The controversial role of mast cells in fibrosis

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ABSTRACT

Fibrosis is a medical condition characterized by an excessive deposition of extracellular matrix compounds such as collagen in tissues. Fibrotic lesions are present in many diseases and can affect all organs, and are also present in many cancers. The excessive extracellular matrix accumulation in these conditions can often have serious consequences and in many cases be life-threatening. A typical event seen in many fibrotic conditions is a profound accumulation of mast cells (MC), suggesting that these cells can contribute to the pathology. Indeed, there is now substantial evidence pointing to an important role of MCs in fibrotic disease. However, investigations based on various clinical settings and different animal models have arrived at partly contradictory conclusions as to how MCs affect fibrosis, with many studies suggesting a detrimental role of MCs whereas others suggest that MCs can be protective. Here we review the current knowledge of how MCs can affect fibrosis.

Key words: mast cell, fibrosis, lung, kidney, heart, skin, liver

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INTRODUCTION

Fibrosis is a common and often serious complication of many diseases, including various kidney disorders, cancer, pulmonary pathologies such as asthma, idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD), liver diseases and cardiac disorders. The hallmark of fibrosis is the excessive accumulation of various extracellular matrix (ECM) components, most notably collagen, but also various other ECM components such as proteoglycans and fibronectin. The extensive accumulation of such compounds can lead to severe disturbances of normal organ function which can have lethal consequences [1].

The exaggerated accumulation of ECM in fibrotic conditions is dependent on an expansion of the fibroblast population, which differentiates into contractile synthetic myofibroblasts characterized by the expression of α-smooth muscle actin (αSMA) and vimentin. Myofibroblasts are the principle cells responsible for the synthesis and deposition of the fibrotic matrix and associated tissue contraction evident within fibrotic tissues [2]. The mechanisms that trigger the excessive proliferation and activation of fibroblasts and their differentiation of fibroblasts to myofibroblasts may vary dependent on the respective pathology, but typically involve effects of pro-fibrotic growth factors such as TGFβ, connective tissue growth factor (CTGF) and platelet-derived growth factor (PDGF), and other triggers such as angiotensin II (Ang II) and pro-inflammatory stimuli [1].

Mast cells (MC) are hematopoietic cells of the immune system, contributing both to innate and adaptive immune responses. They are found as resident cells in all human tissues, with a particularly high density at sites close to the external environment, such as the skin and mucosal surfaces of the lung and gut (reviewed in [3]). MCs are characterized by a high content of secretory granules filled with various preformed compounds including biogenic amines (histamine), proteoglycans of serglycin type, certain preformed cytokines and growth factors

(TNFα, FGF-2, TGFβ, IL-4), lysosomal hydrolases and various MC-restricted proteases [4]. When MCs are activated, for example by high affinity IgE receptor (FcεRI) crosslinking, they degranulate and release their pre-formed granule mediators into the extracellular space. In addition, MC activation leads to the *de novo* generation of numerous compounds including a variety of cytokines, growth factors and chemokines as well as lipid-derived mediators such as platelet activating factor, leukotrienes and prostaglandins [3].

MCs are known to have a beneficial role in protection against a variety of external insults such as various toxins and bacterial infection [5,6], but are predominantly known for their detrimental impact in allergic conditions including asthma [3]. In addition, there is a vast documentation suggesting a role for MCs in various other disorders, including autoimmune diseases [7], cardiac pathologies [8,9], cancer [10,11] and inflammatory kidney disease [12]. Moreover, there is substantial evidence pointing to a prominent role for MCs in fibrotic conditions, based on both clinical findings and studies in animal models. Here we review the current knowledge of how MCs contribute to the manifestations of fibrosis.

We will start by reviewing the circumstantial evidence that MCs contribute to human tissue fibrosis in various organs, before discussing the evidence that MC-derived mediators contribute directly or indirectly to the fibrotic process. We will then discuss the role of MCs in animal models of tissue fibrosis.

Mast cells in human lung fibrosis

The diffuse parenchymal (interstitial) lung diseases form a large, heterogeneous and complex group of conditions associated with the development of lung parenchymal fibrosis. There are many underlying causes or associations including connective tissue and autoimmune disorders (rheumatoid disease, systemic sclerosis, systemic lupus erythematosus), drugs

(amiodarone, nitrofurantoin), organic antigens (from birds, mouldy hay), and inorganic molecules (asbestos, silica), but for many the cause is not known. The most common condition encountered in clinical practice is the condition known as idiopathic pulmonary fibrosis (IPF). This presents with progressive cough and breathlessness, with lung fibrosis and honeycombing present predominantly in a subpleural and basal lung distribution [13]. Its prevalence is increasing and median survival is only 3 years, worse than many cancers [14-16]. Existing treatments are of limited efficacy [17,18]

Histologically, IPF is characterised by patchy fibrosis demonstrating fibroblast foci, interspersed with relatively healthy areas of lung [19]. While the initiating events and role of inflammation in IPF remain unresolved, there is evidence of ongoing damage to the alveolar epithelium and capillary endothelium leading to unopposed activation of repair mechanisms [19]. The chronicity of the insult leads to dysregulated repair with histological evidence of proliferation of type II pneumocytes and the generation of fibroblastic foci. These events are driven by several pro-fibrotic mediators. Epithelial injury stimulates the release of numerous mediators including TGFβ1, which is the key upstream pro-fibrotic growth factor driving IPF [20-24]. Several additional pro-fibrotic mediators, cytokines, chemokines and growth factors including PDGF, FGF-2, IL-4, and IL-13 are released from many cell types and are likely to contribute to the fibrotic process [25-29]. Collectively these cytokines/growth factors contribute to the formation of fibroblast foci and the exaggerated deposition of extracellular matrix (ECM), destroying the lung parenchymal architecture. While different mechanisms may initiate lung fibrosis in other types of interstitial lung disease, the mechanisms leading to the deposition of fibrotic matrix are likely to overlap with those active in IPF.

The key cell depositing the abnormal fibrotic matrix and mediating the associated tissue contraction in all types of lung fibrosis is the myofibroblast [24,30]. Compared to cells from non-

fibrotic lung, myofibroblasts derived from IPF lungs demonstrate enhanced αSMA expression [31], actin stress fibre formation [31], proliferation [32], migration [33], contraction [34] and collagen production [35]. There is also impaired production of PGE_2 [36], an inhibitor of healthy fibroblast proliferation and a known inhibitor of lung MC activation via FcεRI [37].

- **Mast cells infiltrate human fibrotic lung tissue**

There is strong evidence for important bidirectional interactions between MCs and myofibroblasts in fibrotic tissues that appear to exacerbate the fibrotic process in human lungs. MCs are numerous in healthy airways and lung parenchyma, but are increased in the fibrotic areas of alveolar parenchyma in patients with pulmonary fibrosis resulting from several diverse aetiologies including IPF [25,38-46], connective tissue disease-associated lung fibrosis [38,47], chronic hypersensitivity pneumonitis [40,44], sarcoidosis [25,38,44], chronic berylliosis [25], silicosis [48], bronchiolitis obliterans organising pneumonia [44], Langerhans cell histiocytosis [44], and infants with bronchopulmonary dysplasia [49]. Increased numbers of MCs have also been identified in the bronchoalveolar lavage (BAL) fluid of patients with various interstitial lung diseases although studies vary [50-56], and in the alveolar spaces, particularly in hypersensitivity pneumonitis and BOOP [44]. These observations are in keeping with the original electron microscopic images taken by Kawanami et al. demonstrating MCs migrating through the alveolar basement membrane in patients with IPF but not "healthy" controls [38]. In patients with hypersensitivity pneumonitis, BAL MC numbers returned to normal [57] or fell significantly but remained elevated after exposure to the offending agent was prevented [55].

In several studies, the numbers of MCs present in fibrotic lung parenchyma or BAL has correlated strongly with the severity of fibrosis [25,39,44,58]. Negative correlations with lung function have also been recorded [54]. The number of MCs in BAL may also be prognostic. In

patients with sarcoidosis, those with the highest MC counts in BAL at study entry, were the most likely to exhibit disease progression over the subsequent 2 years [56].

- **Mast cell activation in human fibrotic lung tissue**

Morphologically, the MCs in the fibrotic parenchyma in patients with IPF and other interstitial lung diseases often show reduced numbers of granules and disorganised granule content, suggesting there is partial ongoing degranulation [38,40,42,44](**Figure 1**). There is also evidence for the increased release of tryptase within the tissue [45]. In keeping with this, histamine and/or tryptase concentrations are elevated in the BAL fluid of patients with IPF [43,50,51], hypersensitivity pneumonitis [53,57], scleroderma [47] and fibrotic sarcoidosis [43,50]. In patients with active hypersensitivity pneumonitis, BAL MCs appeared degranulated when viewed by electron microscopy [51]. In both normal and IPF lung, the majority of MCs and fibroblasts in the alveolar septae are apposed [39,59] and this, coupled with evidence of ongoing MC activation suggests that there is likely to be intimate cross-talk between the two cell types. The exact mechanisms mast cell activation in human ILDs is not known but potential activation pathways are summarised in **Figure 2**.

- **Mast cell phenotype in human lung fibrosis**

A detailed phenotypic analysis of MCs in IPF lungs by Andersson et al. demonstrated that while total MC numbers are unchanged in healthy areas of IPF lungs, there is a decrease in the density of MCs expressing tryptase only (MC_T) and an increase in the density of MCs expressing both tryptase and chymase (MC_{TC}) in these areas [58]. In the fibrotic parenchyma, there was an increase in both MC_T and MC_{TC}, but this was particularly noteworthy for MC_{TC} cells, which are rarely found in healthy lung parenchyma [58]. Similar observations were made by Hirata et al. [41], who also found a close correlation between MC_{TC} and IL-4^+ cells, perhaps because MC_{TC} commonly express IL-4 unlike MC_T [60]. Andersson [58] also showed that

approximately 10% of MC_T expressed IL-6 in healthy airways, but that this was not present in MC_{TC} in keeping with the work of Bradding et al. [60]. The proportion of IL-6^+ MC_T increased to about 20% in IPF lung parenchyma, but IL-6^+ MC_{TC} were rarely seen [58]. In contrast, there was a marked upregulation of TGFβ1 expression by MCs in the small airways and lung parenchyma in IPF, in both MC_T and MC_{TC} [58]. This was particularly marked for MC_{TC} where TGFβ1 was rarely expressed in health but by almost 100% of MC_{TC} in IPF. This demonstrates the marked plasticity of human lung MCs, and suggests that MCs develop a pro-fibrotic phenotype before the onset of fibrosis, and that MC-derived TGFβ1 is likely to make an important contribution to the fibrotic process. Furthermore, it also suggests that small airway dysfunction also contributes to IPF pathophysiology.

Fibroblast growth factor 2 (FGF-2, known previously as basic FGF) is another potent fibrogenic cytokine produced by human MCs [25,61-64]. In healthy alveolar parenchyma it was present in 30% of MC_T cells but not MC_{TC} cells [64]. Two laboratories have reported that the majority of tryptase$^+$ MCs in IPF and silicosis lung parenchyma express FGF-2 and are the most abundant cells expressing FGF-2 in both conditions [25,48,62]. Furthermore, the distribution of FGF-2^+ MCs matched that of extracellular matrix deposition and correlated with the extent of fibrosis morphometrically, thus providing circumstantial evidence that MCs contribute to the fibrotic process [25].

Stem cell factor (SCF) is an essential growth factor for human MCs, and mRNA expression for the transmembrane form, and SCF protein, are upregulated in IPF tissue where it is localised predominantly to myofibroblasts and hyperplastic alveolar type II cells [42]. Elevated mRNA expression for transmembrane SCF was also observed in IPF-derived fibroblasts compared to non-fibrotic control fibroblasts. This indicates that an appropriate microenvironment exists to account for the MC hyperplasia evident in fibrotic lung tissue.

The presence of increased numbers of activated MCs expressing pro-fibrotic growth factors in IPF suggests that MCs are likely to contribute to fibroblast dysfunction in IPF. This is supported by in vitro studies and model systems as described in later sections.

Mast cells in human chronic kidney disease

Chronic kidney disease (CKD) is common, with an estimated global prevalence of between 11 to 13% [65]. Irrespective of whether the initial pathology is focussed on the renal glomeruli, tubules or interstitium, chronic disease progresses to irreversible tubulointerstitial fibrosis (reviewed in [66] and[67]). As in many fibrotic conditions, it is believed that dysregulated epithelial repair processes result from repeated or chronic injury, driving an unregulated and progressive fibrotic response through the activation of matrix-producing myofibroblasts (reviewed in [68]). This leads to further loss of functioning nephrons and therefore progressive renal failure.

The general consensus is that MCs are present but relatively scarce in healthy human kidneys [69-71]. There are many consistent reports of increased MC numbers in the cortical and medullary interstitium in numerous chronic kidney diseases including membranous nephropathy [70,72,73], lupus nephritis [72,73], focal segmental glomerular sclerosis [72,73], mesangiocapillary glomerulonephritis [72], IgA nephropathy [70,72-75], diabetic nephropathy [70,72], hypertensive nephropathy [76], renal amyloid [77], chronic cyclosporin toxicity [70], and chronic transplant rejection [70,78-80]. MCs were also markedly increased in the corticotubular interstitium of patients with rapidly progressive crescentic glomerulonephritis [69]. MCs are very rarely if ever observed within glomeruli [69,70,73]. The MCs present in diseased interstitium are predominantly of the MC_T phenotype but a significant proportion of MC_{TC} are also present although these vary in number depending on the disease (reviewed in [12], see also[69,74,76,77,81]). Chymase-only MC_C have also been identified in chronic rejection [79].

In numerous studies the number of MCs present in CKD has correlated inversely with renal function [69,74,75,77,79], and positively with the extent of fibrosis [69-71,73-75,77,78], and the number of αSMA$^+$ cells [69,71,77,80]. Furthermore, the number of MCs present in the renal interstitium at day 100 post renal transplantation, was strongly correlated with the decline in renal function at 3 years, and the change in fibrosis index at 3 years [81]. Ultrastructural studies show that the MCs in the fibrotic interstitium are activated [70] and in close approximation to the resident myofibroblasts [74]. MCs in the fibrotic interstitium have been shown to contain FGF-2 [74,77]

Perhaps not surprisingly, SCF expression is increased in parallel with MC numbers in chronic glomerulopathies [71] and hypertensive nephropathy [76], and is likely to play an important role in promoting MC recruitment, survival and activation. SCF expression also correlates with the extent of interstitial fibrosis and αSMA expression [71]. Tryptase in the presence of heparin increased the proliferation of human renal fibroblasts cultured from diseased kidneys [73], and increased the synthesis of fibronectin and collagen type 1. Protease-activated receptor 2 (PAR-2) is upregulated in IgA nephropathy, in both proximal tubular cells and the fibrotic interstitial infiltrate [82]. In cultured renal tubular cells and mesangial cells, activation of PAR-2 stimulated TGFβ1 synthesis [82], implying that activation of PAR-2 by tryptase *in vivo* may be an important pathway promoting renal interstitial fibrosis.

Based on work from other systems, there are several further potential mechanisms whereby MCs might drive kidney fibrosis through the release of pro-fibrotic mediators, modulation of the renin-angiotensin system, cell-cell contact, and transgranulation with fibroblasts. However, there are no studies to-date that have studied this with primary human kidney MCs.

Mast cells in human systemic sclerosis

Systemic sclerosis (scleroderma) is a multi-system connective tissue disorder characterised

by intimal proliferation and fibrosis in small arteries and arterioles, and degenerative changes with fibrosis in the skin and certain internal organs. Healthy skin contains numerous MCs, predominantly of the MC_{TC} phenotype [83]. Hawkins et al. demonstrated that the MC density in clinically involved skin of patients with early stages of scleroderma was significantly greater than in clinically uninvolved skin of the same patients or in the skin of healthy controls [84]. However, MC counts in both clinically involved and uninvolved skin in late scleroderma were within the normal range. The increase in early stage disease occurred in both the papillary and reticular dermis. This increase in MC density in early stage disease was confirmed in further studies [85,86]. Irani et al. found normal or reduced numbers of MCs in the skin of patients with established scleroderma but noted an increased proportion of MC_T cells [87]. In contrast, Seibold *et al.* [88] found elevated MCs numbers in both involved and uninvolved skin in both early and late disease. There was also increased MC degranulation in early but not late disease, and also in currently healthy skin, suggesting that increases in MC numbers and degranulation precede clinically apparent dermal fibrosis. Falanga *et al.* [89] reported raised histamine levels in the plasma of patients with systemic sclerosis providing further evidence of MC activation in this disease.

MCs present in scleroderma skin express TGFβ in their granules and demonstrate morphological features of degranulation when viewed by electron microscopy [90]. MCs and fibroblasts were adherent with numerous gap junctions between MCs and fibroblasts present, and with direct transfer of MC vesicles to fibroblasts evident [91]. Again this suggests the presence of important bi-directional interactions.

While there are inconsistencies in the above studies, overall they suggest that MCs may be important in the pathogenesis of the early cutaneous lesions of progressive systemic sclerosis.

Mast cell in human cardiac fibrosis

Interstitial cardiac fibrosis is a feature of aging and commonly presents in a number of diverse cardiac diseases including ischaemic heart disease, dilated cardiomyopathy, diabetic cardiomyopathy, hypertrophic cardiomyopathy, hypertensive heart disease, and valvular heart disease (reviewed in [92]). This fibrotic response further impairs cardiac function and promotes arrhythmias. As in other fibrotic conditions, the key cell driving the fibrotic response is the myofibroblast.

MCs are present in healthy human hearts, located in the interstitium in close approximation to the muscle fibres and capillaries [93]. These cells are predominantly of the MC_{TC} phenotype [93] and have been isolated and extensively characterised [93,94]. Circumstantial evidence shows that MCs are associated with cardiac fibrosis and may be involved in its pathogenesis. For example, MCs analysed by electron microscopy appeared degranulated in endomyocardial biopsies from patients who had undergone cardiac transplantation, and the MC density correlated with the volume of fibrosis [95]. Furthermore, serial sampling demonstrated that those patients with the most MCs developed more fibrosis over time.

MC density was increased in the hearts of patients with idiopathic dilated or ischaemic cardiomyopathy undergoing cardiac transplantation, and this was accompanied by increased cardiac histamine and tryptase content [96]. MCs from patients with either type of cardiomyopathy released larger quantities histamine, tryptase and LTC_4 than controls following activation with anti-IgE or SCF, suggesting the cells were primed for mediator release [96]. These cardiac MCs also contained SCF suggesting a possible autocrine role, in keeping with observations from other tissues [97]. In another study, the density of MCs correlated with the extent of fibrosis in patients with idiopathic cardiomyopathy [98].

Akgul et al. found a significant increase in MCs in end-stage dilated cardiomyopathy compared to healthy myocardium. MCs were localised particularly within areas of fibrosis and were the predominant source of FGF-2 [99]. Following long term (>40 days) support with a left ventricular assist device, there was a further increase in MC numbers compared to patients who received short term support, but a shift from MC_{TC} to MC_{T}, accompanied by a decrease in myocardial fibrosis and FGF-2 expression [99]. This might suggest the transition from a pro-fibrotic to anti-fibrotic MC phenotype.

Mast cells in human liver fibrosis

Hepatic fibrosis leading to cirrhosis is the final endpoint for many chronic liver diseases. Chronic inflammation in response to ongoing tissue injury is a common precursor, and this leads to accumulation of ECM deposited my myofibroblasts, with TGFβ1 again a central profibrotic mediator driving this response Progression to cirrhosis is variable but usually slow, developing over 20 to 40 years in patients with chronic liver injury (reviewed in [100].

MCs are sparse in healthy human livers where they are found predominantly in the portal tracts associated with blood vessels and bile ducts, and to a lesser degree in the walls of the sinusoids [101,102]. Several studies have shown that MC numbers are increased in fibrotic human livers with numerous MCs in both the portal tracts and the fibrous septae [101-105]. This is evident with liver fibrosis of different aetiologies including alcoholic liver disease [101,102,104,105], hepatitis B [102,104,105], hepatitis C [102,104,105], autoimmune hepatitis [103,105,106], primary biliary cirrhosis (PBC) [101,102,104-106], primary sclerosing cholangitis [102,104], and cirrhosis of unknown cause [102]. In several studies the number of MCs correlated with the severity of fibrosis [101,103,105].

The MCs present in fibrotic livers are predominantly of the MC_{TC} phenotype [102,104,105], and also express basic FGF [104]. In one study, significantly more MCs were present in PBC compared to alcoholic liver disease for any given degree of fibrosis [101]. Histamine has been postulated as a cause of itching in cholestasis, with increased concentrations present in patients with cholestasis including PBC [107]. This is turn suggests that there is increased MC activation in PBC, which might be initiated by exposure to bile acids, which induce MC degranulation in a murine MC line [108].

Human mast cell-fibroblast cross-talk in the pathogenesis of fibrosis

MCs produce a plethora of autacoid mediators, proteases, cytokines, chemokines, growth factors and other molecules which demonstrate pro-fibrotic activity, either directly through effects on fibroblasts and fibrocytes, or indirectly through the recruitment and activation of various inflammatory and structural cell types (summarised in **tables 1-3 and figure 3**). However, MCs also produce molecules that exert potential anti-fibrotic activity, and this, coupled with the ability of certain mediators to change the response to other mediators, makes it difficult to predict exactly how important individual mediators are in regulating fibrosis. While tables 1-3 give an overview of the most consistent effects seen with the different mediators listed, there is nevertheless inconsistent data with regards to their individual effects, even when using cells from the same sources as highlighted in some instances below.

Ex vivo co-culture of MCs with fibroblasts partially addresses the problems of studying individual mediators, although MC heterogeneity between species and tissues may also lead to inconsistencies between studies. However, as combinations of mediators are produced in

potentially physiological concentrations this allows a more global assessment of the MC-fibroblast interaction. The effects of cell-cell contact can also be taken into account.

- **Mast cell mediators acting on human fibroblasts**

Briefly, in humans histamine, FGF-2, and TNFα stimulate fibroblast proliferation [109-112]. TNFα is also a fibroblast chemoattractant [113], and stimulates matrix protein and collagenase production from human lung fibroblasts [112,114]. IL-4 is a chemoattractant for human fibroblasts [115] and both IL-4 and IL-13 promote fibroblast proliferation and differentiation, and trigger the release of matrix proteins, chemokines and inflammatory cytokines from human airway and skin fibroblasts [116-123]. In addition, heparin indirectly potentiates fibroblast activation and proliferation by regulating FGF-2 bioactivity; heparin provides structural stability for FGF-2 and preserves its bioactivity by protecting it from degradation [124]. Furthermore, heparin and/or heparan sulphate facilitate the release of FGF-2 from its basement membrane storage location and the binding of FGF-2 to its receptors [125].

Lung MC-derived TGFβ1 also has the potential to induce fibroblast differentiation into contractile myofibroblasts, as does human lung MC-derived tryptase which induces TGFβ1 release from human airway smooth muscle cells leading to the autocrine upregulation of αSMA, which in turn leads to increased contractility [126]. Tryptase has similar effects on human dermal fibroblasts [127]. Lastly, recombinant amphiregulin induces the proliferation of human airway fibroblasts but not airway smooth muscle (ASM) cells, suggesting a further mechanism whereby MCs might contribute to fibrosis [128].

MC proteases have important effects on fibroblast function, and are favoured as key fibrotic molecules release by MCs. MCs express high levels of a number of proteases that are essentially restricted to this cell type, including tryptase, chymase and carboxypeptidase A3

(CPA3). These proteases are stored in the secretory granules of MCs and are thus released in large quantities when MCs are stimulated to degranulate. Tryptase induces proliferation, chemotaxis, collagen synthesis and migration in human fibroblasts [129-131](see **table 1**). In many of these studies it was suggested that tryptase exerts its effects on fibroblasts by activating PAR-2 receptors on the fibroblast surface, and there is evidence to suggest that tryptase-induced PAR-2 activation and associated proliferation is mediated by prostaglandin 15-deoxy-Delta(12,14)-prostaglandin J(2) acting via PPARγ [132]. There is also clinical evidence linking chymase expression to fibrosis as described above, as well as many potential pro-fibrotic and anti-fibrotic activities (see **table 1**). MC proteases will be discussed further in relation to animal models later.

- **Bi-directional human mast cell-fibroblast cross-talk in co-culture**

MCs interact with fibroblasts via direct contact which activates both cell types with or without additional stimuli. For example, cultured rat fibroblasts use long cytoplasmic processes to phagocytose and internalise rat MC granules, a process termed transgranulation by Greenberg and Woodstock [133]. This uptake of granules is followed by secretion of collagenase and β-hexosaminidase [134].

It is well established that fibroblasts maintain human MC survival. Primary human MCs die rapidly in the absence of exogenous growth factors, but in 1997 Levi-Schaffer and colleagues demonstrated that mouse skin 3T3 fibroblasts maintain the survival of primary human lung MCs over a period of 13 days in the absence of exogenous growth factors [135]. The cells did not appear to form tight junctions and MC proliferation was not detected, but the MCs maintained their phenotype in terms of general morphology, granule morphology, mediator content and their response to secretogogues. The same researchers then showed that human skin MCs also maintain their survival, morphology, histamine content and response to

secretogogues when co-cultured with human foreskin-derived fibroblasts for 8 days [136]. Similar results have been demonstrated for the co-culture of gut-derived human MCs with gut fibroblasts [137], and human lung MCs with primary human lung fibroblasts [138]. However, unlike the original work of Levi-Schaffer, human lung MCs appear to proliferate rapidly in the presence of human lung fibroblasts, confirmed by two independent laboratories [42,138]. The gut MC survival response was interestingly mediated by IL-6 rather than SCF [137,139], while the lung MC survival response was mediated in part by SCF but also required MC-fibroblast contact [42]. The lung MC proliferative response also involved MC-fibroblast adhesion mediated in part by the adhesion molecule CADM1 [138], described further below.

In a 3-cell organotypic skin-equivalent culture system comprising neonatal dermal fibroblasts and the human MC line HMC-1 cells embedded in a collagen gel with keratinocytes cultured above this composite culture, there was a "constitutive" increase in the expression of type a1(I) procollagen mRNA in fibroblasts analysed by in situ hybridisation [131]. Tryptase was the key factor mediating this effect and also promoted fibroblast chemotaxis. Other studies demonstrated that co-culture of human dermal fibroblasts with HMC-1 cells increases the differentiation of fibroblasts to myofibroblasts and increases the contraction of fibroblasts in three-dimensional collagen lattices [127,140]. In one study, addition of culture supernatants of MCs did not enhance the speed of gel contraction unlike co-culture, suggesting the importance of cell-cell contact, supported by the close approximation of the two cell types within the collagen gel [140]. In this study, adding neutralising antibodies against SCF and Kit inhibited gel contraction up to 70%. However, in another study, histamine contributed to the increase in fibroblast αSMA expression, while tryptase increased both αSMA expression and fibroblast contraction [127].

Heterotypic cell-cell contact between either HMC-1 cells or human skin MCs with human skin fibroblasts augmented fibroblast proliferation and required the expression of MC-associated IL-4 [117]. In this study, although IL-4 was present in the lysates of HMC-1 cells it could not be detected in the supernatant of the co-culture system. This suggests that IL-4 was secreted by MCs in low amounts and was strictly limited to cell-cell contacts with fibroblasts. Interestingly, it has previously been proposed that MCs may present IL-4 on their surface in order to confer local cytokine specificity [141] (IL-4 is a heparin-binding cytokine is therefore likely to be retained on the MC surface bound to heparin proteoglycans [142]). This would be analogous to the recently described MC antibody-dependent degranulatory synapse [143].

These findings appear relevant to the in vivo situation. Using immunohistology and in situ hybridization, a five-fold increase in the density of tryptase$^+$ MCs was observed at the fibrotic border in a human skin wound model, which appeared dependent on the increased recruitment/survival of resident MCs or freshly recruited MC progenitors rather than local proliferation [144]. Recruitment of MCs was paralleled by the increased expression CCL2. Notably, approximately 65% of MCs exhibited strong and selective IL-4 immunoreactivity, whereas other resident and passenger cells appeared quiescent. Taken together with the *in vitro* studies of Trautmann et al, these data suggest that MCs make an important contribution to human wound repair via MC-derived CCL2, IL-4 and stimulation of fibroblast proliferation.

A study co-culturing healthy human lung fibroblasts (NHLF) with HMC-1 cells demonstrated contact-dependent secretion of IL-6 into the culture supernatant [145]. HMC-1-derived cellular membranes also stimulated an increase in IL-6 production in the lung fibroblasts. Blockade of ICAM-1, TNF-RI, or surface IL-1β with neutralizing antibodies failed to significantly decrease IL-6 production in these co-cultures. This production of IL-6 is consistent with

subsequent studies examining the interaction of gut fibroblasts with gut MCs [139], and human lung MCs with human ASM cells [146], where IL-6 contributed to the maintenance of MC survival.

In another study the co-culture of HMC-1 cells with primary bronchial fibroblasts isolated from bronchial biopsies of subjects with mild asthma and healthy controls increased procollagen I α1 gene expression by fibroblasts from subjects with asthma [147]. HMC-1 cells expressed both the common IL-4 isoform and IL-4δ2, an alternative splice variant of IL-4. Co-culture significantly increased the expression of IL-4 but not IL-4delta2 by HMC-1 cells when they were cultured with fibroblasts from subjects with asthma compared with cells from healthy controls. Neutralization of IL-4 abrogated collagen mRNA expression. There was no significant change in IL-4Ralpha or IL-13Ralpha1. However, IL-13Ralpha2 gene expression was significantly reduced in fibroblasts from subjects with asthma. This highlights the potential for airway fibroblasts to stimulate IL-4 expression in the HMC-1 cell line leading in turn to increased fibroblast pro-fibrotic activity.

The mechanism(s) by which fibroblasts activate MCs is likely to be facilitated by CADM1 and Kit-dependent adhesion (see below), but the intracellular signalling pathways and mechanisms of degranulation are not known. Carbon-fiber microelectrode amperometry (CFMA) is a useful method to examine the biophysical mechanisms of exocytosis under varied cell culture conditions. While no studies are reported using human cells, degranulation and serotonin exocytosis from mouse peritoneal MCs has been measured in the presence or absence mouse 3T3 fibroblasts using the CFMA technique [148].

More recently, co-culture of human peripheral blood-derived MCs induced human lung fibroblast collagen production and contraction in collagen gels following FcεRI-dependent activation [149]. MMP-2 and -3 derived from MCs were important for these effects, likely via

the activation of latent TGFβ1. In another study, human lung MCs induced the proliferation of human lung fibroblasts in co-culture [42]. This was associated with the contact-dependent activation of MCs by fibroblasts leading to increased tryptase secretion, which was the primary factor responsible for the fibroblast proliferation observed [42]. Gut MCs increased IL-6 and MMP-1 production in co-culture with gut fibroblasts [139], although it is possible that these mediators were derived from both cell types.

Co-culture of human gingival fibroblasts with HMC-1 cells stimulated the release of IL-8 into the culture medium [150]. This study was interesting because not only did the increased IL-8 secretion require HMC-1-fibroblast intercellular contact, it was blocked by the gap junction inhibitor β-glycyrrhetinic. Calcein-dye transfer showed intercellular, gap junction communication between HMC-1 and fibroblasts that was dependent in part on hyaluronic acid on the cell surface of fibroblasts.

MCs in co-culture with fibroblasts may also exhibit anti-fibrotic activity. Adrenomedullin is produced by vascular walls and demonstrates anti-fibrotic activity in the process of cardiovascular remodelling. In patients with abdominal aortic aneurysm, MCs in the outer media and adventitia of the aorta are increased compared to non-aneurysmal atherosclerotic aorta, and express strong adrenomedullin immunoreactivity [151]. When HMC-1 cells were cultured with fibroblasts isolated from aneurysmal patients, they released adrenomedullin into the cultured media, and collagen synthesis by fibroblasts was reduced in an adrenomedullin-sensitive manner.

In summary, MCs secrete many mediators that are pro-fibrotic, and human MCs from various sources become activated and release many of these mediators when in contact with fibroblasts from various tissues. The overall picture is one of pro-fibrotic activity when in co-culture with fibroblasts, with a supportive two-way interaction between the two cell types. Current data

suggest that tryptase released from MCs may be particularly important in promoting fibroblast pro-fibrotic activity, although other mediators undoubtedly contribute. The distribution of MCs, their mediator expression, and morphological features of activation in human fibrotic tissues, coupled with clinical correlates and functional data from co-culture studies, suggests that in many scenarios, interactions between MCs and fibroblasts play a central role in the development and propagation of tissue fibrosis.

The mechanisms and importance of mast cell-fibroblast adhesion via CADM1

Cell adhesion is a fundamental mechanism facilitating cell communication through the delivery of contact-dependent signals together with the targeted release of soluble mediators. Human MCs adhere avidly to human fibroblasts. The two key molecules involved appear to be Kit and CADM1 expressed on MCs, and transmembrane SCF and a ligand for CADM1 (likely CADM1 also) expressed on fibroblasts respectively [3]. However, there is some inconsistency over the contribution made by Kit-SCF.

Several animal studies showed that mouse MCs adhere to mouse fibroblasts through a SCF-Kit-dependent mechanism [152]. However, the situation in humans is more complex. Initial studies examining the adhesion of HMC-1 and primary human skin MCs to human foetal fibroblasts demonstrated avid adhesion, but none of the adhesion molecules including integrins recognised at the time were involved [153]. SCF-Kit did not contribute to adhesion and the authors concluded that the process involved "not-yet-defined heterotypic cell-cell adhesion receptors". Although human airway epithelial cells and ASM cells express transmembrane SCF, we were also unable to find a role for SCF-Kit in the adhesion of human lung MCs to these structural cells using well validated SCF and Kit neutralising antibodies [154,155]. However, we have found that a non function-blocking anti-Kit single chain Fv antibody that was raised against human lung MC and HMC-1 cell membrane proteins

does partially inhibit human lung MC adhesion to both airway epithelial cells and ASM cells [156]. As Kit is a member of the Ig superfamily, it is therefore plausible that it mediates cell-cell adhesion outside the SCF-binding domain.

In 2003 Ito et al. demonstrated that CADM1 (also known as Necl-2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1) plays an important role in the adhesion of mouse MCs to mouse fibroblasts [157]. Subsequent work has demonstrated that CADM1 plays an important role in the adhesion of human lung MCs to both human ASM cells and human lung fibroblasts [138,155].

CADM1 is an adhesion receptor and another member of the immunoglobulin (Ig) superfamily and is particularly important for MC function and for signalling through Kit [3,138,158-160]. Its altered expression is implicated in several diseases including cancer (due to its roles in tumour suppression) [161,162], autism spectrum disorder [163,164], venous thromboembolism [165], and of relevance to this article, pulmonary fibrosis [166]. CADM1 binds to itself (homophilic adhesion) and to heterophilic binding partners including CADM2, CADM3, Nectin-3, and cytotoxic and regulatory T cell molecule (CRTAM) [167-169], all of which are members of the same Ig superfamily. CADM1 is expressed in epithelial cells, nerves, endothelial cells, fibroblasts and MCs, but not other leukocytes. The only exceptions are the malignant T cells in adult T cell leukaemia/lymphoma (ATLL) [170]. The likely binding partner for CADM1 on human lung fibroblasts is CADM1 itself [138].

CADM1 consists of several different splice variants, many of which are expressed by human MCs [159]. The activities of each CADM-1 isoform in MCs differ. For instance, the SP4 isoform mediates homotypic (MC-MC) adhesion through a homophilic (CADM1-CADM1) *trans*-interaction, and also promotes human lung MC and HMC-1 survival in mono-culture, although these two processes are not entirely interdependent [160]. CADM1 downregulation

results in reduced MC viability in mono-culture and decreases expression of the pro-survival protein Mcl-1L, but not Blc-2 or Bcl-XL, and increases caspase-3/7 activity in both HMC-1 cells and human lung MCs [160]. This coincides with decreased basal Kit levels in human lung MCs.

CADM1 is also required for the efficient function of integrin-dependent MC adhesion to extracellular matrix. CADM1 regulates both E-cadherin and $\alpha_6\beta_4$ integrin function in other cell types [171-173], and downregulation of CADM1 in HMC-1 cells results not only in reduced adhesion to human ASM cells, but also reduced adhesion to their extracellular matrix [158]. Time-course studies have demonstrated that CADM1 provides fast initial adhesion to human ASM cells and assists with slower adhesion to the extracellular matrix. However, since CADM1 downregulation, but not antibody-dependent CADM1 inhibition, reduced MC adhesion to the extracellular matrix, it is thought that CADM1 indirectly regulates extracellular matrix adhesion. CADM1 expression positively correlated with surface Kit levels and polymerisation of cortical F-actin in HMC-1 cells and also influenced phosphotyrosine signalling and Kit tyrosine auto-phosphorylation.

The CADM1-dependent adhesive process promotes MC survival and proliferation in co-cultures of ASM cells and fibroblasts in the absence of serum and exogenous SCF or IL-6 [138,146]. More extensive experiments with human ASM cells have suggested that CADM1 and Kit have an intimate relationship in MCs. For example, CADM1 and Kit cooperate and contribute to human lung MC survival and proliferation [146]. In addition, both CADM1 and Kit exist under the transcriptional control of micropthalmia transcription factor (MITF) [157,174], co-immunoprecipitate from HMC-1 cells [146], and co-localise in human lung MCs at points of adhesion to human ASM cells, as determined by confocal imaging [146]. Downregulation or overexpression of CADM1 also reduces or increases, respectively, surface Kit expression in

both human lung MCs and HMC-1 cells [158,160]. Thus, it is thought that CADM-1 plays an important role localising Kit to the surface of MCs at points of cell-cell adhesion. This will facilitate the interaction between Kit and membrane-bound SCF expressed on cells such as fibroblasts and ASM cells, hence promoting MC survival.

In summary, CADM-1 is an important adhesion molecule that interacts with Kit in a co-operative manner to promote MC survival, and is involved in the adhesion of human lung MCs to human lung fibroblasts. Targeting MC-specific CADM1 signal transduction pathways or inhibiting the function of its counter-receptors on fibroblasts has the potential to become a novel strategy for the inhibition of MC function in fibrosis.

Approaches to studying mast cell function in animal models

Based on the strong implications derived from clinical findings and *in vitro* studies that MCs drive fibrosis, investigators have sought to use animal models to address how MCs potentially may impact on fibrotic conditions. Such studies have mainly been conducted in mice and rats, but also models in hamsters, dogs and rabbits have been evaluated.

A particularly powerful strategy to assess the role of MCs in fibrosis has been to use various mouse and rat models in which MCs are absent. Most of these studies have been performed on animals in which MC deficiency is attributed to defects in Kit signaling will result in a profound reduction (although not total eradication) of MCs in these animals. However, as c-kit expression is not exclusive for MCs, animals with defects in Kit also have a number of additional abnormalities, with differing phenotype depending on which type of Kit alteration the animal model harbours [175]. To account for this issue, investigators have developed elegant techniques for reconstitution of the MC niche in these animals, and if such MC reconstitution reverts the phenotype to that of WT mice, this has been taken as evidence that the observed phenotypic effects seen in the MC-deficient animals indeed are due to their MC-deficiency

rather than to off target effects of defective c-kit signaling [175]. More recently, investigators have developed novel models for MC deficiency, by generating mice in which MC deficiency is independent of aberrant c-kit signaling. Typically, such models have utilized the highly MC-restricted expression of certain proteases, especially chymase and CPA3, and investigators have developed different strategies for depleting MCs by utilising the strong promoters for these proteases [175]. Intriguingly, by evaluating these novel models for MC deficiency, it was found that certain findings based on studies of c-kit-defective animals could not be replicated, and it has been suggested that the impact of MCs on various pathological settings therefore needs to be readdressed by using these novel models of MC deficiency [176].

By using various models of MC-deficiency, a large number of studies have addressed the role of MCs in models of fibrosis. In particular, researchers have focused on the impact of MCs on lung, kidney, skin, heart, and liver fibrosis. As detailed below, these studies have provided partly conflicting results as to the contribution of MCs in fibrosis, with some studies indicating a detrimental role for MCs whereas others indicate that MCs are protective, and yet others indicate that MCs do not influence fibrosis in either direction (**Table 4**).

Mast cells in models of lung fibrosis

In an early study on this topic, rats were subjected to bleomycin-induced lung fibrosis. As a sign of MC involvement in this condition, lung histamine levels were dramatically increased and a profound MC hyperplasia of the lung parenchyma was observed [177]. Pulmonary fibrosis can also be induced by radiation, and X-ray irradiation of rats caused progressive lung fibrosis accompanied by dramatic pulmonary MC hyperplasia [178]. As a sign of MC activation, a pronounced elevation of pulmonary histamine and serotonin was seen [178]. Notably, there was a close correlation between MC densities and levels of the respective amines. Increased

numbers of MCs accompanied by elevated histamine levels were also seen in asbestos-induced fibrosis in rats [179].

When evaluating the functional impact of MCs in bleomycin-induced lung fibrosis by using MC-deficient W/W^v mice, Mori et al. [180] showed that the grade of fibrosis was approximately equal in MC-competent compared to MC-deficient mice. Notably though, the content of hydroxyproline (a marker for collagen content) was higher in lungs of MC-deficient versus MC-competent mice, suggesting that MCs actually may confer protection against fibrosis [180]. In agreement with a protective role of MCs in lung fibrosis, Okazaki et al. showed that the extent of bleomycin-induced lung fibrosis was higher in MC-deficient Ws/Ws rats than in MC-competent animals [181]. In contrast, when assessing the airway fibrotic reaction in response to an allergen provocation, Masuda et al. found that the extent of fibrosis was diminished in MC-deficient (W/W^v or Sl/Sld) versus -competent mice [182]. A pro-fibrotic role of MCs in allergic airway inflammation is also supported by another study [183]. A detrimental impact of MCs in lung fibrosis is additionally supported through studies where MC-competent and MC-deficient W^{sh}/W^{sh} [184], W/W^v [185] or mi/mi [186] mice, respectively, were subjected to bleomycin administration.

MCs in models of kidney fibrosis

Increased MC infiltration has been observed in various animal models of kidney fibrosis, including nephropathy induced by protein overload [187] and the unilateral ureteral obstruction model [188]. In a study conducted on MC-competent versus MC-deficient Ws/Ws rats, Miazawa et al. [189] found that fibrosis occurring in conjunction with puromycin aminonucleoside-nephrosis was not MC-dependent. In fact, the MC-deficient animals exhibited more severe fibrosis than did the MC-sufficient control animals, again suggesting that MCs may be protective. In a subsequent study, Kim et al. [190] evaluated MC-deficient W/W^v mice in renal

fibrosis induced by unilateral ureteral obstruction. They also found that the MC-deficient mice developed a more severe fibrosis than did the MC-sufficient control mice, suggesting a protective effect of MCs. Moreover, it was demonstrated that reconstitution of the MC niche reversed this phenotype back to that of the MC-sufficient control animals. Mechanistically, it was found that the MC-deficient animals exhibited higher levels of TGFβ and epithelial to mesenchymal transition (EMT) markers (E-cadherin) than did the control animals, suggesting that MCs protect against fibrosis by regulating the levels of TGFβ and by affecting EMT [190]. At variance with these findings, subsequent studies by Veerappan et al. [188] and Summers et al. [191] indicated that MCs contributed to the fibrotic lesions developing after unilateral ureteral obstruction, and it was suggested that MCs promote renal fibrosis by expressing renin leading to formation of pro-fibrotic Ang II [188], or by enhancing the expression of TGFβ and αSMA [191].

Mast cells in models of skin fibrosis

By inducing graft-versus-host disease in mice, Claman et al. showed that this condition was associated with fibrosis-like lesions of the skin and that MC numbers were substantially reduced, possibly as a consequence of extensive degranulation [192]. To functionally evaluate the role of MCs in skin fibrosis, Everett et al. generated MC-deficient (W/W^v) mice carrying the tight-skin (Tsk) mutation leading to spontaneous skin fibrosis. The authors showed that the early fibrotic lesions did not differ between MC-competent and -deficient mice, but when examining late stages of fibrosis (5-7 months), MCs significantly augmented the skin fibrosis [193]. MCs (using the W/W^v mice and reconstitution approach) were also shown to augment the skin fibrosis occurring in response to biomaterial implantation [194]. However, when addressing the role of MCs in bleomycin-induced skin fibrosis by using novel MC-deficient models

where MC deficiency is independent of defective c-kit signaling, Willenborg et al. found that MCs did not affect the development of fibrotic lesions [195].

MCs in models of cardiac fibrosis

In a model of inflammatory cardiac fibrosis induced by Coxsackievirus B3 virus there was a marked increase in the number of degranulated MCs, suggesting that MCs may contribute to the pathogenesis [196]. Increased numbers of MCs have also been observed in the fibrosis occurring in models of autoimmune myocarditis [197] and in post transplantation hearts [198]. To address the functional impact of MCs on cardiac fibrosis, Liao et al. subjected fully MC-competent mice, as well as mice that were reconstituted with bone marrow from MC-deficient W/W^v mice, to pressure overload leading to atrial fibrosis [199]. They showed that the fibrotic lesions were reduced in mice reconstituted with bone marrow from MC-deficient mice, suggesting that MCs contribute to the pathogenesis. The authors proposed that MCs promote fibrosis by expressing PDGF-A, and that MC-derived PDGF-A induces proliferation and collagen expression in cardiac fibroblasts. In a transgenic model with fibrosis occurring after cardiac-restricted overexpression of TNFα, MC hyperplasia accompanied the development of cardiac fibrosis [200]. Moreover, by backcrossing the TNFα-overexpressing mice to MC-deficient W/W^v mice it was shown that MCs were essential for the development of fibrosis in this model, suggesting that MCs are a primary target for the effects mediated by TNFα. The authors also demonstrated that MC deficiency resulted in attenuation of TGFβ levels, and it was demonstrated that MCs enhanced the proliferation of fibroblasts and induced expression of both αSMA and collagen [200].

MCs in models of liver fibrosis

It was observed that MC numbers increase during experimentally-induced liver fibrosis in rats, and that liver fibroblasts were activated with enhanced collagen synthesis after phagocytosing granules released from liver MCs [201]. MC hyperplasia and a close spatial connection between MCs and fibroblasts was also seen when inducing liver fibrosis by diethylnitrosamine (DEN) [202]. MC hyperplasia was also observed in liver fibrosis induced by pig serum [203], after administration of CCl_4 [102] and after administration of amorphous silica nanoparticles [204]. However, arguing against a major role of MCs in inducing liver fibrosis, Okazaki et al. found that the extent of pig serum-induced liver fibrosis was not suppressed in MC-deficient Ws/Ws rats versus MC-competent animals [181]. In fact, the grade of fibrosis was elevated in the MC-deficient animals, suggesting that MCs actually confer protection [181]. Similarly, Sugihara et al. found that the absence of MCs in Ws/Ws rats or in W/W^v mice did not cause any reduction in the fibrotic lesions occurring in response to CCl_4 or pig serum [205]. Instead, the collagen content (as marked by hydroxyproline content) was somewhat higher in the MC-deficient as compared with the MC-competent rats, suggesting a protective role of MCs [205]. In contrast to these studies, the extent of liver fibrosis developing after bile duct ligation was reduced in MC-deficient W^{sh}/W^{sh} mice in comparison with MC-sufficient controls [206].

MCs in models of lymph node fibrosis

It has been shown that the MC numbers tend to increase in rat lymph nodes during aging and it has also been demonstrated that this increase in MC density is associated with lymph node fibrosis [207]. In keeping with this, MC numbers were increased in the fibrotic lymph nodes of patients with diffuse large B cell lymphoma [208].

MCs in models of adipose tissue fibrosis

In a study by Hirai et al. it was found that MCs accumulate in fibrotic areas of adipose tissue in severely obese diabetic db/db mice [209]. Interestingly, conditioned medium from the adipose tissue enhanced the production of collagen expression in NIH-33T3 fibroblasts, and this induction could be blocked by antagonizing MC tryptase (mMCP6). Hence, this suggests that MCs can promote fibrosis by tryptase-dependent activation of fibroblasts [209].

MC proteases in models of fibrosis

In addition to the clinical and in vitro data supporting a role for MC proteases in fibrosis, animal models have allowed the dynamic manipulation of MC protease expression.

To evaluate the functional impact of chymase on kidney fibrosis, Scandiuzzi et al. subjected wild type (WT) and chymase deficient ($Mcpt4^{-/-}$; also denoted $mMCP4^{-/-}$) mice to immune complex-mediated glomerulonephritis, and showed that the fibrotic scores were lower in the chymase-deficient vs. WT animals [210]. The authors also demonstrated that the chymase-deficient mice had lower levels of Ang II, a pro-fibrotic peptide, than did the WT animals, and they also showed that the expression of collagen type I was reduced in the chymase-deficient mice [210]. A detrimental role of chymase in fibrosis is also supported by a study where $Mcpt4^{-/-}$ mice were evaluated in bleomycin-induced lung fibrosis [201]. In addition, chymase Mcpt4 was shown to contribute to the fibrosis seen after burn injury in mice [211]. On the other hand, when evaluating the $Mcpt4^{-/-}$ mice in the unilateral ureteral obstruction model, it was found that Mcpt4 in fact conferred protection against renal fibrosis, as reflected by higher collagen and fibronectin deposition, higher TGFβ levels, higher αSMA expression and decreased E-cadherin expression in comparison with WT animals [212]. Interestingly, when examining lung and skin tissue taken from naïve $Mcpt4^{-/-}$ mice, i.e. not challenged with pro-

fibrotic stimuli, Tchougounova et al. noted that the mice developed mild fibrosis of the skin and lung spontaneously as manifested by increases in collagen, hydroxyproline and fibronectin deposition [213,214]. Hence, these data are in concordance with a role for chymase in protection against fibrosis.

A number of observations have provided mechanistic insight into how the MC proteases can affect fibrosis. As noted above, several studies have provided a link between MCs and Ang II production in the context of fibrosis. In agreement with this notion, there is substantial evidence to suggest that chymase has major role in the ACE-independent generation of active Ang II from Ang I [215], and it is thus likely that it is the action of chymase that accounts for MC-dependent formation of this pro-fibrotic peptide. There are also several studies supporting a link between MCs and TGFβ. MCs have been shown to express this growth factor in large amounts and to store it within their secretory granules [61,216]. Further, there is evidence that MC chymase can convert the latent form of TGFβ into active growth factor, hence providing a mechanistic explanation for the MC-dependent release of active TGFβ [217].

There are also numerous studies suggesting that MC proteases can have either indirect or direct effects on various connective tissue components, leading to their degradation. An important example of indirects effect of the MC proteases is the ability of chymase to activate pro-MMP9 into active protease [213,218]. Given that MMP9 is known to exert proteolytic activity on various ECM components [219], chymase could thus indirectly promote the degradation of ECM and thereby contribute to anti-fibrotic mechanisms. Chymase has also been reported to activate pro-collagenase [220], thereby providing a mechanism for collagen degradation and amelioration of fibrosis. Another example is the reported ability of tryptase to activate pro-MMP3 and pro-MMP13 [221,222]. As active MMP3 and MMP13 are known to

degrade a number of ECM components such as collagen, proteoglycans and fibronectin [223], it is possible that tryptase thereby could exert anti-fibrotic activity.

A notable example of direct effects of the MC proteases on ECM compounds is the ability of chymase to degrade fibronectin. This has been verified both in purified systems [224], in cell culture settings [214] and also in vivo [212,213]. The collective evidence from these studies suggests that fibronectin is an exceptionally good substrate for chymase, most likely being a preferred target for this protease in vivo [225]. Another example of direct effects of MC proteases on ECM components is the ability of tryptase to degrade denatured collagen (gelatin) [226], which could serve possibly to reduce fibrotic lesions.

Targeting mast cell function in fibrosis

- **Inhibitors of protease activity**

Based on the hypothesis that chymase contributes to the development of fibrotic lesions, investigators have sought to interfere with fibrosis by administrating various chymase inhibitors. Indeed, the chymase inhibitors SUN-C8077, BCEAB and NK3201 reduced bleomycin-induced lung fibrosis in separate studies [227-229]. Mechanistically, it was shown that chymase inhibition led to a decrease in the content of TGFβ and MMP2 in the lungs, and it was demonstrated that chymase inhibition prevented effects of endothelin-1. The latter observation is thus in agreement with a previous study where chymase was shown to be pivotal for the conversion of Big-endothelin-1 to endothelin-1 [230]. Chymase inhibition (using TY-51469) has also been shown to dampen liver fibrosis in hamsters fed on a methionine- and choline-deficient diet, and it was demonstrated that chymase inhibition attenuated the induction of angiotensin II and collagen expression in comparison with placebo-treated animals [231]. In addition, chymase inhibition using various compounds has been demonstrated

to reduce cardiac fibrosis and Ang II production diabetic hamsters [232], cardiac fibrosis after ischemia reperfusion injury [233], silica-induced pulmonary fibrosis in mice [234], CCl_4-induced liver fibrosis in hamsters [235], tubulointerstitial fibrosis in obstructed kidneys in hamsters [236], cardiac fibrosis in rats subjected to ligation of the left anterior descending coronary artery [237], cardiac fibrosis in cardiomyopathic hamsters [238] and cardiac fibrosis in dogs subjected to tachycardia-induced heart failure [239].

As a pharmacological approach to address the role of tryptase in fibrotic conditions in vivo, Li et al. [240] induced cardiac fibrosis in rats by transverse aortic constriction and treated the animals with a selective tryptase inhibitor (nafamostat mesylate). It was found that tryptase inhibition led to ameliorated cardiac fibrosis, in line with a pro-fibrotic impact of MC tryptase. In agreement with these findings, tryptase inhibition by administrating another compound (APC-366) was shown to dampen the bile duct ligation-induced liver fibrosis in rats [241]. The pharmacological approaches used to study the role of mast cells in animal models of fibrosis are summarised in **Table 5**.

- **Other pharmacological approaches to target mast cells in fibrosis**

In addition to targeting specific mediators, there are several other aspects of MC biology potentially suitable for pharmacological targeting (**Figure 4**). However, to develop these it will be important to understand the mechanisms by which fibroblasts and other cells activate MCs in fibrotic tissues.

The traditional approach commonly perceived to prevent MC activation in disease has been the use of drugs labelled as “MC stabilising agents” such as disodium cromoglycate. In a model of 2,4,6,-trinitrobenzene sulfonic acid-induced colitis, Xu et al. demonstrated that nedocromil, a cromoglycate derivative, reduced the extent of fibrosis [242]. Nedocromil has also been demonstrated to ameliorate left ventricular fibrosis in spontaneously hypertensive rats

[243] and to reduce cardiac fibrosis resulting from transverse aortic constriction [240]. Another MC "stabiliser", Tranilast, reduced the extent of experimentally induced fibrosis in mice [180]. However, Tranilast was equally efficient in MC-competent versus -deficient mice [180], suggesting that the impact of Tranilast was not due to effects mediated by MCs. In addition, Tranilast was shown to be without effect against fibrosis in rats with chronic renal failure [244]. In a study by Palaniyandi Selvaraj et al., it was demonstrated that disodium cromoglycate reduced the fibrosis during autoimmune myocarditis [197], and this compound also reduced atrial fibrosis occurring after pressure overload in a mouse model [199], fibrosis after implantation of biomaterials [194], kidney fibrosis in the unilateral ureteral obstruction model [188,191] and the development of liver fibrosis in multidrug resistance 2 knockout mice [245]. However, since cromoglycate has been shown to have effects outside of its impact on MCs [246], it is not certain that effects of this compound are mediated through MCs as opposed to other targets. MC inhibition by ketitofen has also been shown to ameliorate fibrosis in a pig model [247] and joint capsule fibrosis in a rabbit model [248].

In human disease, cromoglycate is used with some efficacy in rhino-conjunctivitis and perhaps asthma, but it is a weak inhibitor of human lung MCs, and demonstrates rapid tachyphylaxis [249-251]. It is therefore unlikely to be helpful in the treatment of lung fibrosis. Beta-2 adrenergic receptor (β2-AR) agonists are currently one of the most commonly used treatments in asthma. β2-AR agonists are more potent at inhibiting FcεRI-dependent MC activation than disodium cromoglycate in vitro, but human lung MCs demonstrate rapid functional desensitisation of the β2-AR [252]. β2-AR agonists have been proposed as a treatment for fibrosis [253], but activation of RTKs including Kit uncouples β2-ARs [254,255]. In fact, β2-AR agonists increased human lung MC mediator release in co-culture with human ASM cells [256], so it is possible that in the lung, β2-AR agonists might make fibrosis worse. Thus more effective inhibitors of MC activation are required.

Alternative targets for preventing MC activation in human lung disease are emerging. In humans and mice, the intermediate conductance Ca^{2+}-activated K^+ channel $K_{Ca}3.1$ is activated following FcεRI-dependent activation [257,258]. These channels support human lung MC influx of Ca^{2+} via Orai 1 channels [259], and thus enhance the activation-dependent influx of Ca^{2+} and histamine release in both human lung MCs and mice [258,260]. Blockers of $K_{Ca}3.1$ are available, and ICA-17043 (Senicapoc) has proved safe in phase III clinical trials for sickle cell disease and attenuated pulmonary fibrosis a sheep model [261]. This drug is also very effective at inhibiting pro-fibrotic human lung myofibroblast activity [31,111,262] and epithelial-mesenchymal transition [263], so $K_{Ca}3.1$ is becoming a highly attractive target for the treatment of fibrosis in several tissues including, lung [111,261], liver [264,265], kidney [265-267], and heart [268-273].

Kit and SCF are also obvious targets. This approach has the potential to target pathological interactions between MCs and airway structural cells mediated by membrane bound Kit which is likely to be important in pulmonary fibrosis. A recent study of the Kit inhibitor imatinib in severe asthma suggested that it exerts biological effects on MCs in the lung although the clinical effects were limited, and side effects limited treatment [274]. Masitinib, another tyrosine kinase inhibitor targeting Kit signalling may also have some efficacy in severe asthma [275]. There is therefore a strong rationale for trialling these in IPF.

Inhibition of MC adhesion to fibroblasts may also be an attractive therapeutic approach. CADM1 plays a central role in the interaction of MCs with fibroblasts [138], regulating MC survival and proliferation, and CADM1 has been implicated in the development of fibrosis [166,276]. CADM1 is also a tumour suppressor in epithelial cells, so targeting would need to be MC-selective. To achieve this, future work should focus on understanding the intracellular signalling pathways connected to CADM1 in MCs and other cells, and the isoforms of CADM1 expressed.

Are mast cells fibrotic or anti-fibrotic?

There is clearly much discrepancy in the results above with both pro-fibrotic and anti-fibrotic roles for MCs described. The human clinical data and in vitro data lean towards a pro-fibrotic role for mast cells, while the animal models manipulating mast cell numbers are inconsistent. Animal models inhibiting chymase in particular suggest that this protease plays a pro-fibrotic role, in keeping with the predominance of NCTC MCs in human fibrotic lesions.

There are likely to be several reasons for these discrepant results. We believe a major reason for this is that the aim of resident tissue MCs is to sense injury and then initiate a co-ordinated program of injury and repair. When this scenario is allowed to progress naturally, and the injurious stimulus is short-lived, then MCs will overall exert anti-fibrotic activity. This is undoubtedly behind the potentially anti-fibrotic activity evident in some short-term animal models. In contrast, when the injurious stimulus is chronic or repeated, then there is great potential for the vast armamentarium of pro-fibrotic MC mediators that are released on a minute-by-minute basis to do harm and lead to tissue fibrosis (**Figure 5**). This seems particularly relevant to human fibrotic diseases which often progress over many years, and is a very different scenario to the short term animal models. The human clinical data associating activated MCs with the progression and severity of fibrosis, the overall pro-fibrotic cross-talk between human MCs and fibroblasts in co-culture, and knowledge that many human MC-derived mediators exert pro-fibrotic effects on human fibroblasts, support this conclusion.

There are without doubt further biological and technical reasons for discrepancies between animal studies, and between human and animal data. These include species and tissue-dependent heterogeneity in MCs and fibroblast biology, and differences in MC numbers and location in mice versus humans; healthy human lung contains numerous MCs whereas these are relatively sparse in healthy mouse lungs. Differences in the animal protocols are also

likely to be very important, for example, mild versus harsh conditions and an acute versus chronic stimulus. Bleomycin lung injury for example is characterised by an initial phase of severe inflammation followed by reversible fibrosis, which is very different to the pathology of human IPF.

Future directions

For further research, we believe it will be important to assess the new generation of MC-deficient mice (non-kit-dependent) in various models of fibrosis. Using more subtle longer term stimuli may be far more informative than the acute inflammatory stimulus delivered by bleomycin for example. More studies on MC protease knockout animals in models of fibrosis will be helpful, in particular tryptase knockouts, considering that many studies implicate tryptase in fibrosis.

Targeting MCs in tissue fibrosis remains an important goal but a major challenge. The diversity and heterogeneity of MC mediators and the multiple mechanisms of MC activation may have important therapeutic implications, and yet we know relatively little about these. Furthermore, different modes of MC activation may be important during different phases of the natural history of a long term fibrotic process, so that treatment effective at an early phase, may not be so effective later. However, our final opinion is that novel therapeutic agents that are able to target pro-fibrotic pathways involving MCs are likely to be effective in many chronic fibrotic disease processes.

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Table 1: Autacoid human mast cell mediators, heparin and proteases, and their biological effects relevant to fibrosis

Mediator	Stored or newly synthesised	Biological effects modulating fibrosis
Histamine	Stored	- ↑ fibroblast proliferation via histamine H2 receptor [110,277] - ↑ fibroblast collagen synthesis [277] and IL-6 production [139] - ↑ dermal skin fibroblast to myofibroblast differentiation [127] or ↓ dermal skin fibroblast to myofibroblast differentiation [278]
Heparin	Stored	- Anticoagulant - Provides proteoglycan-based storage matrix for mediators in MC granules - Stabilises tryptase - Sequesters growth factors to prevent their degradation - ↑ fibroblast proliferation at low concentrations, ↓ proliferation at high concentrations [279] - Enhances proliferative effects of bFGF [279]
Tryptase	Stored	- Indirectly activates collagenase [221,280] - Activates pro-MMP3 and -13[221,222] - Degrades ECM [226,281,282] - ↑ fibroblast proliferation [73,277,283-285] - ↑ fibroblast collagen synthesis [73,130,277] and IL-6 production [139] - ↑ fibroblast chemotaxis [131] - ↑ fibroblast to myofibroblast differentiation [127] - ↑ fibroblast contractility [127] - ↓ fibroblast apoptosis [286] - Activates epithelial cells [287] - Activates TGFβ1 [288] - Promotes angiogenesis [289] - Promotes neutrophil recruitment [290] - Cleaves and inactivates CCL5 and -11 [291] - ↑ IL-33 activity through proteolytic cleavage [292]
Chymase	Stored	- Degrades ECM [293] and several cytokines including IL-5, IL-6 and IL-13 [294] - ↑ IL-33 activity through proteolytic cleavage [292] - Converts type 1 pro-collagen to collagen [295] - Converts angiotensin I to angiotensin II [296] - Reduces adhesion of T cells to airway smooth muscle [293] - Activates IL-1β [297] and IL-18 [298] - Generates soluble SCF from membrane-bound SCF [299] - Releases latent TGFβ1 from extracellular matrix [217] - Protected against fibrosis in a model of renal ureteric obstruction [212]
Carboxypeptidase A3	Stored	- Increases fibroblast proliferation [129]
PGD_2	Newly synthesised	- Stimulates fibroblast contraction [300] - ↓ fibroblast migration [301] - ↓ type I collagen secretion from lung fibroblasts secretion

		[302] - ↑ type I collagen secretion by dermal fibroblasts [129] - Enhances fibroblast proliferation [129] - Inhibits bleomycin-induced pulmonary fibrosis [303]
LTC_4 (and extracellulary derived D_4 and E_4)	Newly synthesised	- Stimulates CCL11 production from fibroblasts in the presence of IL-13 [304] - Induces TGFβ1 expression by epithelial cells [305] - Enhances EGF-dependent fibroblast proliferation [306] - ↑ fibroblast collagen secretion and proliferation [129] - Enhances bleomycin-induced pulmonary fibrosis [307]

Table 2: Human mast cell-derived chemokines with potential roles in tissue fibrosis, either directly through activity on fibroblasts, or indirectly through effects on intermediaries in fibrotic processes

Chemokine and known inducing stimuli in mast cells	Target cells	Important biological effects of chemokine on target cells
-CC-		
CCL1 [308-311] - FcεRI - IgE	T cells	T cell recruitment
CCL2 [308,310-315] - Constitutive - SCF - FcεRI - Dengue virus - IL-1β - Catestatin - Fibroblast contact	Mast cells Epithelial cells T cells Eosinophils Monocytes Basophils Fibroblasts (human IPF) Fibroblasts (human scleroderma) Fibrocytes	Chemotaxis Chemotaxis, proliferation Induces Th2 phenotype Chemotaxis Chemotaxis Activation, mediator release ↑ αSMA, CTGF, TGFβ1, TGFβR2 and pro-collagen I expression CCL2 autocrine loop upregulates αSMA expression Chemotaxis
CCL3 [308-312,315,316] - FcεRI - Nod1 ligand - Dengue virus - catestatin - IgE	Mast cells T cells Macrophages Neutrophils Eosinophils Monocytes Basophils	Activation, mediator release Chemotaxis (selective for Th1), induces Th1 phenotype Differentiation Chemotaxis (in vivo), cytotoxicity Chemotaxis Chemotaxis Activation, mediator release
CCL4 [308,310-312,315,316] - FcεRI - Nod1 ligand - Dengue virus - catestatin	T cells Eosinophils Neutrophils	Chemotaxis (selective for Th1), induces Th1 phenotype Chemotaxis Chemotaxis (in vivo)
CCL5 [308,310,317] - FcεRI - Dengue virus	Mast cells T cells Eosinophils Monocytes Fibroblasts (gingival)	Chemotaxis Chemotaxis (selective for Th1), induces Th1 phenotype Chemotaxis Chemotaxis Chemotaxis, proliferation
CCL7 [308,310,311] - FcεRI	Eosinophils Monocytes Basophils	Chemotaxis Chemotaxis Activation, mediator release
CCL8 [310,312] - Constitutive - Dengue virus	T cells Basophils B cells Eosinophils	Chemotaxis Chemotaxis, histamine release Chemotaxis Chemotaxis
CCL13 [310] - constitutive	Eosinophils Basophils Monocytes	Chemotaxis Histamine release, chemotaxis Chemotaxis

	Immature dendritic cells T cells Endothelial cells Fibroblasts	Chemotaxis Chemotaxis Chemotaxis Inhibits apoptosis
CCL17 [310] - constitutive	T cells	Chemotaxis (selective for Th2)
CCL18 [310,311] - FcεRI	Epithelial cells T cells B cells Immature dendritic cells Basophils Macrophages	Epithelial mesenchymal transition Chemotaxis Modulates activation and chemokine-induced responses Chemotaxis Chemotaxis, histamine release Chemotaxis, activation
CCL19 [310,318] - constitutive	Airway smooth muscle cells	Chemotaxis
CCL20 [310,319] - FcεRI - Pseudomonas	Dendritic cells T cells Fibroblasts (gingival)	Chemotaxis Chemotaxis Chemotaxis, proliferation
CCL22 [308,310] - constitutive	T cells Fibroblasts (gingival)	Chemotaxis (selective for Th2) Chemotaxis, ↑IL-6 secretion
CCL23 [310] - FcεRI	Monocytes T cells Neutrophils Myeloid progenitor cells Dendritic cells	Chemotaxis, adhesion, mediator release Chemotaxis Chemotaxis Suppressed development Chemotaxis
CCL25 [308,310] - constitutive	Periosteal progenitor cells Lymphocytes Mesenchymal stem cells Fibroblasts (gingival)	Chemotaxis Adhesion Chemotaxis Chemotaxis
CCL28 [310] - constitutive	Lymphocytes T cells Haematopoietic progenitor cells Fibroblasts (gingival)	Adhesion Migration Growth and survival Chemotaxis, proliferation, ↑IL-6 secretion
-CXC-		
CXCL2 [310] - FcεRI	Neutrophils Airway smooth muscle cells	Chemotaxis, adhesion Migration
CXCL3 [310,311] - FcεRI	Airway smooth muscle cells	Migration
CXCL4 [310] - constitutive	T cells Endothelial cells Monocytes Neutrophils	Chemotaxis Angiogenesis Impairs chemotaxis Adhesion
CXCL5 [310] - constitutive	Neutrophils	Chemotaxis
CXCL7 [312] - constitutive	Neutrophils	Chemotaxis, degranulation

- Dengue virus		
CXCL8 [310-312,316,320-327] - constitutive - IgE - FcεRI - CXCL12 - substance P - TSLP + IL-1β - IL-33 - Nod1 ligand - Dengue virus	Mast cells Endothelial cells Neutrophils Eosinophils Fibroblasts (human airway)	Reduces chemotaxis and mediator release Chemotaxis, proliferation, survival, angiogenesis Chemotaxis Chemotaxis following priming with IL-3, IL-5 or GM-CSF Chemotaxis
CXCL10 [310,312] - Constitutive - Dengue virus	Mast cells Fibroblasts (human airway)	Chemotaxis Chemotaxis
CXCL14 [310] - constitutive	Bacteria Monocytes Dendritic cells	Anti-microbial action Chemotaxis Chemotaxis
CXCL16 [310] - constitutive	T cells Monocytes	Chemotaxis Chemotaxis
CXCL17 [310] - constitutive	Bacteria Dendritic cells Monocytes	Anti-microbial action Chemotaxis Chemotaxis
-C-		
XCL1 [310]	T cells Fibroblasts (gingival)	Chemotaxis, activation apoptosis Chemotaxis, proliferation
-CX3C-		
CX3CL1 [310]	Mast cells Leukocytes Fibroblasts (synovial) Fibroblasts (gingival)	Chemotaxis Chemotaxis, adhesion ↑ MMP2 production Chemotaxis

Table 3: Human mast cell-derived cytokines with potential roles in tissue fibrosis, either directly through activity on fibroblasts, or indirectly through effects on intermediaries in fibrotic processes

Cytokine and known inducing stimuli in mast cells	Target cells	Important biological effects of cytokine on target cells
GM-CSF [309,327-331] - constitutive - IgE - FcεRI - FcγRI - TLR2 - TSLP + IL-1β - eosinophil contact	Eosinophils	Eosinophil growth, adhesion, transendothelial migration, chemotaxis, activation
	Mast cells	↓ tryptase expression, ↑FcεRI-dependent histamine release
IL-1β [322,329,332,333] - constitutive - FcγRI - TLR2,-4, -5, -9 - IFNγ	Mast cells	Induces histamine release from adenoidal MCs, ↑ uterine MC adhesion
	T cells	Activation
	B cells	Augments proliferation and Ig synthesis
	Hepatocytes	Acute phase protein release
	Endothelial cells	Adhesion molecule expression
	Airway smooth muscle	Uncouples β_2-adrenoceptor
	Airway epithelium	GM-CSF secretion, ↑ ICAM-1 expression, ↑ neutrophil and eosinophil adhesion
IL-3[322,334] - FcεRI	Mast cells	Growth, differentiation, and survival of bone marrow progenitor cells
	Eosinophils	Eosinophil growth, adhesion, transendothelial migration, chemotaxis, activation
	Basophils	Survival, activation
IL-4 [60,117,141,144,335-353] - FcεRI - lectins - proteases - fibroblast contact - constitutive mRNA expression in asthmatic airways	Mast cells	↑FcεRI and releasability, ↑ ICAM-1 expression, ↑sensitivity to adenosine, apoptosis of MC_T but not MC_{TC}
	B cells	Proliferation, increases MHC class II, CD40 and CD25 expression, IgE class switching and secretion, ↑ IL-6 release
	T cells	Proliferation, promotes a Th2 cell phenotype
	Monocytes/macrophages	Reduces H_2O_2 and O_2 production, attenuates parasite killing and tumoricidal activity, induces monocyte to macrophage differentiation, ↓ IL-1, IL-6, IL-8, TNF-α and 15-lipoxygenase production, ↑ MHC class II and CD23 expression
	Eosinophils	Transendothelial migration, activation, primes for CCL5-dependent migration
	Fibroblasts	Proliferation and chemotaxis, transformation to myofibroblasts, ↑ integrin expression and extracellular matrix deposition, ↑ chemokine and cytokine release

	Endothelial cells	Proliferation, ↑ VCAM-1 expression, ↓ ICAM-1 expression
	Airway smooth muscle	Inhibits human ASM cell proliferation, ↑ CCL11 release
	Epithelial cells	↑ NO and periostin production, CCL11, IL-8, GM-CSF, TSLP and TGFβ2 release, ↑15-lipoxygenase expression, ↑mucus production
	Fibrocytes	Promotes differentiation
IL-5 [141,327,329,336,337,344,345,348,354-356] - FcεRI - FcγRI - TLR2, -4 - TSLP + IL-1β	Eosinophils	Growth, adhesion, transendothelial migration, chemotaxis, activation
IL-6 [58,141,322,324,327,333,336,357-360] - constitutive - FcεRI - FcγRI - IgE - TLR4 - IFNγ - SCF - TSLP + IL-1β	B cells	Promotes Ig secretion and enhances IgE production
	T cells	Growth and differentiation
	Mast cells	Supports growth, inhibits IL-4 dependent apoptosis of human lung MC_TS
	Airway smooth muscle	Enhances contractility of human ASM cells
	Epithelial cells/submucosal glands	Mucus hypersecretion
	Fibroblasts (healthy human lung) Fibroblasts (human IPF-derived)	↓ proliferation ↑ proliferation
IL-8 (CXCL8) – see table 2		
IL-9 [322,361] - FcεRI	Mast cells	Induces VEGF expression and release from human LAD2 cell line, promotes MC progenitor growth
	Eosinophils	Enhances differentiation, survival and IL-5 receptor expression
	Neutrophils	Induces IL-8 production and release
	Airway smooth muscle cells	Potentiates ERK-dependent release of IL-8 and CCL11, and thus induces eosinophil chemotaxis
	Airway epithelial cells	Induces goblet cell hyperplasia during repair after injury or during differentiation, and increases mucin expression and release
	Monocytes	Reduces the oxidative burst and LPS-induced TNFα and IL-10 release
	T cells	Promotes growth, prevents apoptosis of a subset of Th2 cells induced by growth factor withdrawal
IL-10 [333,356,360,362,363] - constitutive - FcεRI - TLR2, -4 - IFNγ	Mast cells	↓ FcεRI-dependent lipid mediator and cytokine release, supports progenitor growth
	T cells	Inhibits Th1differentiation
IL-11[353] - FcεR1	T cells	Promotes Th2 differentiation
	Macrophages	Inhibition of LPS-dependent pro-inflammatory activity
	Epithelial cells	Supports tumour growth
IL-13 [326,327,347,364,365] - FcεRI	Mast cells	↑FcεRI and releasability, ↑human lung MC proliferation

- FcγRI - TSLP + IL-1β - IL-33	B cells	IgE class switching and secretion
	T cells	Promotes Th2 responses
	Monocytes/macrophages	Same as IL-4 (see above)
	Eosinophils	Activation, survival
	Endothelial cells	Increases VCAM-1 expression
	Epithelial cells	↑ NO and periostin production, CCL11, GM-CSF, TSLP and TGFβ2 release, ↑mucus production
	Airway smooth muscle	Enhances sensitivity to contractile agonists
	Fibroblasts	Proliferation, transformation to myofibroblasts, ↑ integrin expression and extracellular matrix deposition, ↑chemokine and cytokine release
	Fibrocytes	Promotes differentiation
IL-16 [322,366] - FcεRI	T cells	Th1 chemotaxis, inhibits Th2 activation
IL-17A [367-369] - TNFα - IgG complexes - C5a - TLR4 (LPS)	Epithelial cells	Enhances mucus secretion
	Airway smooth muscle	Enhances sensitivity to contractile agonists
	Neutrophils	Indirectly promotes recruitment through chemokine upregulation
	Fibroblasts	Synergises with TNFα to ↑ IL-6 and IL-6 production
IL-18 [322] - constitutive	Lymphocytes	↑ IFNγ production from Th1, CD8 and NK cells
IL-22 [369] - stimuli not known, mRNA expressed constitutively in psoriatic skin	Keratinocytes	Epidermal hyperplasia, antimicrobial peptide secretion, ↑ VEGF secretion promoting angiogenesis
	Epithelial cells	Proliferation, antimicrobial peptide secretion, inhibits IL-25 release by airway epithelial cells
	Airway smooth muscle	Enhances sensitivity to contractile agonists, prevents apoptosis, ↑migration
IL-24 [370] - T cell-derived microvesicles	Keratinocytes	↑ secretion of pro-inflammatory mediators
TNFα [141,316,333,371-373] - FcεRI - FcγRI - TLR4, -5, -9 - IFNγ - Nod1 ligand	Monocytes/macrophages	Increases cytotoxicity, chemotaxis, and prolonged survival
	T cells	Promotes class II antigen and IL-2 receptor expression, and increases proliferation
	Neutrophils	Increases cytotoxicity and chemotaxis
	Eosinophils	Increases cytotoxicity and oxidant production
	Endothelial cells	Increases E-selectin, ICAM-1 and VCAM-1 expression, and enhances extravasation of most leucocytes
	Fibroblasts	Growth and chemotaxis, ↓ collagen production, ↑ collagenase, IL-6 and IL-8 production
	Epithelial cells	↑ eosinophil adhesion, ↑ mucus production
	Airway smooth muscle	↑ sensitivity to contractile agonists,

		↑cytokine and chemokine release, induces corticosteroid resistance in combination with IFNγ
	Mast cells	Promotes histamine and tryptase release
SCF [97] - constitutive	Mast cells	Growth, differentiation, survival, chemotaxis. Uncouples β_2-adrenoceptor
NGF [374,375] - constitutive	B cells	Differentiation, proliferation, increased Ig synthesis
	T cells	Differentiation, proliferation
	Eosinophils	Proliferation
	Basophils	Activation, mediator release
	Neutrophils	Chemotaxis, survival, mediator release
	Monocytes/macrophages	Proliferation, mediator release
	Fibroblasts	Contraction, migration
	Airway smooth muscle	Migration, contraction, proliferation
	Mast cells	Differentiation, survival, activation, mediator release
TGFβ [58,61,90,216] - constitutive	Airway smooth muscle	Differentiation, ↑ α-smooth muscle actin, ↑ contraction, activation
	Fibroblasts	Myofibroblast differentiation, collagen secretion, contraction
	Epithelial cells	Inhibition of proliferation, EMT
	Endothelial cells	Increases angiogenesis
	Fibrocytes	Promotes differentiation
FGF-2 [25,61-64] - FcεRI	Fibroblasts	Proliferation
	Airway smooth muscle	Proliferation
	Endothelial cells	Increases angiogenesis
PDGF-A [61,376-378] - FcεRI	Fibroblasts	Proliferation and chemotaxis
VEGF [379-382] - substance P - adenosine - PGE_2	Endothelial cells	Angiogenesis
IFNα [329] - TLR3	Natural killer cells	Increases cytotoxicity
	Macrophages	Development, maturation
	Dendritic cells	Activation, maturation, increased IFN-γ production
	T cells	Increases survival of activated T cells, promotes Th1 phenotype, inhibits Th2 phenotype
TSLP [383-385] - FcεRI	Dendritic cells	Induces Th2 immunity
	ILC2	Activation
	Mast cells	Increases IL-5 and -13 expression
Amphiregulin [386,387] - FcεRI	Fibroblasts	Induces proliferation
	Epithelial cells	Increased mucin gene expression

Table 4: Fibrotic responses in animals lacking mast cells or mast cell products

Site of fibrosis	Animals	Model	Role of mast cells/mast cell product	Proposed mechanism	MC reconstitution performed	Reference
lung	W/W^v mice	Bleomycin	Redundant/ protective	Higher hyroxyproline content in MC-deficient mice	no	[180]
lung	Ws/Ws rats	Bleomycin	protective	n.d.[a)]	no	[181]
lung	W/W^v; Sl/Sld mice	allergen	detrimental	MC-dependent promotion of TGFβ/collagen production	yes	[182]
lung	W^{sh}/W^{sh} mice	Allergen (chronic protocol)	detrimental	n.d.	yes	[183]
lung	W^{sh}/W^{sh} mice	Bleomycin	detrimental	Chymase-dependent effects	yes	[184]
lung	W/W^v mice	bleomycin	detrimental	Profibrotic histamine; MC-dependent renin/Ang II production	yes	[185]
lung	mi/mi mice	bleomycin	detrimental	MC-dependent histamine release	no	[186]
lung	$Mcpt4^{-/-}$ (chymase-deficient) mice	bleomycin	detrimental	n.d.	no	[184]
skin	W/W^v mice	Tight skin (Tsk) mice	detrimental	n.d.	no	[193]
skin	W/W^v mice	biomaterial implantation	detrimental	MC-dependent fibrocyte/myofibroblast responses	yes	[194]
skin	$Mcpt5^{-}Cre^{+}$ mice	Bleomycin	redundant	-	no	[195]
skin	$Mcpt4^{-/-}$ (chymase-deficient) mice	spontaneous	protective	Chymase-dependent reduction of collagen and fibronectin	no	[213,214]
liver	Ws/Ws rats	Pig serum-induced	protective	n.d.	no	[181]
liver	Ws/Ws rats; W/W^v mice	CCl_4 or pig serum-induced fibrosis	protective	n.d.	no	[205]
liver	W^{sh}/W^{sh} mice	bile duct ligation	detrimental	MC-dependent TGFβ production and hepatic stellate cell activation	yes	[206]
kidney	Ws/Ws rats	puromycin aminonucleoside-nephrosis	protective	MC-dependent suppression of TGFβ production	no	[189]
kidney	$Mcpt4^{-/-}$	immune complex-	detrimental	Effect on pro-	yes	[210]

	(chymase-deficient) mice	mediated glomerulonephritis		inflammatory cytokines; effect on Ang II		
kidney	Mcpt4-/- (chymase-deficient) mice	ureteral obstruction model	protective	Chymase-dependent fibronectin degradation	yes	[212]
kidney	W/W^v mice	unilateral ureteral obstruction	protective	higher levels of TGFβ and EMT markers in MC-deficient mice	yes	[190]
kidney	W/W^v mice	unilateral ureteral obstruction	detrimental	MC expression of renin, leading to Ang II formation	no	[188]
kidney	W^{sh}/W^{sh} mice	unilateral ureteral obstruction	detrimental	MC enhance the levels of TGFβ and αSMA	yes	[191]
cardiac	WT mice reconstituted with bone marrow from W/W^v mice	pressure overload	detrimental	MC-dependent PDGF-A expression	no	[199]
cardiac	W/W^v mice	TNF overexpression in heart	detrimental	MC-dependent TGFβ expression; MCs targets for TNF	no	[200]

[a)] n.d., not determined

Table 5: **Summary of pharmacological approaches used for targeting mast cells and mast cell products in animal models of fibrosis**

Compound	Type of compound	Model	Outcome/proposed mechanism	Reference
Nedocromil	Mast cell stabiliser	2,4,6,-trinitrobenzene sulfonic acid-induced colitis (rats)	Reduced fibrosis	[242]
Nedocromil	Mast cell stabiliser	left ventricular fibrosis (rats)	Reduced fibrosis	[243]
Nedocromil	Mast cell stabiliser	cardiac fibrosis after transverse aortic constriction (rats)	Reduced fibrosis	[240]
Tranilast	Mast cell stabiliser	Bleomycin-induced pulmonary fibrosis (mice)	Reduced fibrosis (equal effects in MC-sufficient and –deficient mice)	[180]
Tranilast	Mast cell stabiliser	chronic renal failure (rats)	No effect	[244]
Sodium cromoglycate	Mast cell stabiliser	autoimmune myocarditis (rats)	Reduced fibrosis	[197]
Sodium cromoglycate	Mast cell stabiliser	atrial fibrosis occurring after pressure overload (mice)	Reduced fibrosis	[199]
Sodium cromoglycate	Mast cell stabiliser	implantation of biomaterials (mice)	Reduced fibrosis	[194]
Sodium cromoglycate	Mast cell stabiliser	unilateral ureteral obstruction model (mice, rats)	Reduced fibrosis	[188,191]
Sodium cromoglycate	Mast cell stabiliser	liver fibrosis in multidrug resistance 2 knockout mice (mice)	Reduced fibrosis	[245]
SUN-C8077	Chymase inhibitor	bleomycin-induced lung fibrosis (mice)	Reduced fibrosis	[227]
BCEAB (4-[1-([bis-(4-methyl-phenyl)-methyl]-carbamoyl)3-(2-ethoxy-benzyl)-4-oxo-azetidin e-2-yloxy]-benzoic acid)	Chymase inhibitor	bleomycin-induced lung fibrosis (hamsters)	Reduced fibrosis; decrease in TGFβ and MMP2; decreased effects of ET-1	[228]
TY-51469	Chymase inhibitor	liver fibrosis after feeding on a methionine- and choline-deficient diet (hamsters)	Reduced fibrosis; attenuated induction of Ang II and collagen expression	[231]
TEI-E00548; TEI-F0080	Chymase inhibitors	cardiac fibrosis in diabetic hamsters	Reduced fibrosis; Reduced Ang II production; attenuated NOX4-associated oxidative stress	[232]

TY51469	Chymase inhibitor	cardiac fibrosis after ischemia reperfusion injury (pigs)	Reduced fibrosis; reduced MMP9 activation; reduced inflammation	[233]
TY-51469	Chymase inhibitor	silica induced pulmonary fibrosis (mice)	Reduced fibrosis; reduced neutrophil accumulation; reduced TGFβ levels	[234]
TY-51469	Chymase inhibitor	CCl_4-induced liver fibrosis (hamsters)	Reduced fibrosis; reduced Ang II formation; reduced αSMA positivity	[235]
4-[1-(4-methyl-benzo[b]thiophen-3-ylmethyl)-1H-benzimidazol-2-ylsulfanyl]-butyric acid	Chymase inhibitor	tubulointerstitial fibrosis in obstructed kidneys (hamsters)	Reduced fibrosis; reduced Ang II, TGFβ, αSMA	[236]
NK3201	Chymase inhibitor	cardiac fibrosis after ligation of the left anterior descending coronary artery (rats)	Reduced fibrosis	[237]
NK3201	Chymase inhibitor	bleomycin-induced pulmonary fibrosis (hamsters)	Reduced fibrosis	[229]
BCEAB (4-[1-([bis-(4-methyl-phenyl)-methyl]-carbamoyl)3-(2-ethoxy-benzyl)-4-oxo-azetidin e-2-yloxy]-benzoic acid)	Chymase inhibitor	cardiac fibrosis in cardiomyopathy (hamsters)	Reduced fibrosis	[238]
SUNC8257	Chymase inhibitor	cardiac fibrosis after tachycardia (dogs)	Reduced fibrosis; reduced Ang II levels; redced TGFβ mRNA	[239]
Nafamostat mesylate	Tryptase inhibitor	cardiac fibrosis after transverse aortic constriction (rats)	Reduced fibrosis	[240]
APC-366	Tryptase inhibitor	bile duct ligation-induced liver fibrosis (rats)	Reduced fibrosis	[241]

FIGURE LEGENDS

Figure 1. Mast cells immunostained for tryptase in the fibrotic lung of a patient with IPF, demonstrating partial loss of secretory granules.

Figure 2. Potential mechanisms of mast cell activation. Receptors capable of modulating human mast cell activation. Human MCs express a broad range of receptors, allowing them to respond to a diverse range of stimuli, including via IgE-independent mechanisms. Downstream pathways of each individual receptor are not fully understood, but ultimately lead to MC degranulation, gene transcription, arachidonic acid metabolism and cytokine production. Among the well-known signalling pathways are the PLCγ and PI3K pathways which induce an increase in intracellular Ca^{2+} mobilization and Ca^{2+} influx. PAF, platelet activatingfactor; PI3K, phosphoinositide 3-kinase; PLCγ, phospholipase Cγ; IP3, inositol-1,4,5-triphosphate; TLR, toll-like receptor; LPS, lipopolysaccharide; PGN, peptidoglycan; MBP-1, major basic protein-1; FcR, Fc receptor; CRH, corticotropin-releasing hormone; CRH-R, CRH receptor; PAR, protease-activated receptor.

Figure 3. A summary of the potential pro-fibrotic interactions between mast cells and fibroblasts. Dotted arrows = stimuli acting on MCs; solid arrows = MC-derived stimuli or effects; red text = structural cell-derived cytokines.

Figure 4. Potential points for therapeutic intervention aimed at inhibiting mast cell function.

Figure 5. The pro-fibrotic versus anti-fibrotic role of mast cells.