SPOTLIGHT REVIEW

Crosstalk between fibroblasts and inflammatory cells

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Fibroblasts, which are traditionally recognized as a quiescent cell responsible for extracellular matrix production, are more and more appreciated as an active key player of the immune system. This review describes how fibroblasts and immune cells reciprocally influence the pathogenesis of fibrosis. An overview is given how fibroblasts are triggered by components of the innate and adaptive immunity on the one hand and how fibroblasts modulate immune cell behaviour via conditioning the cellular and cytokine microenvironment on the other hand. Finally, latest insights into the role of cardiac fibroblasts in the orchestration of inflammatory cell infiltration in the heart, and their impact on heart failure, are outlined.

Keywords   Fibroblast • Myofibroblast • Innate and adaptive immune response • Heart failure

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1. Introduction

Fibrosis is a scarring process, which is characterized by excess deposition of collageneous and non-collagenous extracellular matrix (ECM) due to the accumulation, proliferation, and activation of fibroblasts and myofibroblasts. Consequently, fibrosis leads to dysregulated organ architecture and function.[1] Inflammatory and immunological reactions underlie the fibrosis process, by which both components of the innate and adaptive immune system are involved (*Figure 1*),[2] as well as the renin−angiotensin−aldosterone system and metabolic derangements.

Resolution of the inflammatory response requires the elimination of the major part of immune cells, which were recruited and actively proliferated during the acute phase of inflammation. Though, the presence of aberrant activated fibroblasts, myofibroblasts, lead to chronic inflammation via induction of a dysregulated homeostatic balance between leucocyte recruitment, proliferation, emigration, and death.[3] This review gives an overview of the multiple interactions of the components of the innate and adaptive immunity on the fibroblast leading to fibroblast activation, and how fibroblasts upon activation can lead to chronic inflammation. Finally, the significance of cardiac inflammation and fibrosis and their mutual interaction in the development of heart failure is briefly discussed.

2. Fibroblasts
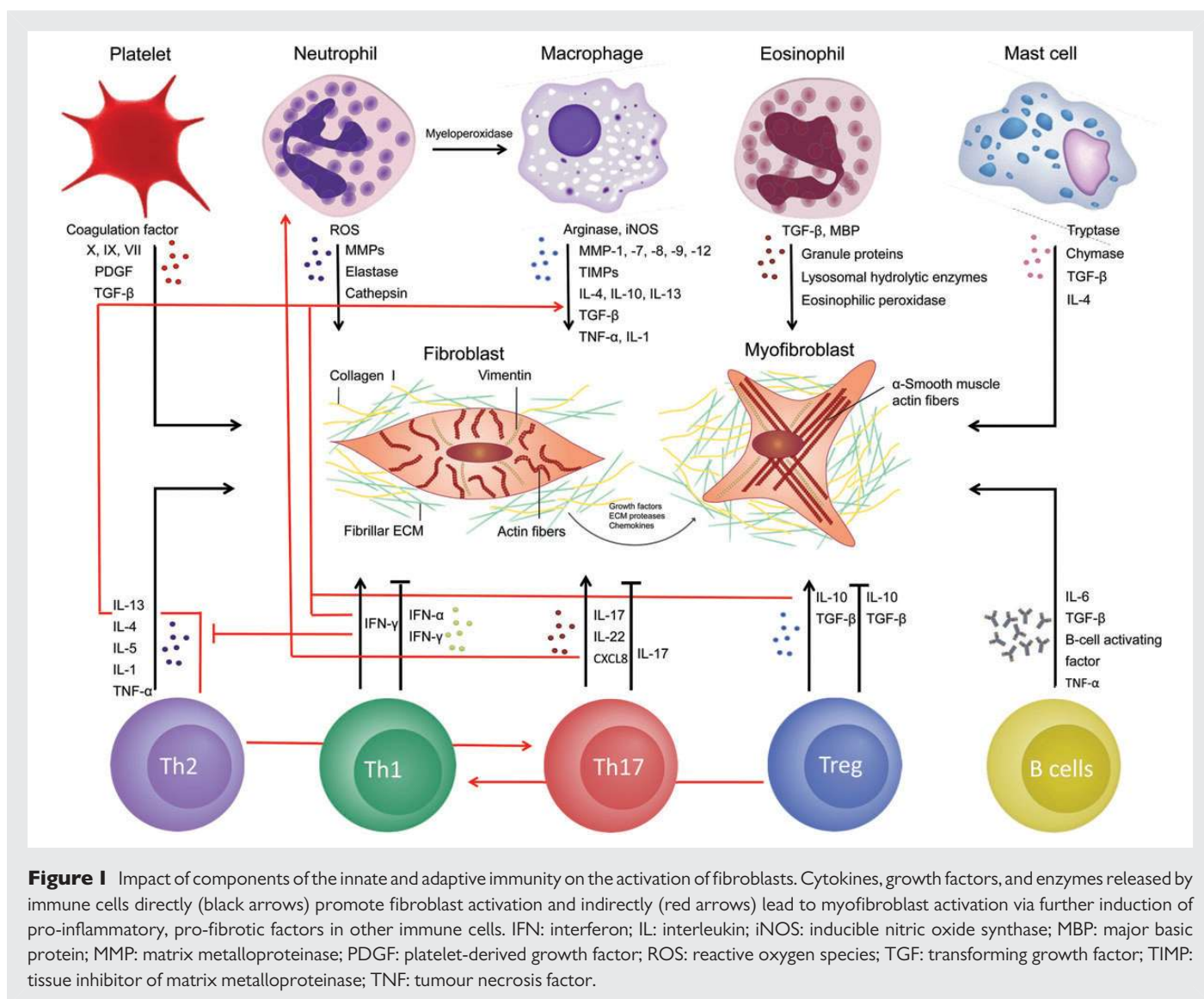### 2.1 Definition, function, and diversity

Fibroblasts are a heterogeneous population of stromal cells characterized by a spindle-shaped morphology and flat, oval nuclei, the lack of epithelial, vascular, and leucocyte lineage markers, as well as the ability to adhere to plastic. So far, no universal fibroblast marker has been identified. Fibroblasts are traditionally recognized for their structural role in synthesizing and remodelling the ECM in tissues. Though, beyond their role in structural support, fibroblasts are able to secrete and respond to cytokines, chemokines, and growth factors. They maintain the homeostasis of adjacent cells and orchestrate the maintenance of inflammatory infiltrates, indicating their importance in tissue development, differentiation, remodelling, and repair. In the heart, where fibroblasts account for 60−70% of the cells,[4,5] compared with cardiomyocytes which only constitute 30−40%, the crosstalk between cardiac fibroblasts and cardiomyocytes is important for both cardiac development and remodelling in response to tissue injury. Cardiac fibroblasts provide contractile co-ordination and electrical coupling between cardiomyocytes, allow for mechanical force distribution throughout the myocardium, and contribute to angiogenesis, all of which are extensively reviewed elsewhere.[6,7]

Fibroblasts differ from the anatomical site,[8,9] the disease status, and even within the same tissue. Consistent with the varying biophysical requirements of different tissues, fibroblasts from distinct tissues differ in proliferation, collagen and matrix metalloproteinase (MMP) production,[9] contractility, and immunomodulatory function.[8] Importantly, these differences in characteristic phenotypes among fibroblasts from distinct tissues are maintained after extended *in vitro* culture, supporting the concept that fibroblasts possess positional identity. This concept is corroborated by comparative transcriptome analysis, which revealed that the transcriptional profiles of fibroblasts can be clustered into groups according to the anatomical site.[10,11]

The diversity of fibroblasts (within the same tissue) can be explained by their distinct cellular origins. Fibroblasts mainly originate from

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Figure I Impact of components of the innate and adaptive immunity on the activation of fibroblasts. Cytokines, growth factors, and enzymes released by immune cells directly (black arrows) promote fibroblast activation and indirectly (red arrows) lead to myofibroblast activation via further induction of pro-inflammatory, pro-fibrotic factors in other immune cells. IFN: interferon; IL: interleukin; iNOS: inducible nitric oxide synthase; MBP: major basic protein; MMP: matrix metalloproteinase; PDGF: platelet-derived growth factor; ROS: reactive oxygen species; TGF: transforming growth factor; TIMP: tissue inhibitor of matrix metalloproteinase; TNF: tumour necrosis factor.

primary mesenchymal cells, but can also arise through epithelial–mesenchymal transition (as seen in the liver[12] and kidney[13]), or endothelial–mesenchymal transition (as seen in the lung,[14] heart,[15] and cancer[16]) and can be derived from circulating cells, including mesenchymal stromal cells (MSCs) and fibrocytes. Fibrocytes are defined as circulating monocyte-derived cells that are capable of expressing a fibroblastic phenotype.[17] Although they only comprise a small percentage of circulating leucocytes under non-pathological conditions in humans, their number increases by chronic inflammatory and fibrotic derangements including autoimmune[18] and cardiovascular disorders,[19,20] and contribute to the fibrotic process.

3. Innate and adaptive immune mechanisms rule the development of fibrosis

Substantial evidence postulates that fibrosis develops as a complication of inflammatory processes, in which both innate and adaptive immune mechanisms play a considerable role. Tissue damage, infections with bacteria, viruses, fungi, and parasites, foreign body implants, autoimmune disease, or tumours could progress to an adverse chronic inflammation, which subsequently leads to fibrotic disease. Moreover, even a low-grade, but persistent inflammation promotes fibrosis in cardiovascular diseases and hypertension.[21] Therefore, the elimination of an inflammatory trigger and the resolution of inflammation are of critical importance for the prevention of fibroblasts activation, excessive accumulation of ECM, and tissue fibrosis.

3.1 Innate immunity triggers of fibrosis

The first-line defence of the innate immune system largely depends on a sophisticated array of pattern recognition receptors, which recognize conserved pathogen-associated molecular patterns.[22] Interestingly, fibroblasts have been shown to express a variety of pattern recognition receptors, including Toll-like receptors (TLRs), and the subsequent ligand activation of those receptors can directly activate fibroblasts and promote their differentiation into collagen-producing myofibroblasts.[23,24]

3.1.1 Platelets

Also platelets express a range of TLRs,[25,26] contributing to their immune cell function in the state of infection and inflammation, besides their substantial role in the coagulation response via fibrin clot formation.

Furthermore, a substantial body of evidence has indicated that platelets promote systemic and cardiac inflammatory responses, and ventricular remodelling.[27] Activated platelets release several growth factors promoting healing including platelet-derived growth factor, a potent chemotactic agent, and transforming growth factor (TGF)-β, which stimulates the deposition of ECM.[28] Moreover, dysregulation in the coagulation signalling cascade may contribute to tissue fibrosis.[29] The pro-fibrotic effects of coagulation factor X have been shown in a model of acute lung injury,[30] while overexpression of human blood co-agulation factor IX is associated with myocardial fibrosis.[31] However, deficiency of coagulation factor VII could result in spontaneous cardiac fibrosis[32] and coagulation insufficiency is typical for cirrhosis patients.[33,34] Therefore, a balanced coagulation response seems to be of critical importance for the prevention of tissue fibrosis.

3.1.2 Neutrophils

Upon tissue injury, damaged epithelial and endothelial cells not only release inflammatory factors triggering the coagulation cascade, but also a cocktail of growth factors and chemokines, which subsequently initiate an influx of neutrophils and monocytes to the site of the damaged tissue. Neutrophils are the first cells attracted to the injured site followed by monocytes, and finally lymphocytes and mast cells.[35] Neutrophils act as a first-line defence and initiate an acute inflammatory response to engulf dead cells and tissue debris in order to facilitate tissue repair. However, excessive and persistent neutrophil infiltration or their delayed elimination exacerbates the tissue injury via the release of inflammatory mediators and proteinases.[36] Neutrophils count has been utilized as a prognostic biomarker of chronic remodelling of the left ventricle (LV).[37]

Neutrophils release large amounts of reactive oxygen species during the respiratory burst[38] via the multicomponent enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Reactive oxygen species-releasing neutrophils are a proven pro-fibrotic mediator in systemic sclerosis,[39] pulmonary fibrosis,[40] and hepatic fibrosis.[41] Inhibition of NADPH oxidase attenuates cardiac fibrosis post-myocardial infarction.[42,43] Besides numerous pro-inflammatory cytokines, neutrophils secrete granules containing enzymes such as MMPs, elastase, and cathepsins capable of cleaving collagenous and non-collagenous connective tissue components, involved in tissue remodelling during the fibrotic process.[44] In addition, neutrophils-derived elastase has been shown to play a pivotal role in the pathogenesis of pulmonary fibrosis.[45] During the acute phase of inflammation, neutrophils play an indirect pro-fibrotic role by activating further cellular components of the innate immune system such as macrophages promoting fibrogenesis.[46]

3.1.3 Macrophages

Macrophages are indispensable effector cells involved in tissue remodelling and fibrosis. They are the main source of several types of MMPs (MMP-1, -7, -8, -9, and -12) as well as their endogenous suppressors, tissue inhibitors of MMPs (TIMPs).[47] The proper balance of MMPs and TIMPs is crucial for the normal deposition and degradation of the ECM. The MMPs released by macrophages mediate not only signalling through proteolysis of both ECM and non-matrix substrates, they also amplify the inflammatory response and influence the progression of tissue remodelling. MMP-9 is the most relevant pro-fibrotic MMP,[48] which inhibition or deletion has been shown to reduce fibrosis in models of dilated cardiomyopathy[49] and myocardial infarction.[50] MMP-2 is not only responsible for the degradation of matrix proteins. It also cleaves monocyte chemotactic protein (MCP)-3, lowering its

chemotactic activity and diminishing the invasion of inflammatory cells and subsequently the inflammatory response and fibrosis in viral-induced myocarditis.[51]

In addition to MMPs, macrophages are a leading producer of TGF-β, considered the most significant pro-fibrotic agent involved in the progression of chronic fibrotic diseases affecting skin, liver, lung, kidney, and heart.[52] Inhibition of alternatively activated macrophages (M2 macrophages) has been shown to abrogate TGF-β-driven lung fibrosis.[53] TGF-β has several isoforms (TGF-β1, TGF-β2, and TGF-β3), which are synthesized as latent precursors bound to latent TGF-β-binding proteins (LTBP-1, -3, and -4). LTBPs are removed extracellularly via proteolytic cleavage releasing active TGF-β.[54] Activated TGF-β binds to TGF-β receptors and the signalling within the cell is mediated via the SMAD family of transcriptional activators.[55] TGF-β induces the expression of ECM genes and suppresses the activity of genes encoding MMPs, which are capable of degrading ECM.[56,57] TGF-β induces the expression of pro-fibrotic genes such as Type I collagen and connective tissue growth factor (CTGF) in a SMAD3-dependent manner.[58,59] Moreover, TGF-β can promote collagen synthesis in SMAD-independent pathways via mitogen-activated protein kinase cascades, including p38 MAPK, Jun N-terminal kinase, and extracellular signal-regulated kinase.[60] TGF-β1 and -β3 as well as LTBPs are up-regulated in patients with cardiac fibrosis, highlighting the implication of active TGF-β in human cardiac fibrogenesis.[61] Heart failure patients with preserved ejection fraction exhibit high TGF-β1 expression accompanied by reduced MMP-1 and elevated TIMP-1 favouring the collagen synthesis in the heart of those patients.[62] Furthermore, numerous studies have demonstrated the direct effect of TGF-β on fibroblast differentiation to myofibroblasts.[63,64] Inhibition of TGF-β via neutralizing antibodies has been shown to be well tolerated and to successfully reduce the development of fibrosis in different experimental models.[65–67] For example, TGF-β neutralization prevented cardiac fibrosis and improved diastolic dysfunction in pressure-overloaded rats.[68] In contrast, TGF-β-deficient mice exhibit profoundly diminished collagen deposition, though they suffer from a severe wasting syndrome in addition to extensive inflammatory, tissue necrosis, resulting in organ failure, and death.[69,70] Early inhibition of TGF-β is associated with increased mortality, leucocyte infiltration, and chemokine expression,[71] while the later elimination of TGF-β results in improved survival and reduced tissue fibrosis.[72] In addition, TGF-β1-producing regulatory T cells (Tregs) have been demonstrated to reduce bleomycin-induced fibrosis in an interleukin (IL)-10-dependent manner.[73] These findings indicate that TGF-β-mediated effects in fibrosis are complex, and timing of action as well as cell environment are of critical importance. In fact, a miscalculated inhibition has led to adverse side effects in human.[55,74] Therefore, the development of anti-fibrotic therapies targeting TGF-β and its signalling should be carefully considered and properly evaluated in suitable animal models.

The complex role of macrophages in fibrosis is evident from a study, demonstrating that deletion of the macrophage population either during injury or during repair and resolution has dramatically different effects on the overall fibrotic outcome. During a progressive inflammatory injury, macrophage depletion results in amelioration of fibrosis, while depletion during recovery results in a failure of resolution with persistence of pro-fibrotic cellular and matrix components.[75] Furthermore, macrophage depletion in the early phase post-myocardial injury markedly impairs the wound healing and increases remodelling and mortality, indicating that macrophages are a key player in myocardial wound healing.[76]

Macrophages stimulated by TLR ligands and interferon (IFN)-γ undergo classical M1 activation, while those stimulated by IL-4 and -13 become M2 macrophages.[77,78] M2 macrophages are involved in wound healing, tissue remodelling, fibrosis, and inflammatory responses.[79,80] They contribute to cardiac fibrosis[81] and are shown to directly promote collagen I expression in cardiac fibroblasts.[82] M2 macrophages release arginase 1 capable of controlling L-proline production essential for the collagen synthesis of activated myofibroblasts.[83] For example, inflammatory Gr1+ monocytes, recruited to the injured liver in a CCR2-dependent manner, give rise to CD11b+F4/80+ macrophages producing TGF-β and inducible nitric oxide synthase directly promoting the progression of liver fibrosis.[84] Furthermore, suppression of cardiac monocyte/macrophage infiltration in deoxycorticosterone acetate/salt hypertensive rats subsides myocardial fibrosis.[85] Interestingly, in Coxsackievirus-induced myocarditis, two distinct M2 macrophage populations have a completely different role in the development of cardiac fibrosis. TLR4+casp-1+IL-1β+ M2 macrophages exacerbate inflammation and fibrosis, whereas Tim-3+ M2 macrophages decrease the inflammatory and fibrotic response.[86]

Activated macrophages produce, in addition to TGF-β, cytokines such as IL-4, IL-10, IL-13,[87] tumour necrosis factor (TNF)-α, and IL-1, which have been shown to activate fibroblasts, overproducing proteins of the ECM. Furthermore, TNF-α has been found to activate the extracellular regulated kinase-specific pathway in fibroblasts resulting in increased expression of TGF-β[88] and support the excessive production of pro-inflammatory cytokines via the nuclear factor-kappa B (NF-κB) pathway.[89] Once activated, macrophages not only secrete pro-fibrotic factors, but also recruit myofibroblasts and exacerbate inflammatory cell infiltration to sites of tissue injury, leading to profound production of a variety of chemokines, cytokines, and growth factors, which endpoint of repair turns to excessive and poorly ordered matrix deposition and fibrosis.[23,90−93]

3.1.4 Eosinophils

Eosinophils secrete TGF-β, granule proteins, major basic protein (MBP), lysosomal hydrolytic enzymes, and eosinophilic peroxidase, factors implicated in tissue remodelling and fibrosis.[94] Eosinophil-derived TGF-β induces fibroblast activation and transdifferentiation to myofibroblasts overexpressing ECM proteins.[94] MBP-1 has been shown to exert pro-fibrotic effects in muscular dystrophy,[95] while granule proteins directly stimulate fibroblast proliferation. Importantly, TGF-β, MBP, and eosinophilic peroxidase induce epithelial−mesenchymal transition further contributing to myofibroblast generation and fibrosis.[94] Moreover, toxic eosinophil granular proteins have been involved in the development of endomyocardial fibrosis in the hypereosinophilic heart syndrome.[96,97]

3.1.5 Mast cells

Mast cells produce a variety of proteases, cytokines, growth factors, vasoactive agents, and other biologically active mediators such as tryptase, chymase, and TGF-β, which are known to activate fibroblasts and subsequently support the development of cardiac fibrosis.[98,99] Volume overload is associated with increased mast cell density in the LV, paralleled with activation of MMPs, subsequent collagen degradation and LV dilatation, and cardiac fibrosis.[100] Furthermore, a recent study demonstrated that IL-4, most likely produced by mast cells in the heart during pressure overload, is also a significant contributor to cardiac fibrosis.[101] In contrast, mast cell deficiency was shown to be protective in pulmonary[102] and cardiac fibrosis.[103]

3.2 Adaptive immunity triggers of fibrosis
3.2.1 Th1 and Th2 cells

While a number of studies suggest a pro-fibrotic role of T cells in fibrosis, it became also evident that T cells are indispensable, proved by the finding that T-cell-deficient mice develop prominent fibrosis.[104] The pro-fibrotic effect of T lymphocytes seems to be context-dependent and the factors present in the environment trigger specific T-cell populations, which subsequently determine the fibrotic outcome.

A substantial pile of evidence links T-helper type 2 (Th2) cells, characterized by the secretion of the cytokines IL-4, IL-5, and IL-13, with wound healing and fibrosis.[105,106] IL-13 is one of the most noticeable mediators of fibrosis[107,108] and in combination with IL-4 is capable of inducing the phenotypic transition of human fibroblasts to myofibroblasts in a c-Jun NH_2-terminal kinase-dependent manner.[109] Furthermore, IL-13 has been found to inhibit fibroblast MMP synthesis and subsequently down-regulates the matrix degradation, which results in excessive collagen deposition.[110] IL-13 has been shown to induce TGF-β1 in macrophages via the IL-13Rα2 receptor, which elimination in vivo reduced the production of TGF-β1 and collagen deposition in bleomycin-induced lung fibrosis.[111] Moreover, a very recent study has demonstrated that disturbance of IL-13/TGF-β1 interaction by IL-13Rα2 siRNA prevents cardiac allograft fibrosis.[112] Therefore, therapeutic targeting of IL-13Rα2 receptor in the course of extended inflammation could prevent TGF-β1-associated fibrosis. Moreover, IL-3 influences Th17-mediated inflammation and Th2-driven fibrosis[113] and facilitates its direct pro-fibrotic activity[114] via IL-13Rα1 and IL-13Rα2 expressed directly on the reactive myofibroblasts.

The Th1 cytokine IFN-γ plays a controversial role in inflammation and fibrosis, with numerous reports showing pro-fibrotic and anti-fibrotic effects. The pro-fibrotic effects of IFN-γ follow from IFN-γ-deficient mice having attenuated lung inflammation and fibrosis after intratracheal bleomycin administration.[115] In addition, a recent study by Marko et al.[116] demonstrated that angiotensin II-treated IFN-γR knockout mice exhibited reduced cardiac hypertrophy, decreased infiltration of cardiac macrophages and T cells, and less cardiac fibrosis. IFN-γ mediates its pro-fibrotic activities via intensifying the production of pro-inflammatory and pro-fibrotic mediators such as TNF-α.[117,118] IFN-γ-producing T cells control the differentiation, migration, and activation of macrophages as well as their MCP-1 expression, which subsequently leads to inflammation and fibrosis.[106,119] A role of IFN-γ signalling in the differentiation of resident fibroblasts to myofibroblasts has not been elucidated so far.

The anti-fibrotic effects of IFN-γ have been discovered long time ago. IFN-α and -γ potently inhibit the collagen production of human fibroblasts, regulating the normal and pathological fibrogenesis.[120] In a bleomycin-induced mouse model of lung fibrosis, IFN-γ initially down-regulated the bleomycin-induced overexpression of TGF-β and subsequently the procollagen expression, resulting in reduced collagen content.[121] The exact mechanism of the IFN-γ-mediated suppression of TGF-β has been revealed. IFN-γ signalling via the Jak/STAT1 pathway induces an instant expression of SMAD7, which prevents the TGF-β-mediated phosphorylation of SMAD3 and consequently abrogates the TGF-β signalling to the nucleus.[122] Transcriptional modification via STAT1 is another mechanism via which IFN-γ antagonizes TGF-β signalling and collagen deposition in lung fibrosis.[123] In addition, IFN-γ inhibits the IL-4- and IL-13-promoted differentiation from fibrocytes into myofibroblasts.[124] In a model of a chronic viral myocarditis, IFN-γ reduced TGF-β1, IL-1β, and IL-4-associated inflammation and fibrosis.[125]

3.2.2 Th17 cells

Th17 cells expressing IL-17A and IL-22 are another essential player in the development and progression of fibrotic disease in lung,[126] cardiac,[127] and hepatic fibrosis.[128] IL-17A is characterized by its ability to induce the expression of a variety of pro-inflammatory mediators, such as IL-1, IL-6, TNF-α, CXCL8, granulocyte colony-stimulating factor, and granulocyte−macrophage colony-stimulating factor by endothelial and epithelial as well as stromal cells, which ultimately results in the recruitment and activation of neutrophils.[129] Moreover, Th17 cells can directly chemoattract neutrophils, known for their pro-fibrotic effect, through the production and release of CXCL8.[130] Furthermore, IL-17 promotes MMP-1 expression in cardiac fibroblasts via NF-κB, activating protein-1, and CCAAT-enhancer-binding protein (C/EBP)-β activation[131] and induces cardiac fibrosis via activation of the protein kinase C (PKC)β/Erk1/2/NF-κB pathway.[132] During the course of viral myocarditis, IL-17 causes the proliferation of cardiac fibroblasts and, in parallel, induces the degradation of collagen Type I and III via up-regulation of MMP-2.[133] The pro-fibrotic role of IL-22, which is up-regulated in patients with chronic hepatitis B virus infection and liver fibrosis, follows from the finding that IL-22 blockade in hepatitis B virus transgenic mice with T-cell-mediated liver fibrosis restricted the progression of liver fibrosis.[134]

However, IL-17 and -22 have besides pro-fibrotic also anti-fibrotic features. Nakashima *et al.*[135] have shown that IL-17A down-regulated the expression of CTGF and collagen I in fibroblasts of healthy patients. Th17 cells elicited MCP-1, IL-8, and a MMP-1 response, while simultaneously inhibited Type I collagen production in dermal fibroblasts of healthy and systemic sclerosis patients.[136] In models of hypersensitive pneumonitis, IL-17 and -22 even exhibit a dual role in fibrosis, where their pro- or anti-inflammatory/fibrotic effects depend on the particular antigen.[137] Taking the above-discussed studies into account, it seems that Th17 cells play a dual role in the fibrosis process. Probably, the presence of regulatory mediators, such as chemokines, transcription factors, and receptors in the particular inflammatory environment, can guide the Th17 response in a pro-fibrotic or anti-fibrotic direction.

3.2.3 Regulatory T cells

Th17 cells are not the only dual cell population. Tregs can also either suppress or promote fibrosis. Tregs release important immunosuppressive cytokines including IL-10 and TGF-β that have control over the inflammatory response and contribute to the maintenance of self-tolerance and host immune defence. Tregs play a considerable role during the inflammatory process and the subsequent progression of fibrosis contributing[138] or suppressing[139] the development of fibrosis. Adoptive transfer of Tregs in hypertensive hearts has been shown to attenuate cardiac fibrosis and inflammation, to reduce the interstitial myofibroblast numbers, and to decrease the activity of the TGF-β1 system.[139] Moreover, IL-10 produced by Tregs *in vivo* and *in vitro* significantly inhibits the collagen synthesis by cardiac fibroblasts.[140] This is in line with other reports showing pronounced anti-fibrotic features of IL-10 in models of wound healing and Crohn's disease.[141,142] IL-10 exerts its anti-fibrotic effects via reduction of STAT3 activity[143] and via inhibition of the NF-κB pathway.[144] In contrast, long-term overexpression of IL-10 promotes lung fibrosis via fibrocyte recruitment and M2 macrophage activation.[145] Moreover, Tregs releasing TGF-β1 have been shown to affect CD4+ T-cell homeostasis in an HIV model by inducing collagen deposition in lymphatic tissues.[146] In contrast, depletion of Tregs attenuated the progress of silica-induced lung fibrosis and enhanced the Th1 response.[138] Therefore, before considering Tregs application as a suitable anti-fibrotic strategy, efforts should be focused on understanding the exact mechanisms and the particular tissue environment factors modulating whether Tregs would exhibit an anti-fibrotic or pro-fibrotic effect.

3.2.4 B cells

B cells are involved in antigen presentation, produce autoantibodies and various cytokines, and are essential players in immune-mediated disorders.[147,148] In addition, B cells have been shown to play a role in hepatic fibrosis in an antibody- and T-cell-independent manner.[149] B cells release the pro-fibrotic cytokine IL-6 and trigger liver fibrosis by inducing differentiation of hepatic stellate cells into myofibroblasts, promoting fibroblast proliferation, and augmenting the collagen and TIMP synthesis.[150] In addition, Zhou *et al.*[151] have demonstrated that anti-fibrillin-1 autoantibodies from systemic sclerosis patients have a potent pro-fibrotic effect in normal dermal fibroblasts, indicated by the increased expression of ECM components, the phosphorylation and nuclear translocation of SMAD3, as well as the induction of TGF-β1. A recent study illustrated that B cells releasing B-cell-activating factor are prominent inducers of excessive collagen, TIMP1, MMP-9, α-SMA expression in human dermal fibroblasts, as well as of pro-inflammatory and pro-fibrotic cytokines IL-6, CCL2, and TGF-β.[152] Furthermore, TNF-α-secreting B cells have been shown to contribute to myocardial fibrosis and fibrosis-related cardiac dysfunction in patients with dilated cardiomyopathy.[153] In conclusion, the role of B cells in (cardiac) fibrosis has so far been neglected and requires further investigation.

4. Fibroblasts are key players in the control of tissue damage

Fibroblasts modify the quantity, quality, and duration of the inflammatory infiltrate and play a critical role in the switch of acute resolving to chronic persistent inflammation[154] by several means. An overview of their impact on immune cell chemotaxis, infiltration, transendothelial migration, retention, and apoptosis, and underlying mechanisms, is outlined below and illustrated in *Figure 2*.

The vascular endothelium is an anatomical defence barrier. Circulating leucocytes from the blood flow have to pass the endothelium to reach the underlying tissue. Traditionally, vascular inflammation has been described as an event whereby, upon endothelial cell activation, leucocytes extravasate and induce an inflammatory response onto the microenvironment via the release of pro-inflammatory stimuli. Though, it is more and more recognized that also the fibroblasts from the stromal microenvironment drive homing of circulating leucocytes[155] and promote activation of the endothelium,[156−158] indicating a bidirectional activation in response to tissue injury. In detail, fibroblasts are a major source of constitutive and cytokine-induced C−C and C−X−C chemokines including MCP-1, macrophage inflammatory protein (MIP)-1, RANTES, and IP-10 and express chemokine receptors.[159] Fibroblasts derived from an environment with cell-mediated inflammatory responses demonstrate a dramatic alteration in their cytokine profile and produce high levels of MCP-1, when compared with normal fibroblasts.[160] MCP-1 itself also stimulates collagen expression and endogenous up-regulation of TGF-β expression in fibroblasts, leading to autocrine and/or juxtacrine stimulation of collagen gene expression.[161] The induction in chemokine production following activation of fibroblasts with inflammatory stimuli such as TNF-α depends
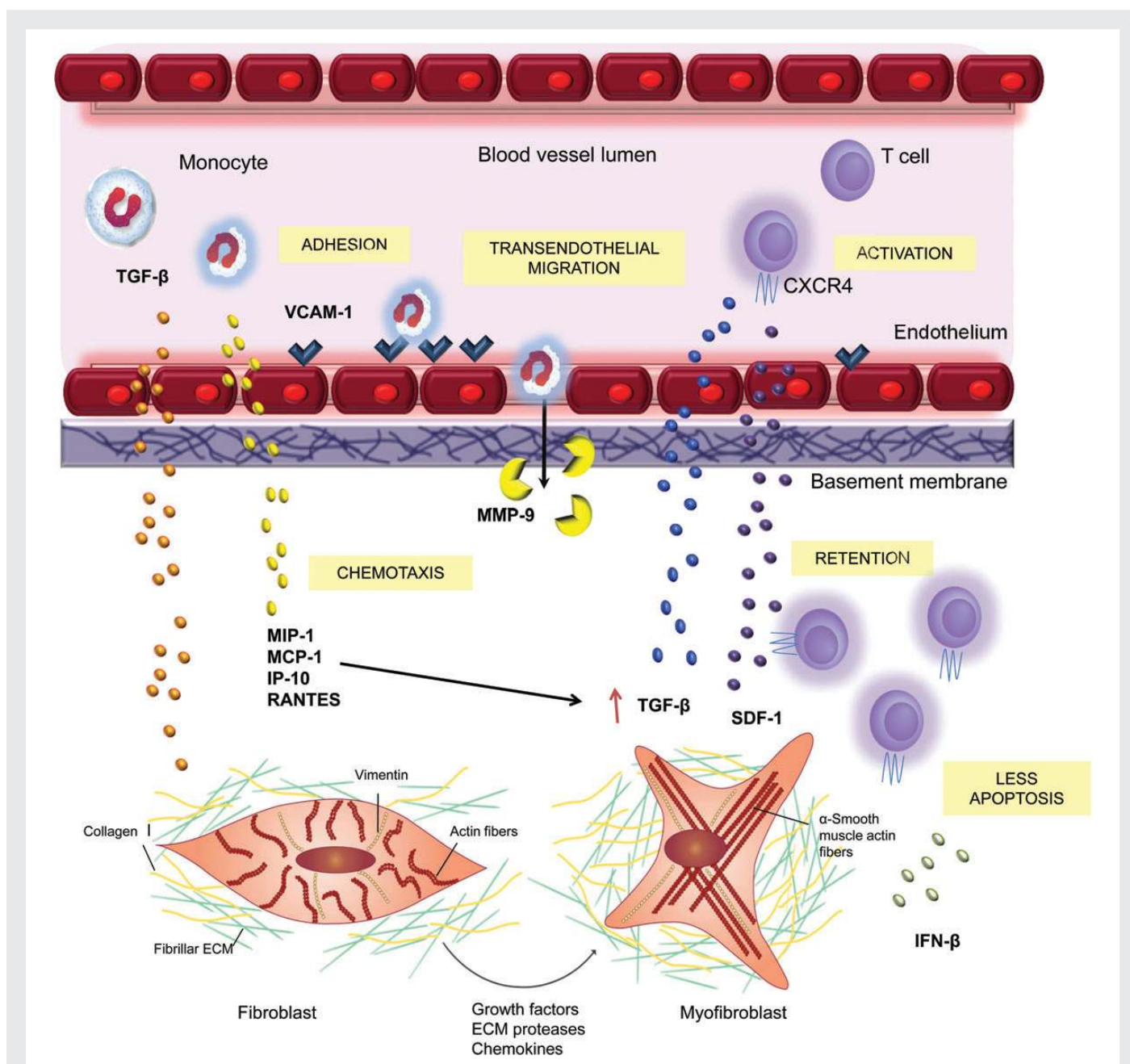
Figure 2 Role of (activated) fibroblasts in the inflammatory process. (Activated) fibroblasts from the stromal microenvironment drive homing of circulating leucocytes via the release of chemokines and promote the recruitment of circulating leucocytes on endothelial cells via the induction of adhesion molecules on the endothelium. Furthermore, myofibroblasts activate leucocytes to produce the gelatinase MMP-9, allowing degradation of the basal membrane and subsequent transendothelial migration. Activated fibroblasts enhance local T-cell persistence via (i) up-regulation of CXCR4 on T cells via TGF-β and induced expression of its ligand stromal-derived factor-1 (SDF-1), promoting the SDF-1/CXCR4 axis and via (ii) the reduction of T-cell apoptosis involving the release of IFN-β.

on the transcription factor RelB, which is capable of stabilizing IκB, the endogenous NF-κB inhibitor. In the absence of RelB expression in fibroblasts, but not in macrophages, NF-κB activity is uncontrolled leading to continuous chemokine generation. This has been elegantly demonstrated by experiments with RelB$^{-/-}$ fibroblasts, which exhibited a dramatic persistent induction of chemokine production, including MCP-1, MIP-1α, MIP-1β, MIP-2, IP-10, and RANTES, following lipopolysaccharide. In contrast, upon lipopolysaccharide activation of normal fibroblasts, only a transient production of chemokines, closely followed by induction

of RelB expression, was induced.[162] *In vivo*, activated RelB$^{-/-}$ fibroblasts dramatically increased the recruitment of granulocytes into tissues,[162] further supporting the importance of chemokine production by fibroblasts in tissue inflammation. Interestingly, fibroblasts from fibrotic lesions express higher levels of the MCP-1 (CCL2) receptor, CCR2, compared with those from non-fibrotic lesions,[160] further enhancing hereby the inflammatory and fibrotic process. Furthermore, co-culture of macrophages with fibroblasts revealed that a contact-dependent expression of chemokines, especially of the macrophage-derived MIP-1α,

is induced in macrophages. Further experiments unravelled that MIP-1 production in macrophages was dependent on the TNF-α-induced expression of intercellular adhesion molecule-1 (ICAM-1) by the fibroblast.[163] On the other hand, the CCL2/CCR2 axis enhances vascular cell adhesion molecule-1 (VCAM-1) expression in human synovial fibroblasts promoting monocyte adhesion to human fibroblasts,[164] indicating a reciprocal enhancement of monocyte−fibroblast adhesion and chemokine production. Taken together, fibroblasts have the ability to participate in the maintenance of an inflammatory response via the expression of chemokines.[160] The interaction between fibroblasts and macrophages may be an important early event in the recruitment of monocytes and may facilitate a cytokine network that maintains the activation of tissue inflammation.

Besides attracting immune cells to the site of injury via the release of chemokines, fibroblasts can promote or inhibit the recruitment of circulating leucocytes on endothelial cells via the induction or decrease of cytokine-induced expression of adhesion molecules on endothelial cells.[165] This influence of fibroblasts on the cytokine sensitivity of the vascular endothelium depends on the tissue origin of the fibroblasts and is restricted to normal stromal fibroblasts. Fibroblasts associated with chronic inflammation bypass this and develop a direct inflammatory phenotype.[165] Lindner *et al.*[166] further demonstrated that cardiac fibroblasts also activate leucocytes to produce the gelatinase MMP-9, allowing easier transendothelial migration through the basal membrane.

Fibroblasts not only modulate the recruitment of immune cells, but also regulate their behaviour, retention, and survival in damaged tissue. In general, the crosstalk between fibroblasts and leucocytes depends on the interaction between the leucocyte surface antigen CD40 on fibroblasts and its ligand, CD40L, which is expressed on immune cells. This interaction induces, among others, the up-regulation of ICAM-1 and VCAM-1 on fibroblasts,[167,168] which in turn are important for the induction of chemokine production (see below) and the reduction in T-cell apoptosis (see above). Furthermore, fibroblasts also express the co-stimulatory molecule B7,[169] suggesting that similar to interactions between lymphocytes and antigen-presenting cells, CD40-CD40L and co-stimulatory CTLA4-B7 interactions play an important role in the fibroblast−leucocyte crosstalk.

Activated fibroblasts up-regulate CXCR4 on T cells via TGF-β[170] and express its ligand stromal-derived factor-1 (SDF-1),[171] suggesting that fibroblasts alter the migratory phenotype of the leucocytes towards a stationary phenotype via SDF-1/CXCR4 interactions, leading to retention of the infiltrated cells. The importance of CXCR4 expression on T cells for the recruitment of activated T cells towards inflammatory sites follows from T-cell-specific CXCR4-deficient mice, which exhibit less T cells into affected joints and a lower incidence of collagen-induced arthritis compared with wild-type littermates.[172] Intriguingly, SDF is also an important attractant for fibrocytes. Its significance for fibrocyte recruitment has recently been demonstrated by Garibaldi *et al.*[173] They showed a reduction in acute lung injury by decreasing fibrocyte recruitment and subsequent fibroproliferation involving a down-regulation in SDF expression. On the other hand, also the recruitment of MSC depends on SDF/CXCR4 signalling.[174–176] Though, in contrast to fibrocytes and leucocytes, which exert pro-inflammatory and pro-fibrotic effects, MSCs have immunomodulatory[177–179] and anti-fibrotic[179,180] features. Depending on the local concentration of SDF-1α, this chemokine can act as a chemo-attractant as well as a repellent for leucocytes.[3,181] The above-mentioned observations illustrate the delicate and fine-tuned regulation of SDF/CXCR4 interactions and their influence on the retention of leucocytes/fibrocytes as well as of MSC,

promoting or reducing the inflammatory/fibrotic process. Besides raising the retention of infiltrated T cells via inducing the SDF/CXCR4 axis, fibroblasts promote local T-cell persistence via reducing T-cell apoptosis. This occurs in an integrin-ligand-dependent manner[182] and via the release of IFN-β,[183] bringing the cell hereby in a resting G0/G1 state.

5. Link of inflammation and cardiac fibrosis in the development of heart failure

With (i the spleen being a reservoir of monocytes, which are recruited to the inflammatory heart,[184] and the findings that (ii) splenectomy improves the outcome of myocardial infarction,[185] (iii) the use of an antibody against T cells decreases cardiac damage in myocarditis,[186] and (iv) our observation that *ex vivo* supplementation of splenocytes isolated from mice with virus-induced inflammatory cardiomyopathy to fibroblasts induces more collagen production in fibroblasts compared with splenocytes from control mice,[179] we postulated the hypothesis that the cardiosplenic axis is important for cardiac inflammation, fibrosis, and the subsequent development of heart failure.[175,187] This hypothesis has recently been elegantly demonstrated and detailed by Ismahil *et al.*[188] Via splenectomy experiments and adoptive transfer of splenocytes from mice with heart failure, but not from sham-operated mice in naive recipients, they showed that activation of mononuclear phagocytes is central to the progression of cardiac remodelling in heart failure and heightened antigen processing in the spleen plays a critical role in this process. Furthermore, the authors illustrated that splenocytes promote immune-mediated fibrosis responses in the failing heart, and retain this memory upon adoptive transfer.[188]

Via up-regulated chemokine expression, e.g. MCP-1, immune cells from the spleen are attracted to the heart. In a model of suprarenal aortic constriction, increased cardiac expression of MCP-1 was observed preceding TGF-β1 up-regulation and subsequent cardiac fibrosis and diastolic dysfunction.[189] The significance of TGF-β1 follows, among others, from experiments in pressure-overloaded rats where administration of an anti-TGF-β1 neutralizing antibody 1 day before operation inhibited fibroblast activation and subsequently prevented collagen mRNA induction and myocardial fibrosis, but not myocyte hypertrophy.[68] Westermann *et al.*[62] provided insights into the role of cardiac inflammation as a pro-fibrogenic stimulus in subjects with heart failure with preserved ejection fraction (HFPEF). In endomyocardial biopsy specimens from these patients, they identified increased inflammatory cells and higher TGF-β1 mRNA levels associated with both reduced levels of MMP-1, the major collagenase in the human heart, and elevated TIMP-1 levels. In accordance with these findings indicating TGF-β1-induced collagen synthesis in human subjects with HFPEF, *in vitro* stimulation of primary human cardiac fibroblasts from HFPEF patients with TGF-β1 resulted in transdifferentiation of fibroblasts to myofibroblasts, which produced more CTGF and more collagen and expressed less MMP-1. Double staining further confirmed the expression of TGF-β1 in immune cells present in endomyocardial biopsies of HFPEF patients, whereas activated human acute monocytic leukemia cell line (THP-1) monocytes were found to express TGF-β1 in a time-dependent manner *in vitro*. Collectively, these data provide accumulating evidence linking cardiac inflammation with TGF-β1-induced collagen synthesis and diastolic dysfunction.[190] The pro-fibrotic factor CTGF, which is induced in cardiac fibroblasts upon TGF-β1 stimulation,
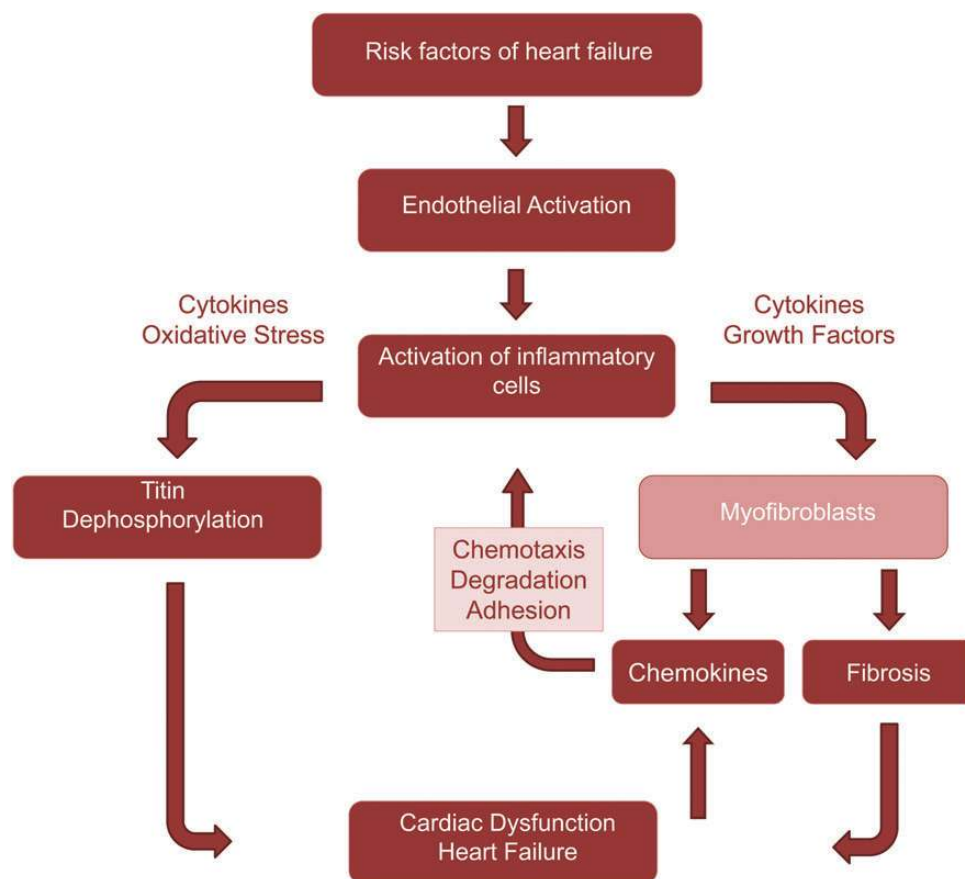
Figure 3 Pivotal role of myofibroblasts in the pathogenesis of heart failure. Risk factors of heart failure (diabetes mellitus, age, smoking, hypertension, etc.) lead to endothelial cell activation and the induction of inflammatory responses, which trigger the transdifferentiation of fibroblasts to myofibroblasts via the release of cytokines and growth factors. Myofibroblasts, in turn, lead to extensive collagen production and the production of chemokines and further activate inflammatory cells to adhere to the endothelium, to release MMP-9 enabling transendothelial migration, and to induce chemokine expression, attracting other inflammatory cells. Besides triggering fibroblasts, inflammatory cells also lead to titin dephosphorylation in cardiomyocytes via the induction of oxidative stress leading to cardiomyocyte stiffness and further contributing to the pathogenesis of heart failure. Figure has been adapted from Tschöpe and Lam[193] with kind permission of Springer Science+Business Media.

also enhances the migration of monocytes.[191] The ability of cardiac fibroblasts to induce the chemotaxis of monocytes via their production of CTGF[191] and/or chemokines[166] and to facilitate transendothelial migration through the basal membrane[166] indicate a self-maintaining mechanism supporting the inflammatory and fibrotic process.[192] It is suggested that these mechanisms also affect the cardiomyocyte, including the function of titin (*Figure 3*).[193] Further studies still have to prove whether thereby differences occur between HFPEF and heart failure with reduced ejection fraction.

6. Conclusions and perspectives

There is accumulating evidence showing that fibroblasts are - in contrast to their traditional view of being solely matrix-producing cells - cells with important immunomodulatory properties, playing a pivotal role in the switch to chronic inflammation. The complex interaction among different immune cells triggering the fibroblast on the one hand, and the multifactorial enhancement of tissue inflammation by fibroblasts on the other hand (as summarized in this review) might explain why uni-directed strategies blocking inflammation[194] or fibrosis have failed to abrogate

the fibrotic process, and indicate the need for therapies with more broaden immunomodulatory effects. In that view, MSC and the recently identified cardiac-derived adherent proliferating cells (CardAPs)[195] having both immunomodulatory[177–179,196] and anti-fibrotic[179,180,197] features are attractive tools to counteract the inflammatory/fibrotic process. They home to the site of injury and their cardioprotective effects are exerted via the cardiosplenic axis.[179] CardAPs might further profit from their cardiac niche origin. Especially HFPEF, where the mechanisms triggering inflammation and fibrosis probably represent multifactorial stressors, including metabolic derangements (diabetes mellitus), might profit from a multi-directed strategy like the iv application of MSC. Finally, the differences in functionality among fibroblasts from healthy and diseased tissues, which underlie their diverse intrinsic susceptibility to inflammation, indicate the need to investigate the inflammation/fibrosis status via (endomyocardial) biopsies allowing an optimal stratified strategy.

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References

1. Weber KT. Fibrosis and hypertensive heart disease. *Curr Opin Cardiol* 2000;**15**: 264–272.

2. Wick G, Grundtman C, Mayerl C, Wimpissinger TF, Feichtinger J, Zelger B, Sgonc R, Wolfram D. The immunology of fibrosis. *Ann Rev Immunol* 2013;**31**:107–135.

3. Buckley CD, Pilling D, Lord JM, Akbar AN, Scheel-Toellner D, Salmon M. Fibroblasts regulate the switch from acute resolving to chronic persistent inflammation. *Trends Immunol* 2001;**22**:199–204.

4. Nag AC. Study of non-muscle cells of the adult mammalian heart: a fine structural analysis and distribution. *Cytobios* 1980;**28**:41–61.

5. Camelliti P, Borg TK, Kohl P. Structural and functional characterisation of cardiac fibroblasts. *Cardiovasc Res* 2005;**65**:40–51.

6. Ottaviano FG, Yee KO. Communication signals between cardiac fibroblasts and cardiac myocytes. *J Cardiovasc Pharmacol* 2011;**57**:513–521.

7. Martin ML, Blaxall BC. Cardiac intercellular communication: are myocytes and fibroblasts fair-weather friends? *J Cardiovasc Transl Res* 2012;**5**:768–782.

8. Brouty-Boye D, Pottin-Clemenceau C, Doucet C, Jasmin C, Azzarone B. Chemokines and cd40 expression in human fibroblasts. *Eur J Immunol* 2000;**30**:914–919.

9. Lindner D, Zietsch C, Becher PM, Schulze K, Schultheiss HP, Tschope C, Westermann D. Differential expression of matrix metalloproteases in human fibroblasts with different origins. *Biochem Res Int* 2012;**2012**:875742.

10. Parsonage G, Falciani F, Burman A, Filer A, Ross E, Bofill M, Martin S, Salmon M, Buckley CD. Global gene expression profiles in fibroblasts from synovial, skin and lymphoid tissue reveals distinct cytokine and chemokine expression patterns. *Thromb Haemost* 2003;**90**:688–697.

11. Chang HY, Chi JT, Dudoit S, Bondre C, van de Rijn M, Botstein D, Brown PO. Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc Natl Acad Sci USA* 2002;**99**:12877–12882.

12. Zeisberg M, Yang C, Martino M, Duncan MB, Rieder F, Tanjore H, Kalluri R. Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition. *J Biol Chem* 2007;**282**:23337–23347.

13. Zeisberg M, Kalluri R. Fibroblasts emerge via epithelial-mesenchymal transition in chronic kidney fibrosis. *Front Biosc* 2008;**13**:6991–6998.

14. Li Z, Wermuth PJ, Benn BS, Lisanti MP, Jimenez SA. Caveolin-1 deficiency induces spontaneous endothelial-to-mesenchymal transition in murine pulmonary endothelial cells in vitro. *Am J Pathol* 2013;**182**:325–331.

15. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, Chandraker A, Yuan X, Pu WT, Roberts AB, Neilson EG, Sayegh MH, Izumo S, Kalluri R. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 2007;**13**:952–961.

16. Potenta S, Zeisberg E, Kalluri R. The role of endothelial-to-mesenchymal transition in cancer progression. *Br J Cancer* 2008;**99**:1375–1379.

17. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med* 1994;**1**:71–81.

18. Galligan CL, Fish EN. Circulating fibrocytes contribute to the pathogenesis of collagen antibody-induced arthritis. *Arthritis Rheum* 2012;**64**:3583–3593.

19. Keeley EC, Mehrad B, Janardhanan R, Salerno M, Hunter JR, Burdick MM, Field JJ, Strieter RM, Kramer CM. Elevated circulating fibrocyte levels in patients with hypertensive heart disease. *J Hypertens* 2012;**30**:1856–1861.

20. Reilkoff RA, Bucala R, Herzog EL. Fibrocytes: emerging effector cells in chronic inflammation. *Nat Rev Immunol* 2011;**11**:427–435.

21. Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. *Hypertension* 2001;**38**:581–587.

22. Creagh EM, O'Neill LA. TLRs, NLRs and RLRs: a trinity of pathogen sensors that co-operate in innate immunity. *Trends Immunol* 2006;**27**:352–357.

23. Meneghin A, Hogaboam CM. Infectious disease, the innate immune response, and fibrosis. *J Clin Invest* 2007;**117**:530–538.

24. Otte JM, Rosenberg IM, Podolsky DK. Intestinal myofibroblasts in innate immune responses of the intestine. *Gastroenterology* 2003;**124**:1866–1878.

25. Shiraki R, Inoue N, Kawasaki S, Takei A, Kadotani M, Ohnishi Y, Ejiri J, Kobayashi S, Hirata K, Kawashima S, Yokoyama M. Expression of toll-like receptors on human platelets. *Thromb Res* 2004;**113**:379–385.

26. Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional toll-like receptor-4. *Blood* 2005;**106**:2417–2423.

27. Liu Y, Gao XM, Fang L, Jennings NL, Su Y, Xu Q, Samson AL, Kiriazis H, Wang XF, Shan L, Sturgeon SA, Medcalf RL, Jackson SP, Dart AM, Du XJ. Novel role of platelets in mediating inflammatory responses and ventricular rupture or remodeling following myocardial infarction. *Arterioscler Thromb Vasc Biol* 2011;**31**:834–841.

28. Sinno H, Prakash S. Complements and the wound healing cascade: an updated review. *Plastic Surgery Int* 2013;**2013**:146764.

29. Chambers RC. Procoagulant signalling mechanisms in lung inflammation and fibrosis: novel opportunities for pharmacological intervention? *Br J Pharmacol* 2008; **153**(Suppl 1):S367–S378.

30. Scotton CJ, Krupiczojc MA, Konigshoff M, Mercer PF, Lee YC, Kaminski N, Morser J, Post JM, Maher TM, Nicholson AG, Moffatt JD, Laurent GJ, Derian CK, Eickelberg O, Chambers RC. Increased local expression of coagulation factor X contributes to the fibrotic response in human and murine lung injury. *J Clin Invest* 2009;**119**:2550–2563.

31. Ameri A, Kurachi S, Sueishi K, Kuwahara M, Kurachi K. Myocardial fibrosis in mice with overexpression of human blood coagulation factor ix. *Blood* 2003;**101**:1871–1873.

32. Xu H, Noria F, Sandoval-Cooper MJ, Menchen H, Donahue DL, Ploplis VA, Castellino FJ. Severe deficiency of coagulation factor vii results in spontaneous cardiac fibrosis in mice. *J Pathol* 2009;**217**:362–371.

33. Cahill PA, Redmond EM, Sitzmann JV. Endothelial dysfunction in cirrhosis and portal hypertension. *Pharmacol Ther* 2001;**89**:273–293.

34. Hugenholtz GG, Porte RJ, Lisman T. The platelet and platelet function testing in liver disease. *Clin Liver Dis* 2009;**13**:11–20.

35. Stramer BM, Mori R, Martin P. The inflammation-fibrosis link? A Jekyll and Hyde role for blood cells during wound repair. *J Invest Dermatol* 2007;**127**:1009–1017.

36. Bratton DL, Henson PM. Neutrophil clearance: when the party is over, clean-up begins. *Trends Immunol* 2011;**32**:350–357.

37. Meissner J, Irfan A, Twerenbold R, Mueller S, Reiter M, Haaf P, Reichlin T, Schaub N, Winkler K, Pfister O, Heinisch C, Mueller C. Use of neutrophil count in early diagnosis and risk stratification of ami. *Am J Med* 2011;**124**:534–542.

38. Ciz M, Denev P, Kratchanova M, Vasicek O, Ambrozova G, Lojek A. Flavonoids inhibit the respiratory burst of neutrophils in mammals. *Oxid Med Cell Longev* 2012;**2012**: 181295.

39. Barnes TC, Anderson ME, Edwards SW, Moots RJ. Neutrophil-derived reactive oxygen species in SSC. *Rheumatol (Oxford)* 2012;**51**:1166–1169.

40. Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med* 2011;**208**: 1339–1350.

41. Svegliati-Baroni G, Saccomanno S, van Goor H, Jansen P, Benedetti A, Moshage H. Involvement of reactive oxygen species and nitric oxide radicals in activation and proliferation of rat hepatic stellate cells. *Liver* 2001;**21**:1–12.

42. Liu XH, Pan LL, Deng HY, Xiong QH, Wu D, Huang GY, Gong QH, Zhu YZ. Leonurine (SCM-198) attenuates myocardial fibrotic response via inhibition of NADPH oxidase 4. *Free Radic Biol Med* 2013;**54**:93–104.

43. Qin F, Simeone M, Patel R. Inhibition of NADPH oxidase reduces myocardial oxidative stress and apoptosis and improves cardiac function in heart failure after myocardial infarction. *Free Radic Biol Med* 2007;**43**:271–281.

44. Ma Y, Yabluchanskiy A, Lindsey ML. Neutrophil roles in left ventricular remodeling following myocardial infarction. *Fibrogenesis Tissue Repair* 2013;**6**:11.

45. Takemasa A, Ishii Y, Fukuda T. A neutrophil elastase inhibitor prevents bleomycin-induced pulmonary fibrosis in mice. *Eur Respir J* 2012;**40**:1475–1482.

46. Lefkowitz DL, Lefkowitz SS. Macrophage-neutrophil interaction: a paradigm for chronic inflammation revisited. *Immunol Cell Biol* 2001;**79**:502–506.

47. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* 2011;**11**:723–737.

48. Lim DH, Cho JY, Miller M, McElwain K, McElwain S, Broide DH. Reduced peribronchial fibrosis in allergen-challenged MMP-9-deficient mice. *Am J Physiol Lung Cell Mol Physiol* 2006;**291**:L265–L271.

49. Matsumoto Y, Park IK, Kohyama K. Matrix metalloproteinase (MMP)-9, but not MMP-2, is involved in the development and progression of c protein-induced myocarditis and subsequent dilated cardiomyopathy. *J Immunol* 2009;**183**:4773–4781.

50. Ducharme A, Frantz S, Aikawa M, Rabkin E, Lindsey M, Rohde LE, Schoen FJ, Kelly RA, Werb Z, Libby P, Lee RT. Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. *J Clin Invest* 2000;**106**:55–62.

51. Westermann D, Savvatis K, Lindner D, Zietsch C, Becher PM, Hammer E, Heimesaat MM, Bereswill S, Volker U, Escher F, Riad A, Plendl J, Klingel K, Poller W, Schultheiss HP, Tschope C. Reduced degradation of the chemokine MCP-3 by matrix metalloproteinase-2 exacerbates myocardial inflammation in experimental viral cardiomyopathy. *Circulation* 2011;**124**:2082–2093.

52. Verrecchia F, Mauviel A. Transforming growth factor-beta and fibrosis. *World J Gastroenterol* 2007;**13**:3056–3062.

53. Murray LA, Chen Q, Kramer MS, Hesson DP, Argentieri RL, Peng X, Gulati M, Homer RJ, Russell T, van Rooijen N, Elias JA, Hogaboam CM, Herzog EL. TGF-beta driven lung fibrosis is macrophage dependent and blocked by serum amyloid p. *Int J Biochem Cell Biol* 2011;**43**:154–162.

54. Annes JP, Munger JS, Rifkin DB. Making sense of latent tgfbeta activation. *J Cell Sci* 2003; **116**:217–224.

55. Leask A, Abraham DJ. Tgf-beta signaling and the fibrotic response. *FASEB J* 2004;**18**: 816–827.

56. Varga J, Jimenez SA. Stimulation of normal human fibroblast collagen production and processing by transforming growth factor-beta. *Biochem Biophys Res Commun* 1986; **138**:974–980.

57. Overall CM, Wrana JL, Sodek J. Independent regulation of collagenase, 72-kDa progelatinase, and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-beta. *J Biol Chem* 1989;**264**:1860–1869.

58. Chen SJ, Yuan W, Mori Y, Levenson A, Trojanowska M, Varga J. Stimulation of Type I collagen transcription in human skin fibroblasts by TGF-beta: involvement of SMAD 3. *J Invest Dermatol* 1999;**112**:49–57.

59. Verrecchia F, Chu ML, Mauviel A. Identification of novel TGF-beta/SMAD gene targets in dermal fibroblasts using a combined CDNA microarray/promoter transactivation approach. *J Biol Chem* 2001;**276**:17058–17062.

60. Ruiz-Ortega M, Rodriguez-Vita J, Sanchez-Lopez E, Carvajal G, Egido J. TGF-beta signaling in vascular fibrosis. *Cardiovasc Res* 2007;**74**:196–206.

61. Waltenberger J, Lundin L, Oberg K, Wilander E, Miyazono K, Heldin CH, Funa K. Involvement of transforming growth factor-beta in the formation of fibrotic lesions in carcinoid heart disease. *Am J Pathol* 1993;**142**:71–78.

62. Westermann D, Lindner D, Kasner M, Zietsch C, Savvatis K, Escher F, von Schlippenbach J, Skurk C, Steendijk P, Riad A, Poller W, Schultheiss HP, Tschope C. Cardiac inflammation contributes to changes in the extracellular matrix in patients with heart failure and normal ejection fraction. *Circ Heart Fail* 2011;**4**:44–52.

63. Bronnum H, Eskildsen T, Andersen DC, Schneider M, Sheikh SP. IL-1beta suppresses TGF-beta-mediated myofibroblast differentiation in cardiac fibroblasts. *Growth Fact* 2013;**31**:81–89.

64. Meyer-Ter-Vehn T, Gebhardt S, Sebald W, Buttmann M, Grehn F, Schlunck G, Knaus P. P38 inhibitors prevent TGF-beta-induced myofibroblast transdifferentiation in human tenon fibroblasts. *Invest Ophthalmol Vis Sci* 2006;**47**:1500–1509.

65. Shah M, Foreman DM, Ferguson MW. Control of scarring in adult wounds by neutralising antibody to transforming growth factor beta. *Lancet* 1992;**339**:213–214.

66. Sharma K, Jin Y, Guo J, Ziyadeh FN. Neutralization of TGF-beta by anti-TGF-beta antibody attenuates kidney hypertrophy and the enhanced extracellular matrix gene expression in STZ-induced diabetic mice. *Diabetes* 1996;**45**:522–530.

67. Koyanagi M, Egashira K, Kubo-Inoue M, Usui M, Kitamoto S, Tomita H, Shimokawa H, Takeshita A. Role of transforming growth factor-beta1 in cardiovascular inflammatory changes induced by chronic inhibition of nitric oxide synthesis. *Hypertension* 2000;**35**:86–90.

68. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, Imaizumi T. Transforming growth factor-beta function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. *Circulation* 2002;**106**:130–135.

69. Kulkarni AB, Karlsson S. Transforming growth factor-beta 1 knockout mice. A mutation in one cytokine gene causes a dramatic inflammatory disease. *Am J Pathol* 1993;**143**:3–9.

70. Bottinger EP, Letterio JJ, Roberts AB. Biology of TGF-beta in knockout and transgenic mouse models. *Kidney Int* 1997;**51**:1355–1360.

71. Ikeuchi M, Tsutsui H, Shiomi T, Matsusaka H, Matsushima S, Wen J, Kubota T, Takeshita A. Inhibition of TGF-beta signaling exacerbates early cardiac dysfunction but prevents late remodeling after infarction. *Cardiovasc Res* 2004;**64**:526–535.

72. Okada H, Takemura G, Kosai K, Li Y, Takahashi T, Esaki M, Yuge K, Miyata S, Maruyama R, Mikami A, Minatoguchi S, Fujiwara T, Fujiwara H. Postinfarction gene therapy against transforming growth factor-beta signal modulates infarct tissue dynamics and attenuates left ventricular remodeling and heart failure. *Circulation* 2005;**111**:2430–2437.

73. Kitani A, Fuss I, Nakamura K, Kumaki F, Usui T, Strober W. Transforming growth factor (TGF)-beta1-producing regulatory T cells induce SMAD-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-beta1-mediated fibrosis. *J Exp Med* 2003;**198**:1179–1188.

74. McCartney-Francis NL, Frazier-Jessen M, Wahl SM. TGF-beta: a balancing act. *Int Rev Immunol* 1998;**16**:553–580.

75. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, Wu S, Lang R, Iredale JP. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. *J Clin Invest* 2005;**115**:56–65.

76. van Amerongen MJ, Harmsen MC, van Rooijen N, Petersen AH, van Luyn MJ. Macrophage depletion impairs wound healing and increases left ventricular remodeling after myocardial injury in mice. *Am J Pathol* 2007;**170**:818–829.

77. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010;**11**:889–896.

78. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 2002;**23**:549–555.

79. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* 2008;**13**:453–461.

80. Martinez FO, Helming L, Gordon S. Alternative activation of macrophages: an immunologic functional perspective. *Ann Rev Immunol* 2009;**27**:451–483.

81. Bagnost T, Ma L, da Silva RF, Rezakhaniha R, Houdayer C, Stergiopulos N, Andre C, Guillaume Y, Berthelot A, Demougeot C. Cardiovascular effects of arginase inhibition in spontaneously hypertensive rats with fully developed hypertension. *Cardiovasc Res* 2010;**87**:569–577.

82. Meznarich J, Malchodi L, Helterline D, Ramsey SA, Bertko K, Plummer T, Plawman A, Gold E, Stempien-Otero A. Urokinase plasminogen activator induces pro-fibrotic/M2 phenotype in murine cardiac macrophages. *PLoS ONE* 2013;**8**:e57837.

83. Hesse M, Modolell M, La Flamme AC, Schito M, Fuentes JM, Cheever AW, Pearce EJ, Wynn TA. Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. *J Immunol* 2001;**167**:6533–6544.

84. Karlmark KR, Weiskirchen R, Zimmermann HW, Gassler N, Ginhoux F, Weber C, Merad M, Luedde T, Trautwein C, Tacke F. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. *Hepatology* 2009;**50**:261–274.

85. Ishimaru K, Ueno H, Kagitani S, Takabayashi D, Takata M, Inoue H. Fasudil attenuates myocardial fibrosis in association with inhibition of monocyte/macrophage infiltration in the heart of DOCA/salt hypertensive rats. *J Cardiovasc Pharmacol* 2007;**50**:187–194.

86. Fairweather D, Cihakova D. Alternatively activated macrophages in infection and autoimmunity. *J Autoimmunity* 2009;**33**:222–230.

87. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* 2012;**122**:787–795.

88. Sullivan DE, Ferris M, Nguyen H, Abboud E, Brody AR. TNF-alpha induces TGF-beta1 expression in lung fibroblasts at the transcriptional level via AP-1 activation. *J Cell Mol Med* 2009;**13**:1866–1876.

89. Luedde T, Schwabe RF. NF-κB in the liver—linking injury, fibrosis and hepatocellular carcinoma. *Nat Rev Gastrol Hepatol* 2011;**8**:108–118.

90. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol* 2008;**214**:199–210.

91. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007;**117**:524–529.

92. Szabo G, Mandrekar P, Dolganiuc A. Innate immune response and hepatic inflammation. *Semin Liver Dis* 2007;**27**:339–350.

93. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007;**13**:1324–1332.

94. Aceves SS, Ackerman SJ. Relationships between eosinophilic inflammation, tissue remodeling, and fibrosis in eosinophilic esophagitis. *Immunol Allergy Clin North Am* 2009;**29**:197–211, xiii-xiv.

95. Wehling-Henricks M, Sokolow S, Lee JJ, Myung KH, Villalta SA, Tidball JG. Major basic protein-1 promotes fibrosis of dystrophic muscle and attenuates the cellular immune response in muscular dystrophy. *Hum Mol Genet* 2008;**17**:2280–2292.

96. Sasano H, Virmani R, Patterson RH, Robinowitz M, Guccion JG. Eosinophilic products lead to myocardial damage. *Hum Pathol* 1989;**20**:850–857.

97. Tai PC, Ackerman SJ, Spry CJ, Dunnette S, Olsen EG, Gleich GJ. Deposits of eosinophil granule proteins in cardiac tissues of patients with eosinophilic endomyocardial disease. *Lancet* 1987;**1**:643–647.

98. Levick SP, McLarty JL, Murray DB, Freeman RM, Carver WE, Brower GL. Cardiac mast cells mediate left ventricular fibrosis in the hypertensive rat heart. *Hypertension* 2009;**53**:1041–1047.

99. Zhao XY, Zhao LY, Zheng QS, Su JL, Guan H, Shang FJ, Niu XL, He YP, Lu XL. Chymase induces profibrotic response via transforming growth factor-beta 1/SMAD activation in rat cardiac fibroblasts. *Mol Cell Biochem* 2008;**310**:159–166.

100. Stewart JA Jr, Wei CC, Brower GL, Rynders PE, Hankes GH, Dillon AR, Lucchesi PA, Janicki JS, Dell'Italia LJ. Cardiac mast cell- and chymase-mediated matrix metalloproteinase activity and left ventricular remodeling in mitral regurgitation in the dog. *J Mol Cell Cardiol* 2003;**35**:311–319.

101. Kanellakis P, Ditiatkovski M, Kostolias G, Bobik A. A pro-fibrotic role for interleukin-4 in cardiac pressure overload. *Cardiovasc Res* 2012;**95**:77–85.

102. Veerappan A, O'Connor NJ, Brazin J, Reid AC, Jung A, McGee D, Summers B, Branch-Elliman D, Stiles B, Worgall S, Kaner RJ, Silver RB. Mast cells: a pivotal role in pulmonary fibrosis. *DNA Cell Biol* 2013;**32**:206–218.

103. Levick SP, Melendez GC, Plante E, McLarty JL, Brower GL, Janicki JS. Cardiac mast cells: the centrepiece in adverse myocardial remodelling. *Cardiovasc Res* 2011;**89**:12–19.

104. McLarren KW, Cole AE, Weisser SB, Voglmaier NS, Conlin VS, Jacobson K, Popescu O, Boucher JL, Sly LM. Ship-deficient mice develop spontaneous intestinal inflammation and arginase-dependent fibrosis. *Am J Pathol* 2011;**179**:180–188.

105. Henderson NC, Iredale JP. Liver fibrosis: cellular mechanisms of progression and resolution. *Clin Sci (Lond)* 2007;**112**:265–280.

106. Wynn TA. Fibrotic disease and the T(h)1/T(h)2 paradigm. *Nat Rev Immunol* 2004;**4**:583–594.

107. Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol* 2000;**164**:2585–2591.

108. Yang G, Volk A, Petley T, Emmell E, Giles-Komar J, Shang X, Li J, Das AM, Shealy D, Griswold DE, Li L. Anti-IL-13 monoclonal antibody inhibits airway hyperresponsiveness, inflammation and airway remodeling. *Cytokine* 2004;**28**:224–232.

109. Hashimoto S, Gon Y, Takeshita I, Maruoka S, Horie T. IL-4 and IL-13 induce myofibroblastic phenotype of human lung fibroblasts through c-Jun NH$_2$-terminal kinase-dependent pathway. *J Allergy Clin Immunol* 2001;**107**:1001–1008.

110. Bailey JR, Bland PW, Tarlton JF, Peters I, Moorghen M, Sylvester PA, Probert CS, Whiting CV. IL-13 promotes collagen accumulation in Crohn's disease fibrosis by down-regulation of fibroblast MMP synthesis: a role for innate lymphoid cells? *PLoS ONE* 2012;**7**:e52332.

111. Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13alpha2 receptor is involved in induction of TGF-beta1 production and fibrosis. *Nat Med* 2006;**12**:99–106.

112. Brunner SM, Schiechl G, Kesselring R, Martin M, Balam S, Schlitt HJ, Geissler EK, Fichtner-Feigl S. IL-13 signaling via IL-13ralpha2 triggers TGF-beta1-dependent allograft fibrosis. *Transplant Res* 2013;**2**:16.

113. Mentink-Kane MM, Wynn TA. Opposing roles for IL-13 and IL-13 receptor alpha 2 in health and disease. *Immunol Rev* 2004;**202**:191–202.

114. Ramalingam TR, Pesce JT, Sheikh F, Cheever AW, Mentink-Kane MM, Wilson MS, Stevens S, Valenzuela DM, Murphy AJ, Yancopoulos GD, Urban JF Jr, Donnelly RP,

Wynn TA. Unique functions of the Type II interleukin 4 receptor identified in mice lacking the interleukin 13 receptor alpha1 chain. *Nat Immunol* 2008;**9**:25–33.

115. Chen ES, Greenlee BM, Wills-Karp M, Moller DR. Attenuation of lung inflammation and fibrosis in interferon-gamma-deficient mice after intratracheal bleomycin. *Am J Respir Cell Mol Biol* 2001;**24**:545–555.

116. Marko L, Kvakan H, Park JK, Qadri F, Spallek B, Binger KJ, Bowman EP, Kleinewietfeld M, Fokuhl V, Dechend R, Muller DN. Interferon-gamma signaling inhibition ameliorates angiotensin II-induced cardiac damage. *Hypertension* 2012;**60**:1430–1436.

117. Nathan CF, Prendergast TJ, Wiebe ME, Stanley ER, Platzer E, Remold HG, Welte K, Rubin BY, Murray HW. Activation of human macrophages. Comparison of other cytokines with interferon-gamma. *J Exp Med* 1984;**160**:600–605.

118. Piguet PF, Collart MA, Grau GE, Kapanci Y, Vassalli P. Tumor necrosis factor/cachectin plays a key role in bleomycin-induced pneumopathy and fibrosis. *J Exp Med* 1989;**170**: 655–663.

119. Han YL, Li YL, Jia LX, Cheng JZ, Qi YF, Zhang HJ, Du J. Reciprocal interaction between macrophages and T cells stimulates IFN-gamma and MCP-1 production in Ang II-induced cardiac inflammation and fibrosis. *PLoS ONE* 2012;**7**:e35506.

120. Jimenez SA, Freundlich B, Rosenbloom J. Selective inhibition of human diploid fibroblast collagen synthesis by interferons. *J Clin Invest* 1984;**74**:1112–1116.

121. Gurujeyalakshmi G, Giri SN. Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: downregulation of TGF-beta and procollagen I and III gene expression. *Exp Lung Res* 1995;**21**:791–808.

122. Ulloa L, Doody J, Massague J. Inhibition of transforming growth factor-beta/SMAD signalling by the interferon-gamma/stat pathway. *Nature* 1999;**397**:710–713.

123. Eickelberg O, Pansky A, Koehler E, Bihl M, Tamm M, Hildebrand P, Perruchoud AP, Kashgarian M, Roth M. Molecular mechanisms of TGF-(beta) antagonism by interferon (gamma) and cyclosporine a in lung fibroblasts. *FASEB J* 2001;**15**:797–806.

124. Shao DD, Suresh R, Vakil V, Gomer RH, Pilling D. Pivotal advance: Th-1 cytokines inhibit, and Th-2 cytokines promote fibrocyte differentiation. *J Leukoc Biol* 2008;**83**: 1323–1333.

125. Fairweather D, Frisancho-Kiss S, Yusung SA, Barrett MA, Davis SE, Gatewood SJ, Njoku DB, Rose NR. Interferon-gamma protects against chronic viral myocarditis by reducing mast cell degranulation, fibrosis, and the profibrotic cytokines transforming growth factor-beta 1, interleukin-1 beta, and interleukin-4 in the heart. *Am J Pathol* 2004;**165**:1883–1894.

126. Wilson MS, Madala SK, Ramalingam TR, Gochuico BR, Rosas IO, Cheever AW, Wynn TA. Bleomycin and IL-1beta-mediated pulmonary fibrosis is IL-17a dependent. *J Exp Med* 2010;**207**:535–552.

127. Feng W, Li W, Liu W, Wang F, Li Y, Yan W. IL-17 induces myocardial fibrosis and enhances RANKl/OPG and MMP/TIMP signaling in isoproterenol-induced heart failure. *Exp Mol Pathol* 2009;**87**:212–218.

128. Wang L, Chen S, Xu K. IL-17 expression is correlated with hepatitis b related liver diseases and fibrosis. *Int J Mol Med* 2011;**27**:385–392.

129. Ouyang W, Kolls JK, Zheng Y. The biological functions of T helper 17 cell effector cytokines in inflammation. *Immunity* 2008;**28**:454–467.

130. Pelletier M, Maggi L, Micheletti A, Lazzeri E, Tamassia N, Costantini C, Cosmi L, Lunardi C, Annunziato F, Romagnani S, Cassatella MA. Evidence for a cross-talk between human neutrophils and Th17 cells. *Blood* 2010;**115**:335–343.

131. Cortez DM, Feldman MD, Mummidi S, Valente AJ, Steffensen B, Vincenti M, Barnes JL, Chandrasekar B. IL-17 stimulates MMP-1 expression in primary human cardiac fibroblasts via p38 MAPK- and ERK1/2-dependent C/EBP-beta, NF-κB, and AP-1 activation. *Am J Physiol Heart Circ Physiol* 2007;**293**:H3356–H3365.

132. Liu Y, Zhu H, Su Z, Sun C, Yin J, Yuan H, Sandoghchian S, Jiao Z, Wang S, Xu H. IL-17 contributes to cardiac fibrosis following experimental autoimmune myocarditis by a PKCbeta/Erk1/2/NF-κβ-dependent signaling pathway. *Int Immunol* 2012;**24**:605–612.

133. Xie Y, Li M, Wang X, Zhang X, Peng T, Yang Y, Zou Y, Ge J, Chen H, Chen R. In vivo delivery of adenoviral vector containing interleukin-17 receptor a reduces cardiac remodeling and improves myocardial function in viral myocarditis leading to dilated cardiomyopathy. *PLoS ONE* 2013;**8**:e72158.

134. Zhang Z, Zhao J, Fu Y, Wang FS. Increased IL-22-producing cells contribute to liver fibrosis through promoting Th17 migration in chronic HBV patients. *J Immunol* 2013; **190**:202–204.

135. Nakashima T, Jinnin M, Yamane K, Honda N, KKajihara I, Makino T, Masuquchi S, Fukushima S, Okamoto Y, Hasegawa M, Fujimoto M, Ihn H. Impaired IL-17 signaling pathway contributes to the increased collagen expression in scleroderma fibroblasts. *J Immunol* 2012;**188**:3573–3583.

136. Brembilla NC, Montanari E, Truchetet ME, Raschi E, Meroni P, Chizzolini C. Th17 cells favor inflammatory responses while inhibiting type I collagen deposition by dermal fibroblasts: differential effects in healthy and systemic sclerosis fibroblasts. *Arthritis Res Ther* 2013;**15**:R151.

137. Simonian PL, Roark CL, Born WK, O'Brien RL, Fontenot AP. Gammadelta T cells and Th1 cytokines in hypersensitivity pneumonitis and lung fibrosis. *Trans Res* 2009;**154**: 222–227.

138. Liu F, Liu J, Weng D, Chen Y, Song L, He Q, Chen J. CD4+CD25+FOXp3+ regulatory T cells depletion may attenuate the development of silica-induced lung fibrosis in mice. *PLoS ONE* 2010;**5**:e15404.

139. Kanellakis P, Dinh TN, Agrotis A, Bobik A. CD4(+)CD25(+)FOXp3(+) regulatory t cells suppress cardiac fibrosis in the hypertensive heart. *J Hypertens* 2011;**29**: 1820–1828.

140. Cao Y, Xu W, Xiong S. Adoptive transfer of regulatory T cells protects against Coxsackievirus B3-induced cardiac fibrosis. *PLoS ONE* 2013;**8**:e74955.

141. Shi JH, Guan H, Shi S, Cai WX, Bai XZ, Hu XL, Fang XB, Liu JQ, Tao K, Zhu XX, Tang CW, Hu DH. Protection against TGF-beta1-induced fibrosis effects of IL-10 on dermal fibroblasts and its potential therapeutics for the reduction of skin scarring. *Arch Dermatol Res* 2013;**305**:341–352.

142. Yuan C, Chen WX, Zhu JS, Chen NW, Lu YM, Ou YX, Chen HQ. IL-10 treatment is associated with prohibitin expression in the Crohn's disease intestinal fibrosis mouse model. *Med Inflamm* 2013;**2013**:617145.

143. Krishnamurthy P, Rajasingh J, Lambers E, Qin G, Losordo DW, Kishore R. IL-10 inhibits inflammation and attenuates left ventricular remodeling after myocardial infarction via activation of STAT3 and suppression of HUR. *Circ Res* 2009;**104**:e9–18.

144. Verma SK, Krishnamurthy P, Barefield D, Singh N, Gupta R, Lambers E, Thal M, Mackie A, Hoxha E, Ramirez V, Qin G, Sadayappan S, Ghosh AK, Kishore R. Interleukin-10 treatment attenuates pressure overload-induced hypertrophic remodeling and improves heart function via signal transducers and activators of transcription 3-dependent inhibition of nuclear factor-κβ. *Circulation* 2012;**126**:418–429.

145. Sun L, Louie MC, Vannella KM, Wilke CA, LeVine AM, Moore BB, Shanley TP. New concepts of IL-10-induced lung fibrosis: fibrocyte recruitment and M2 activation in a CCL2/CCR2 axis. *Am J Physiol Lung Cell Mol Physiol* 2011;**300**:L341–L353.

146. Estes JD, Wietgrefe S, Schacker T, Southern P, Beilman G, Reilly C, Milush JM, Lifson JD, Sodora DL, Carlis JV, Haase AT. Simian immunodeficiency virus-induced lymphatic tissue fibrosis is mediated by transforming growth factor beta 1-positive regulatory T cells and begins in early infection. *J Infect Dis* 2007;**195**:551–561.

147. Yanaba K, Bouaziz JD, Matsushita T, Magro CM, St Clair EW, Tedder TF. B-lymphocyte contributions to human autoimmune disease. *Immunol Rev* 2008;**223**:284–299.

148. Lund FE. Cytokine-producing b lymphocytes-key regulators of immunity. *Curr Opin Immunol* 2008;**20**:332–338.

149. Novobrantseva TI, Majeau GR, Amatucci A, Kogan S, Brenner I, Casola S, Shlomchik MJ, Koteliansky V, Hochman PS, Ibraghimov A. Attenuated liver fibrosis in the absence of B cells. *J Clin Invest* 2005;**115**:3072–3082.

150. Xue H, McCauley RL, Zhang W. Elevated interleukin-6 expression in keloid fibroblasts. *J Surg Res* 2000;**89**:74–77.

151. Zhou X, Tan FK, Milewicz DM, Guo X, Bona CA, Arnett FC. Autoantibodies to fibrillin-1 activate normal human fibroblasts in culture through the TGF-beta pathway to recapitulate the 'scleroderma phenotype'. *J Immunol* 2005;**175**:4555–4560.

152. Franc?ois A, Chatelus E, Wachsmann D, Sibilia J, Bahram S, Alsaleh G, Gottenberg JE. B lymphocytes and B-cell activating factor promote collagen and profibrotic markers expression by dermal fibroblasts in systemic sclerosis. *Arthritis Res Ther* 2013;**15**:R168.

153. Yu M, Wen S, Wang M, Liang W, Li HH, Long Q, Guo HP, Liao YH, Yuan J. TNF-alpha-secreting B cells contribute to myocardial fibrosis in dilated cardiomyopathy. *J Clin Immun* 2013;**33**:1002–1008.

154. Parsonage G, Filer AD, Haworth O, Nash GB, Rainger GE, Salmon M, Buckley CD. A stromal address code defined by fibroblasts. *Trends Immunol* 2005;**26**:150–156.

155. Tieu BC, Lee C, Sun H, Lejeune W, Recinos A III, Ju X, Spratt H, Guo DC, Milewicz D, Tilton RG, Brasier AR. An adventitial IL-6/MCP1 amplification loop accelerates macrophage-mediated vascular inflammation leading to aortic dissection in mice. *J Clin Invest* 2009;**119**:3637–3651.

156. Nash GB, Buckley CD, Ed Rainger G. The local physicochemical environment conditions the proinflammatory response of endothelial cells and thus modulates leukocyte recruitment. *FEBS Lett* 2004;**569**:13–17.

157. Rainger GE, Stone P, Morland CM, Nash GB. A novel system for investigating the ability of smooth muscle cells and fibroblasts to regulate adhesion of flowing leukocytes to endothelial cells. *J Immunol Methods* 2001;**255**:73–82.

158. Lukacs NW, Chensue SW, Smith RE, Strieter RM, Warmington K, Wilke C, Kunkel SL. Production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1 alpha by inflammatory granuloma fibroblasts. *Am J Pathol* 1994;**144**:711–718.

159. Lukacs NW, Kunkel SL, Allen R, Evanoff HL, Shaklee CL, Sherman JS, Burdick MD, Strieter RM. Stimulus and cell-specific expression of C-X-C and C-C chemokines by pulmonary stromal cell populations. *Am J Physiol* 1995;**268**:L856–L861.

160. Hogaboam CM, Steinhauser ML, Chensue SW, Kunkel SL. Novel roles for chemokines and fibroblasts in interstitial fibrosis. *Kidney Int* 1998;**54**:2152–2159.

161. Gharaee-Kermani M, Denholm EM, Phan SH. Costimulation of fibroblast collagen and transforming growth factor beta1 gene expression by monocyte chemoattractant protein-1 via specific receptors. *J Biol Chem* 1996;**271**:17779–17784.

162. Xia Y, Pauza ME, Feng L, Lo D. Relb regulation of chemokine expression modulates local inflammation. *Am J Pathol* 1997;**151**:375–387.

163. Steinhauser ML, Kunkel SL, Hogaboam CM, Evanoff H, Strieter RM, Lukacs NW. Macrophage/fibroblast coculture induces macrophage inflammatory protein-1alpha production mediated by intercellular adhesion molecule-1 and oxygen radicals. *J Leukoc Biol* 1998;**64**:636–641.

164. Lin YM, Hsu CJ, Liao YY, Chou MC, Tang CH. The CCL2/CCR2 axis enhances vascular cell adhesion molecule-1 expression in human synovial fibroblasts. *PLOS ONE* 2012;**7**: e49999.

165. McGettrick HM, Smith E, Filer A, Kissane S, Salmon M, Buckley CD, Rainger GE, Nash GB. Fibroblasts from different sites may promote or inhibit recruitment of flowing lymphocytes by endothelial cells. *Eur J Immunol* 2009;**39**:113–125.

166. Lindner D, Zietsch C, Schultheiss H-P, Tschöpe C, Westermann D. Cardiac fibroblasts as a stimulator for cardiac inflammation. *Cardiovasc Res* 2012;**93**(Suppl 1):S102.

167. Smith RS, Smith TJ, Blieden TM, Phipps RP. Fibroblasts as sentinel cells. Synthesis of chemokines and regulation of inflammation. *Am J Pathol* 1997;**151**:317–322.

168. Yellin MJ, Winikoff S, Fortune SM, Baum D, Crow MK, Lederman S, Chess L. Ligation of CD40 on fibroblasts induces CD54 (ICAM-1) and CD106 (VCAM-1) up-regulation and IL-6 production and proliferation. *J Leukoc Biol* 1995;**58**:209–216.

169. Lee SK, Seo SH, Kim BS, Kim CD, Lee JH, Kang JS, Maeng PJ, Lim JS. IFN-gamma regulates the expression of B7-H1 in dermal fibroblast cells. *J Derm Sci* 2005;**40**:95–103.

170. Buckley CD, Amft N, Bradfield PF, Pilling D, Ross E, Arenzana-Seisdedos F, Amara A, Curnow SJ, Lord JM, Scheel-Toellner D, Salmon M. Persistent induction of the chemokine receptor CXCR4 by TGF-beta 1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. *J Immunol* 2000;**165**:3423–3429.

171. Li M, Riddle SR, Frid MG, El Kasmi KC, McKinsey TA, Sokol RJ, Strassheim D, Meyrick B, Yeager ME, Flockton AR, McKeon BA, Lemon DD, Horn TR, Anwar A, Barajas C, Stenmark KR. Emergence of fibroblasts with a proinflammatory epigenetically altered phenotype in severe hypoxic pulmonary hypertension. *J Immunol* 2011;**187**: 2711–2722.

172. Chung SH, Seki K, Choi BI, Kimura KB, Ito A, Fujikado N, Saijo S, Iwakura Y. CXC chemokine receptor 4 expressed in T cells plays an important role in the development of collagen-induced arthritis. *Arthritis Res Ther* 2010;**12**:R188.

173. Garibaldi BT, D'Alessio FR, Mock JR, Files DC, Chau E, Eto Y, Drummond MB, Aggarwal NR, Sidhaye V, King LS. Regulatory T cells reduce acute lung injury fibroproliferation by decreasing fibrocyte recruitment. *Am J Resp Cell Mol Biol* 2013;**48**:35–43.

174. Ponte AL, Marais E, Gallay N, Langonne A, Delorme B, Herault O, Charbord P, Domenech J. The in vitro migration capacity of human bone marrow mesenchymal stem cells: comparison of chemokine and growth factor chemotactic activities. *Stem Cells* 2007;**25**:1737–1745.

175. Van Linthout S, Stamm C, Schultheiss HP, Tschope C. Mesenchymal stem cells and inflammatory cardiomyopathy: cardiac homing and beyond. *Cardiol Res Pract* 2011; **2011**:757154.

176. Escher F, Schmidt-Lucke C, Van Linthout S, Savvatis K, Schultheiss H-P, Tschöpe C. Cardiac migration of mesenchymal stem cells in patients with inflammatory cardiomyopathy. *Eur Heart J Suppl* 2010;**31**:S464–S465.

177. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell* 2008;**2**:141–150.

178. Van Linthout S, Savvatis K, Miteva K, Peng J, Ringe J, Warstat K, Schmidt-Lucke C, Sittinger M, Schultheiss HP, Tschope C. Mesenchymal stem cells improve murine acute Coxsackievirus B3-induced myocarditis. *Eur Heart J* 2011;**32**:2168–2178.

179. Savvatis K, van Linthout S, Miteva K, Pappritz K, Westermann D, Schefold JC, Fusch G, Weithauser A, Rauch U, Becher PM, Klingel K, Ringe J, Kurtz A, Schultheiss HP, Tschope C. Mesenchymal stromal cells but not cardiac fibroblasts exert beneficial systemic immunomodulatory effects in experimental myocarditis. *PLoS ONE* 2012;**7**: e41047.

180. Mias C, Lairez O, Trouche E, Roncalli J, Calise D, Seguelas MH, Ordener C, Piercecchi-Marti MD, Auge N, Salvayre AN, Bourin P, Parini A, Cussac D. Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction. *Stem Cells* 2009;**27**: 2734–2743.

181. Poznansky MC, Olszak IT, Foxall R, Evans RH, Luster AD, Scadden DT. Active movement of T cells away from a chemokine. *Nat Med* 2000;**6**:543–548.

182. Salmon M, Scheel-Toellner D, Huissoon AP, Pilling D, Shamsadeen N, Hyde H, D'Angeac AD, Bacon PA, Emery P, Akbar AN. Inhibition of T cell apoptosis in the rheumatoid synovium. *J Clin Invest* 1997;**99**:439–446.

183. Pilling D, Akbar AN, Girdlestone J, Orteu CH, Borthwick NJ, Amft N, Scheel-Toellner D, Buckley CD, Salmon M. Interferon-beta mediates stromal cell rescue of T cells from apoptosis. *Eur J Immunol* 1999;**29**:1041–1050.

184. Shen Y, Xu W, Chu YW, Wang Y, Liu QS, Xiong SD. Coxsackievirus group B type 3 infection upregulates expression of monocyte chemoattractant protein 1 in cardiac myocytes, which leads to enhanced migration of mononuclear cells in viral myocarditis. *J Virology* 2004;**78**:12548–12556.

185. Leuschner F, Panizzi P, Chico-Calero I, Lee WW, Ueno T, Cortez-Retamozo V, Waterman P, Gorbatov R, Marinelli B, Iwamoto Y, Chudnovskiy A, Figueiredo JL, Sosnovik DE, Pittet MJ, Swirski FK, Weissleder R, Nahrendorf M. Angiotensin-converting enzyme inhibition prevents the release of monocytes from their splenic reservoir in mice with myocardial infarction. *Circ Res* 2010;**107**:1364–1373.

186. Kishimoto C, Abelmann WH. Monoclonal antibody therapy for prevention of acute Coxsackievirus B3 myocarditis in mice. *Circulation* 1989;**79**:1300–1308.

187. Miteva K, Van Linthout S, Volk HD, Tschope C. Immunomodulatory effects of mesenchymal stromal cells revisited in the context of inflammatory cardiomyopathy. *Stem Cells Int* 2013;**2013**:353097.

188. Ismahil MA, Hamid T, Bansal SS, Patel B, Kingery JR, Prabhu SD. Remodeling of the mononuclear phagocyte network underlies chronic inflammation and disease progression in heart failure: critical importance of the cardiosplenic axis. *Circ Res* 2014;**114**:266–282.

189. Kai H, Mori T, Tokuda K, Takayama N, Tahara N, Takemiya K, Kudo H, Sugi Y, Fukui D, Yasukawa H, Kuwahara F, Imaizumi T. Pressure overload-induced transient oxidative stress mediates perivascular inflammation and cardiac fibrosis through angiotensin ii. *Hypertens Res* 2006;**29**:711–718.

190. Kapur NK. Transforming growth factor-beta: governing the transition from inflammation to fibrosis in heart failure with preserved left ventricular function. *Circ Heart Fail* 2011;**4**:5–7.

191. Liu SC, Hsu CJ, Fong YC, Chuang SM, Tang CH. CTGF induces monocyte chemoattractant protein-1 expression to enhance monocyte migration in human synovial fibroblasts. *Biochim Biophys Acta* 2013;**1833**:1114–1124.

192. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol* 2013;**62**:263–271.

193. Tschope C, Lam CS. Diastolic heart failure: what we still don't know. Looking for new concepts, diagnostic approaches, and the role of comorbidities. *Herz* 2012;**37**: 875–879.

194. Javed Q, Murtaza I. Therapeutic potential of tumour necrosis factor-alpha antagonists in patients with chronic heart failure. *Heart Lung Circ* 2013;**22**:323–327.

195. Haag M, Van Linthout S, Schroder SE, Freymann U, Ringe J, Tschope C, Sittinger M. Endomyocardial biopsy derived adherent proliferating cells—a potential cell source for cardiac tissue engineering. *J Cell Biochem* 2010;**109**:564–575.

196. Haag M, Stolk M, Ringe J, Linthout SV, Tschope C, Sittinger M, Seifert M. Immune attributes of cardiac-derived adherent proliferating (CAP) cells in cardiac therapy. *J Tissue Eng Regen Med* 2013;**7**:362–370.

197. Miteva K, Haag M, Peng J, Savvatis K, Becher PM, Seifert M, Warstat K, Westermann D, Ringe J, Sittinger M, Schultheiss HP, Tschope C, Van Linthout S. Human cardiac-derived adherent proliferating cells reduce murine acute Coxsackievirus B3-induced myocarditis. *PLoS ONE* 2011;**6**:e28513.