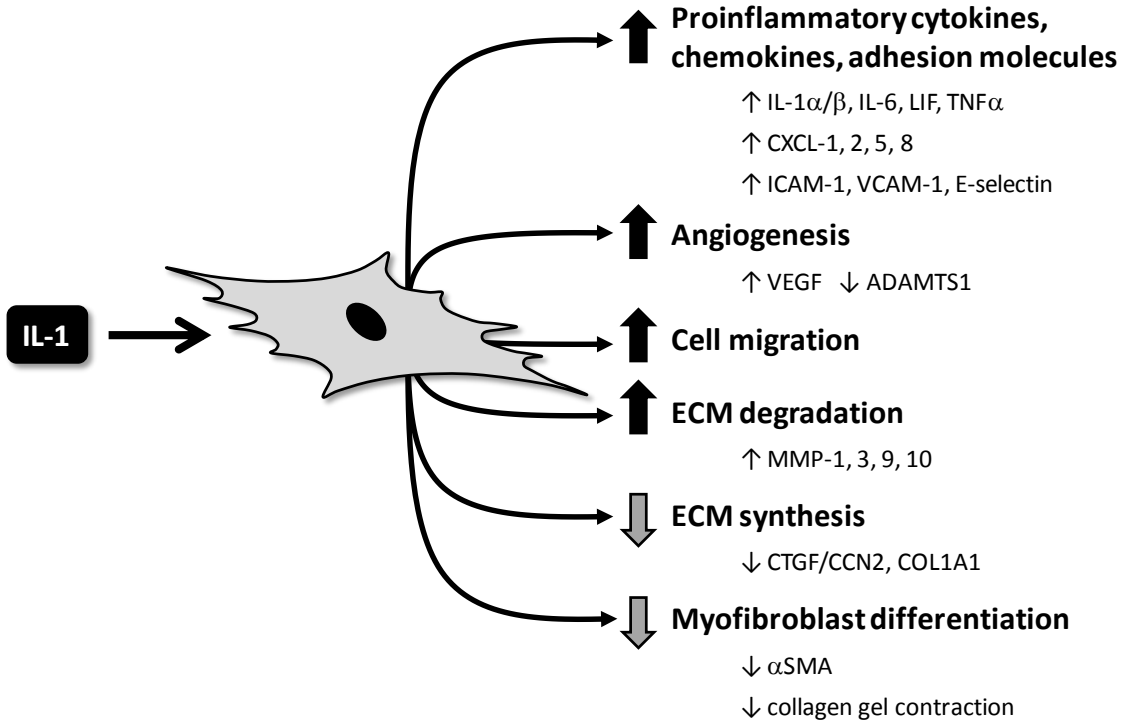


Figure 2


IL-1
Proinflammatory cytokines, chemokines, adhesion molecules
↑ IL-1α/β, IL-6, LIF, TNFα
↑ CXCL-1, 2, 5, 8
↑ ICAM-1, VCAM-1, E-selectin
Angiogenesis
↑ VEGF ↓ ADAMTS1
Cell migration
ECM degradation
↑ MMP-1, 3, 9, 10
ECM synthesis
↓ CTGF/CCN2, COL1A1
Myofibroblast differentiation
↓ αSMA
↓ collagen gel contraction