Cardiovascular Research (2014) **102**, 258–269
doi:10.1093/cvr/cvu062

SPOTLIGHT REVIEW

Crosstalk between fibroblasts and inflammatory cells

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Received 3 January 2014; revised 27 February 2014; accepted 27 February 2014

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

This article is part of the Spotlight Issue on: Heterocellular signalling and crosstalk in the heart in ischaemia and heart failure.

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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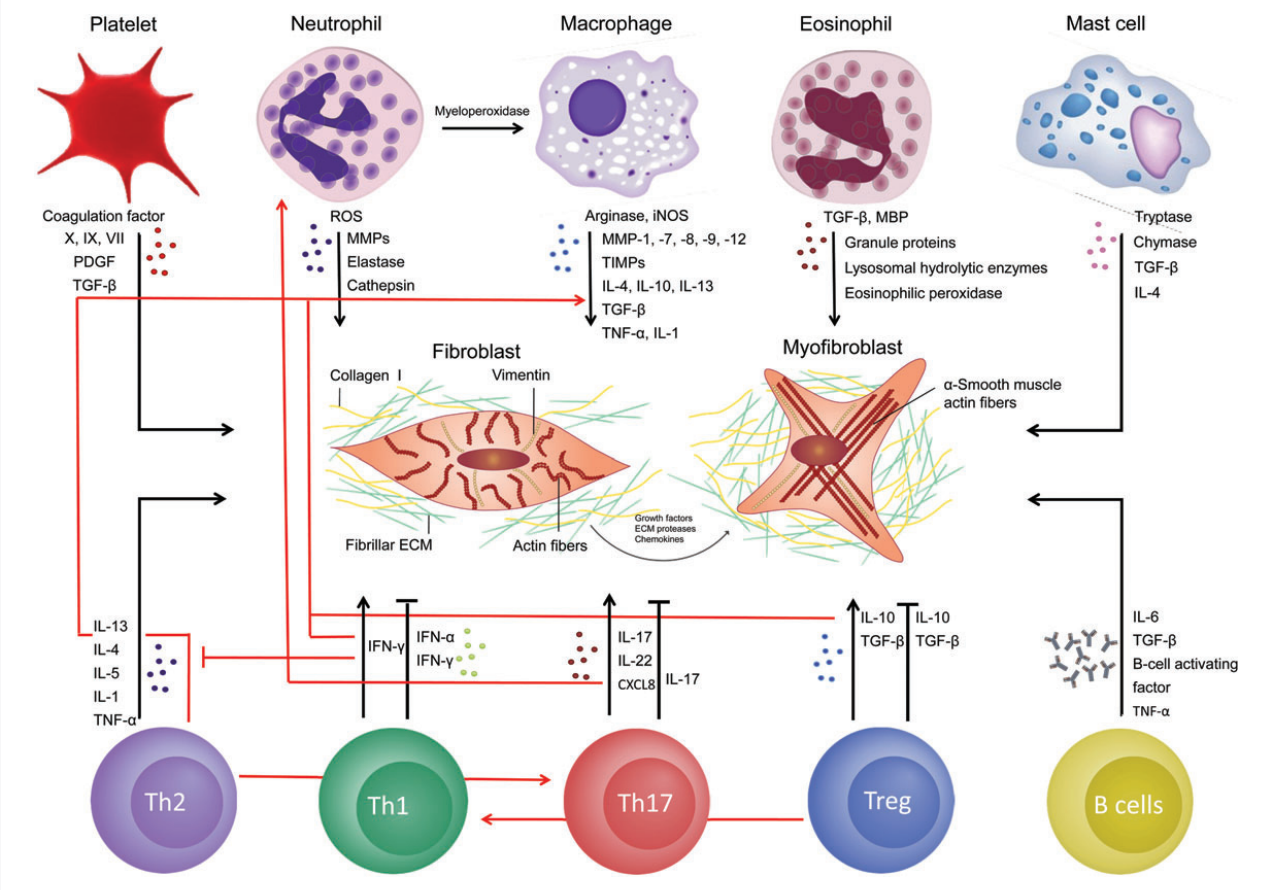

Figure 1 Impact of components of the innate and adaptive immunity on the activation of fibroblasts. Cytokines, growth factors, and enzymes released by immune cells directly (black arrows) promote fibroblast activation and indirectly (red arrows) lead to myofibroblast activation via further induction 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 coagulation 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

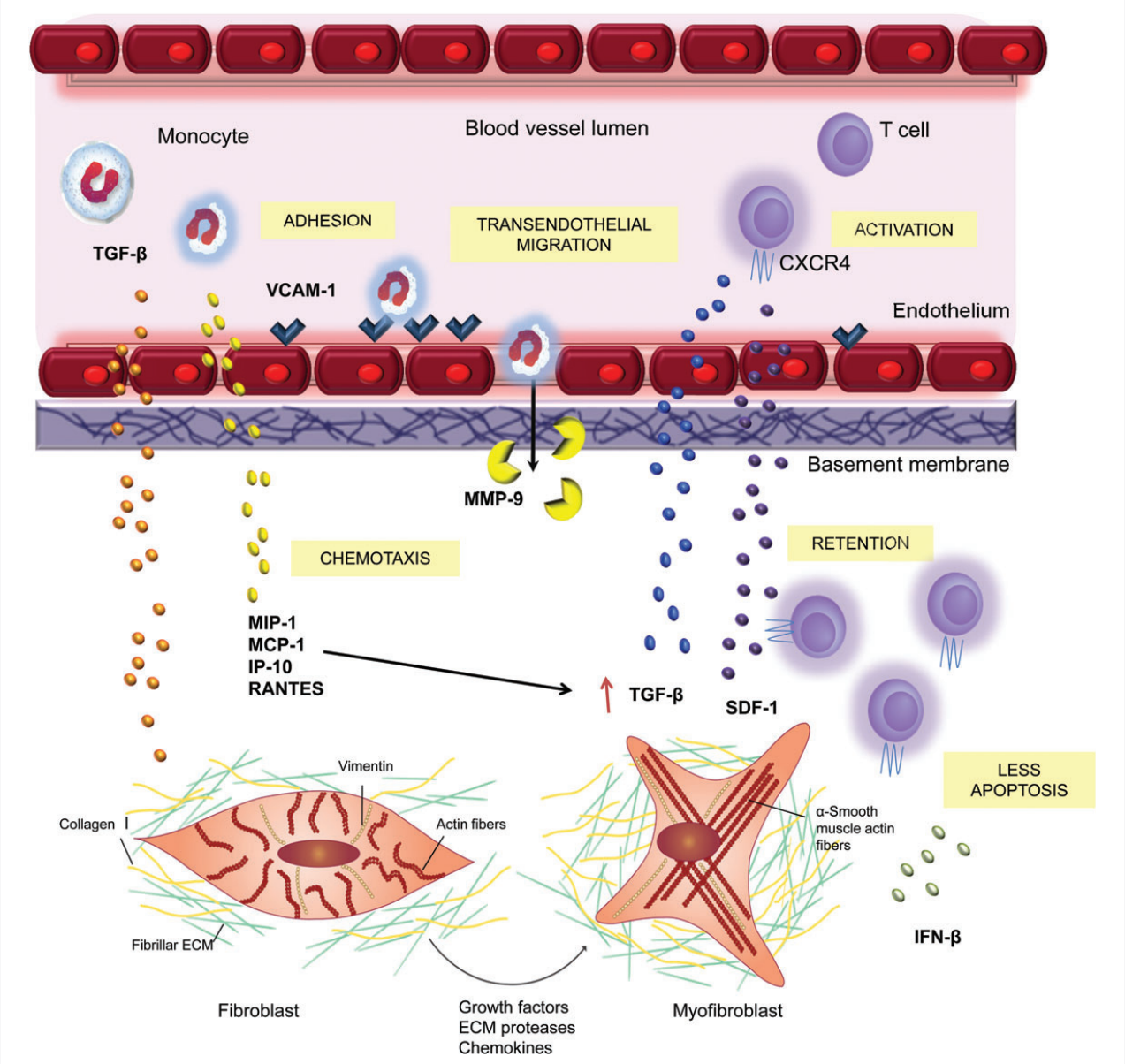

Figure 2 Role of (activated) fibroblasts in the inflammatory process. (Activated) fibroblasts from the stromal microenvironment drive homing of circulating leucocytes via the release of chemokines and promote the recruitment of circulating leucocytes on endothelial cells via the induction of adhesion 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,

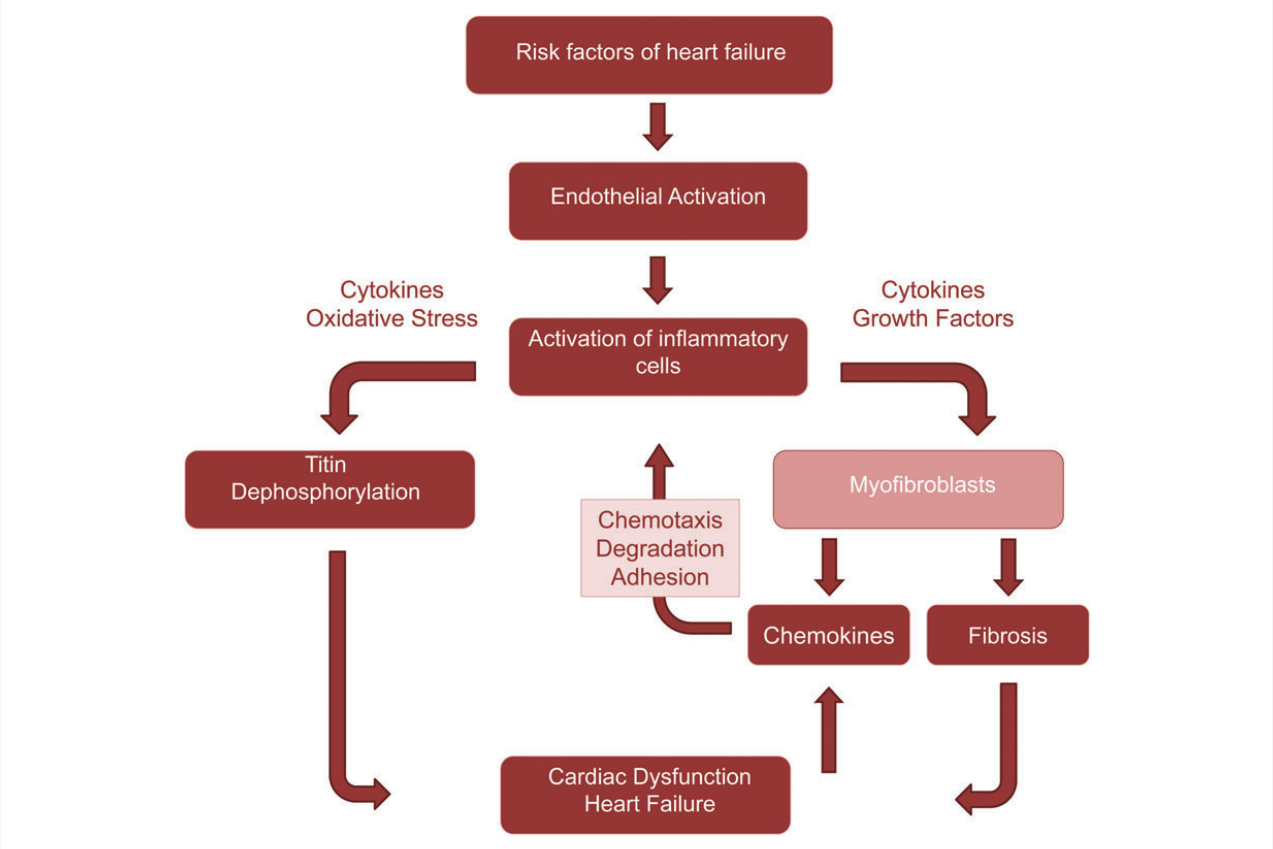

Figure 3 Pivotal role of myofibroblasts in the pathogenesis of heart failure. Risk factors of heart failure (diabetes mellitus, age, smoking, hypertension, etc.) lead to endothelial cell activation and the induction of inflammatory responses, which trigger the transdifferentiation of fibroblasts to myofibroblasts 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.

Funding

This study is supported by the DZHK to S.V.L. and C.T., and by the European 7th Framework Consortia MEDIA and REDDSTAR to C.T.

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