Immune Mechanisms in Pulmonary Fibrosis

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Abstract

Pulmonary fibrosis, particularly idiopathic pulmonary fibrosis, represents a chronic and progressive disease with high mortality and limited therapeutic options. Excessive deposition of extracellular matrix proteins results in fibrotic remodeling, alveolar destruction, and irreversible loss of lung function. Both innate and adaptive immune mechanisms contribute to fibrogenesis at several cellular and noncellular levels. Here, we summarize and discuss the role of immune cells (T cells, neutrophils, macrophages, and fibrocytes) and soluble mediators (cytokines and chemokines) involved in pulmonary fibrosis, pointing toward novel immune-based therapeutic strategies in the field.

Keywords: fibrosis; immunity; lung; T cells; neutrophils

Clinical Relevance

In this review, we discuss the emerging role of immune cells (T cells, neutrophils, macrophages, and fibrocytes) and soluble mediators (cytokines and chemokines) involved in pulmonary fibrosis, pointing toward novel immune-based therapeutic strategies in the field.

Pulmonary Fibrosis

Pulmonary fibrosis represents a chronic and progressive tissue repair response, which leads to irreversible scarring and remodeling of the lung. The fibrogenic triggers that initiate and maintain fibrotic pulmonary remodeling remain controversial, but probably include infections (1), cigarette smoke (2), radiotherapy (3), chemotherapy (4), environmental and occupational pollutants (5, 6), obesity (7), diabetes mellitus (8), gastroesophageal reflux (8), pulmonary hypertension (9), obstructive sleep apnea (10), chronic graft-versus-host disease (11), and connective tissue diseases/autoimmune disorders (12), such as rheumatoid arthritis (13), scleroderma (14), and Sjögren's syndrome (15). However, pulmonary fibrosis can also manifest without any known etiology. Idiopathic pulmonary fibrosis (IPF) is the prototypic age-related and irreversible fibrotic disease, with a median survival of 2–6 years after diagnosis, and is largely refractory to current pharmacological treatments (16). To date, the highest genetic risk factor for developing IPF is a polymorphism in the MUC5B gene (17–19). Lung transplantation is the only effective treatment approach for patients with IPF (20).

Fibrogenesis is thought to represent dysregulated and perpetuated wound healing/connective tissue repair in response to recurring alveolar microinjuries. A hallmark of this fibrotic repair process is the excessive deposition of extracellular matrix (ECM) components, such as hyaluronan, fibronectin, and interstitial collagens, which irreversibly remodel the lung tissue structure, leading to thickening of the alveolar and peribronchial walls, thus impairing gas exchange (21, 22). During wound healing, fibroblasts are key cells responsible for the synthesis and deposition of ECM in that they provide an initial scaffold for tissue regeneration (21, 22). When aberrant wound healing and fibrosis develops, fibroblasts respond by hyperproliferating at sites of injury, acquire a "profibrotic" phenotype resistant to apoptosis, and differentiate into contractile

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myofibroblasts that perpetuate the fibrotic process (21, 22). This activated fibroblast/myofibroblast is highly responsive to growth factors/cytokines, such as connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β1, IL-1β, IL-6, IL-13, and IL-33 (23), as well as aberrantly activated profibrotic pathways, including TGF-β (24), wingless-type MMTV integration site family member (Wnt/WNT) (25), Sonic Hedgehog (26, 27), or Notch (28), that maintain fibrotic tissue transformation. Furthermore, recent work showed that the interplay between perivascular fibroblasts, epithelial cells, endothelial cells, and perivascular macrophages regulates the fine tuning between alveolar repair and fibrosis through Wnt and Notch signaling interaction (29). Concisely, endothelial expression of CXC chemokine receptor (CXCR) 7 prevents epithelial damage by Jagged1 inhibition, whereas recruitment of vascular endothelial growth factor (VEGF) receptor 1–expressing macrophages stimulates Wnt/β-catenin–dependent up-regulation of Jagged1, thereby stimulating Notch signaling in fibroblasts and enhancing fibrosis (29).

Recently, subtypes of skin fibroblasts with intrinsic fibrogenic potential that express engrailed-1 (En1) were identified. These fibroblasts trigger increased ECM deposition during development and repair, and contribute to tissue fibrosis in multiple mouse models (30). CD26/dipeptidyl peptidase-4 was identified as a surface marker of En1-positive fibroblasts. Depletion of En1-positive fibroblasts or small molecule–based inhibition of CD26/dipeptidyl peptidase-4 leads to decreased connective tissue deposition and fibrosis (30).

Proteases play a key role in ECM remodeling (31). In particular, matrix metalloproteinases (MMPs) and their inhibitor, tissue inhibitors of metalloproteinase (TIMP)-1 have been involved in the pathogenesis of IPF and sarcoidosis (32). MMPs and TIMPs, mainly derived from macrophages, can either act in a pro- or antifibrotic manner, depending on the protease/antiprotease net balance and the microenvironmental tissue context (33–35). MMP-3 was found to initiate epithelial–mesenchymal transition (EMT) in IPF by activation of the β-catenin signaling pathway through cleavage of E-cadherin (36). Gene expression studies further provided evidence of an up-regulation and potential role of MMP-1, MMP-2, MMP-7, and MMP-9 in IPF (37, 38). Lung epithelial cells are critically involved in fibrogenesis through a sequence ranging from early epithelial damage to fibrogenic EMT (39). EMT causes epithelial cells to lose their canonical features, particularly cell-to-cell adherence, and to acquire migratory and mesenchymal properties, increasing their capability to convert to fibroblasts and to finally undergo transdifferentiation into myofibroblasts that synthesize ECM (40). During EMT, epithelial cells also lose their distinct marker expression profile, including E-cadherin, thyroid transcription factor-1, aquaporin-5, zonula occludens-1, and cytokeratins, and acquire a mesenchymal morphology associated with expression of fibroblastic markers, particularly fibronectin extra domain A, α-smooth muscle actin (α-SMA), type I and III collagen, CTGF, vimentin, and desmin (39–41).

Among the cytokines studied so far, primarily the profibrotic cytokine TGF-β1 has been described to play a central role in promoting EMT (22, 24). TGF-β1 drives EMT via SMAD2/3-dependent downstream mechanisms (42, 43) and promotes the transition of epithelial cells to fibroblasts through the transcription factors, zinc finger protein SNAI1 (SNAI) and TWIST (44, 45). Inflammation has a modulatory effect on TGF-β1–mediated pathways, as the proinflammatory cytokines, IL-1β, TNF-α, and IFN-γ, were found to enhance TGF-β1–induced EMT via up-regulation of TGF-β receptor type I (46). Furthermore, the damage/danger-associated molecular pattern/alarmin high-mobility group box 1, released upon tissue injury by necrotic cells, enhanced EMT through the TGF-β1/SMAD2/3 pathway (47). The integrin-α3β1, expressed on epithelial cells, phosphorylates β-catenin and activates pβ-catenin to form a complex with SMAD2 to initiate EMT (48). Li and colleagues (49) further showed that prostaglandin E2 could modulate cell migration after EMT through activation of E prostanoid (EP) 2 and EP4 as well as inhibition of EP1 and EP3 receptors. Recently, it has been shown that p63-positive lung epithelial basal cells overlying fibroblastic foci could act as EMT progenitors (50). Other EMT inducers include cigarette smoke (51), radiation (52), oxidative stress (53), mechanical stretch (54), and IL-17A (55). In contrast, other studies, including lineage-tracing approaches, found no evidence of EMT in fibrotic settings (56–60). Additional translational research studies are warranted to solve these discrepancies.

Besides TGF-β, dysregulated activation of the WNT-1–inducible signaling protein plays a key role in IPF (25), promoting lung fibrogenesis by increasing the release of profibrotic cytokines and proteases, including secreted phosphoprotein 1, MMP-7, MMP-9, and plasminogen activator inhibitor 1, from the alveolar epithelium, as well as by inducing EMT and increasing collagen production by fibroblasts (25). Wnt1/β-catenin signaling further promoted human embryonic pulmonary fibroblast to convert into myofibroblasts and enhanced ECM deposition upon tissue injury (61). Low-density lipoprotein receptor-related protein 5, a WNT coreceptor, was identified as a driver of lung fibrosis in mice and a marker of pulmonary fibrosis disease severity in humans with IPF (62). Therapeutically, WNT/β-catenin pathway inhibitors reversed established fibrosis and significantly improved survival in bleomycin-induced pulmonary fibrosis (63, 64). Recently, Wang and colleagues (65) showed that inhibition of WNT/β-catenin signaling promoted the differentiation of bone marrow–derived mesenchymal stem cells into alveolar type II epithelial cells and inhibited fibroblast-to-myofibroblast transdifferentiation, as well as ECM accumulation in bleomycin-induced pulmonary fibrosis.

Immune Cells in Pulmonary Fibrosis

Both innate and adaptive immune cell responses have been linked to (myo) fibroblast biology and fibrogenesis. Figure 1 and Table 1 summarize the main effects reported for key adaptive (T cell subsets) and innate (macrophages, neutrophils) immune cell types. The immune cell skewing in pulmonary fibrosis probably affects antimicrobial host defense functions and infection susceptibilities, a topic that is beyond the scope of this review and is discussed in reviews dedicated to fibrosis and infections (1). In the chapters presented subsequently here, we discuss the main studies published to date on distinct

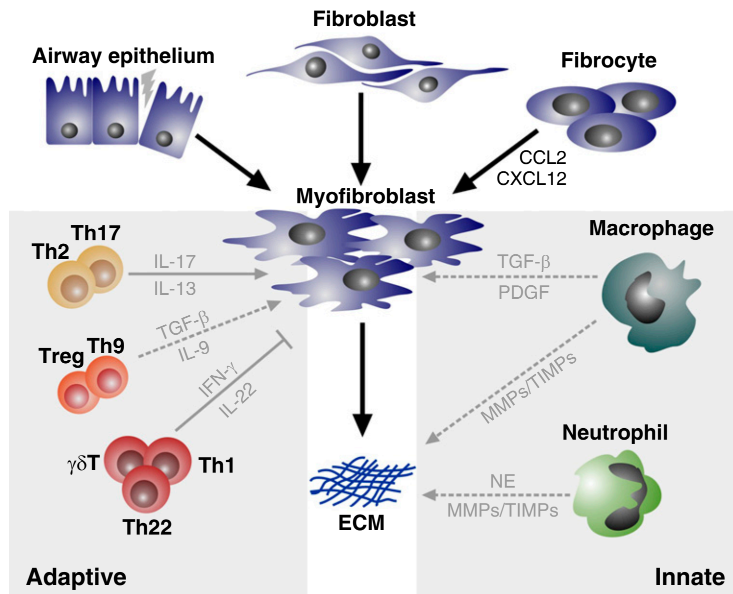

Figure 1. Effect of immune cells on myofibroblasts and fibrogenesis. Airway epithelial cell injury/epithelial–mesenchymal transition and fibroblast transdifferentiation and/or chemokine (CC chemokine ligand [CCL] 2, CXC chemokine ligand [CXCL] 12)–mediated fibrocyte recruitment contribute to the generation of myofibroblasts, which represent the major producers of extracellular matrix (ECM) components. Both adaptive (T cells, *left box*) and innate (macrophages and neutrophils, *right box*) immune cells modulate fibrogenesis through various mechanisms. Adaptive immunity: Th2 and Th17 cells promote pulmonary fibrosis, whereas Th1, Th22, and γδ-T cells inhibit fibrogenesis. Regulatory T cells (Tregs) and Th9 cells have been associated with both anti- and profibrotic effects. Innate immunity: macrophages might enhance pulmonary fibrosis through production of transforming growth factor (TGF)-β and platelet-derived growth factor (PDGF), or ameliorate pulmonary fibrosis by enhancing ECM degradation through matrix metalloproteinase (MMP) activities. Macrophages further represent a source of tissue inhibitors of metalloproteinases (TIMPs) that can antagonize MMP-mediated ECM degradation. Neutrophils produce various proteases, particularly serine proteases (neutrophil elastase [NE]) and MMPs, which degrade matrix components but can also activate TGF-β through NE and produce TIMPs, thereby promoting ECM accumulation. *Dashed lines* represent effects/interactions that are complex/multifaceted or not firmly established.

immune cell subsets and their potential involvement in pulmonary fibrosis.

T Cells

There is emerging evidence that a skewed Th1/Th2 balance plays a modulatory role during the inflammatory phase of pulmonary fibrosis (22, 66). Systemic depletion of T cells using anti-CD3 monoclonal antibodies dampened ECM accumulation and fibrosis in a murine model of bleomycin-induced pulmonary fibrosis (67). The Th1 cytokines, IFN-γ and IL-12, attenuated fibrosis (68), whereas the prototypical Th2 cytokines, IL-4, IL-5, and IL-13, have been linked to fibrogenesis (69, 70), leading to the view that Th1 responses are protective, whereas Th2 responses are harmful (22, 70). At the transcriptional level, overexpression of the Th2 transcription factor, GATA binding protein 3, or inhibiting the Th1 transcription factor, T-bet, modulated pulmonary fibrosis (71, 72). Whereas, in one study, Th17 cells (T cells characterized by production of IL-17) showed no direct impact on fibrosis (73), other studies supported a role for IL-17 and Th17 cells by demonstrating that blocking/neutralization of IL-17A delayed the progression and promoted the resolution of pulmonary fibrosis in different murine fibrosis models (55, 74, 75). The potential role of regulatory T cells (Tregs; $CD4^{+}CD25^{high}FOXP3^{+}$) in IPF remains controversial. Whereas, on the one hand, increased Tregs were reported (76), others demonstrated a reduction in Tregs in peripheral blood and bronchoalveolar lavage (BAL) fluid of patients with IPF (77). Other findings support a profibrotic role of Tregs in early stages of pulmonary fibrosis by increasing TGF-β1 release and collagen deposition (78), whereas, at late stages, Tregs were found to dampen lung fibrosis (78). Xiong and colleagues (79) showed that Treg depletion provided protection from radiation-induced lung fibrosis by increasing Th17 responses and shifting the Th1/Th2 balance toward Th1. Other studies, however, showed that Tregs attenuated fibrocyte recruitment and pulmonary fibrosis via suppression of fibroblast growth factor (FGF)-9 and CXC chemokine ligand (CXCL) 12 (80, 81). Viewing these studies in combination, the potential role of Tregs in pulmonary fibrosis remains incompletely defined. Tregs can probably exert both anti- and profibrotic roles, depending on the stage of pulmonary fibrosis and mutual interactions with other T cell subtypes, an issue requiring further investigations. Th9 and Th22 cells, T cell subsets producing IL-9 or IL-22, were also involved in fibrosis, with dual pro- and antifibrotic effects described for Th9 (82–84) and protective effects for Th22 (85). In particular, IL-9 overexpression *in vivo* yielded profibrotic effects associated with high collagen and fibronectin deposition in bronchial areas (82), whereas other studies provided evidence for an antifibrotic role of IL-9 by showing that IL-9 mitigated silica-induced lung fibrosis and type-2 immunity (83), and was protective in a bleomycin-induced lung fibrosis model through a prostaglandin E_2–dependent mechanism (84). γδ T cells were found to attenuate fibrotic responses via production of CXCL10 (86). Collectively, the role of T cells in pulmonary fibrosis seems to be complex and substantially dependent on the subtype of T cells.

Macrophages

Aside from their role as antimicrobial phagocytes, alveolar macrophages have been involved in the pathogenesis of fibrotic lung diseases. Alveolar macrophages represent a potent source of profibrotic cytokines (such as TGF-β1 and PDGF), chemokines, and proteases (MMPs) (87). However, conditional depletion of TGF-β1 from macrophages did not affect fibrosis (88). Depending on their polarization, the local micromilieu, and the stage of fibrotic disease, alveolar macrophages have been reported to exert both pro- and antifibrotic effects (22, 87, 89). Particularly, the two contrasting macrophage phenotypes, M1 (classically activated) and M2 (alternatively activated), are keys to understanding the beneficial versus harmful roles of alveolar macrophages in fibrotic diseases (90, 91). The prototypical Th2

Table 1. Immune Cells and Mediators Involved in Pulmonary Fibrosis

Cells and Mediators	Description
Immune cells	
T cells	Th1 cytokines (IFN-γ and IL-12) attenuate PF, Th2 cytokines (IL-4, IL-5 and IL-13) enhance PF, Th17 cells enhance PF, Tregs and Th9 (IL-9) have both pro- and antifibrotic roles in PF; Th22 (IL-22) and γδ-T cells have an antifibrotic role in PF.
Macrophages	M1 macrophages induce myofibroblast apoptosis and digest ECM by activation of MMPs. M2 macrophages recruit and activate fibroblasts through TGF-β1 and PDGF secretion. M2 macrophages further produce TIMPs and inhibit degradation of ECM. Both macrophage phenotypes (M1/M2) can exert pro- and antifibrotic effects.
Neutrophils	Neutrophils produce elastase, MMPs, and TIMPs. Neutrophil elastase activates TGF-β and recruits inflammatory cells to the lung, thereby promoting PF.
Fibrocytes	Fibrocytes produce ECM, cross-linking enzymes, chemokines, growth factors, and MMPs, and promote PF. Fibrocytes secrete paracrine mediators, which activate resident fibroblasts to promote PF. Fibrocytes can differentiate into fibroblasts and myofibroblasts.
Cytokines	
IL-1β	Profibrotic effects of IL-1β, mediated through IL-1R1/MyD88 signaling pathway.
IL-13	IL-13 differentiates human lung fibroblast to myofibroblast through a JNK-dependent pathway.
IL-17	IL-17 interacts/cooperates with TGF-β signaling to promote PF.
TGF-β1	TGF-β promotes EMT through SMAD-2/3 signaling pathways. TGF-β1 induces PF through ERK, MAPK, PI3K/Akt, and Rho-like GTPase pathways. TGF-β1 differentiates fibroblasts into myofibroblasts and increases ECM accumulation.
PDGF	PDGF stimulates fibroblasts and increases ECM gene expression in fibroblasts.
Chemokines	
CCL2	CCL2 increases fibrocyte recruitment and differentiation into fibroblasts, resulting in excessive collagen deposition. CCL2 activates M2 macrophage activation and promotes PF.
CCL17	CCL17 promotes PF through the recruitment of $CCR4^+$ Th2 cells and alveolar macrophages.
CCL18	CCL18 increases collagen production in lung fibroblasts through ERK1/2, PKCα, and Sp1/Smad3 signaling pathways.
CXCL12	CXCL12 recruits fibrocytes and activates the Rac1/ERK and JNK signaling pathways to induce AP-1 activation and CTGF expression in fibroblasts.

Definition of abbreviations: AP-1, activator protein 1; CCL, CC chemokine ligand; CTGF, connective tissue growth factor; CXCL, CXC chemokine ligand; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; ERK, extracellular signal–regulated kinase; IL-1R1, IL-1 receptor 1; JNK, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MyD88, myeloid differentiation primary response gene 88; PDGF, platelet-derived growth factor; PF, pulmonary fibrosis; PI3K, phosphatidyl inositol 3-kinase; PKC, protein kinase C; Rac1, Ras-related C3 botulinum toxin substrate 1; SMAD, SMA/MAD homology; Sp1, specificity protein 1; TGF, transforming growth factor; TIMP, tissue inhibitors of metalloproteinase; Tregs, regulatory T cells.

cytokines, IL-4 and IL-13, induce M2 macrophage polarization, characterized by production of IL-10, arginase-1, found in inflammatory zone 1, and distinct chemokines, particularly CC chemokine ligand (CCL) 17 and CCL18 (92). Although M2 macrophages accumulate in fibrotic lungs and have been broadly associated with profibrotic activities (91), their precise functional role in fibrotic environments remains uncertain and poorly understood. M2 macrophages were also linked to antifibrotic activities, as they were found to break down ECM by employing MMP-10 (93). Furthermore, M1 macrophages have been associated with profibrotic roles, as supported by *in vivo* depletion studies (94). Collagen was found to induce M2 macrophages via the profibrotic chemokine, CCL18, thereby feeding a positive loop between fibroblasts and alveolar macrophages (95, 96). Macrophage receptor with collagenous structure has been further involved in polarization of macrophages toward a profibrotic M2 phenotype and promoting fibrotic responses to lung injury (97). Src homology phosphotyrosyl phosphatase 2, a cytoplasmic tyrosine phosphatase associated with IL-4Rα, inhibited Janus kinase 1/signal transducer and activator of transcription signaling through its phosphatase activity, inhibited macrophage skewing toward M2 phenotype, and prevented pulmonary fibrosis (98). A central pathway for macrophage infiltration, MMP production, and promotion of pulmonary fibrosis is CCL2 and its receptor, CCR2 (*see* subsection CCL2 in section IV. Chemokines in Pulmonary Fibrosis for details) (99). TNF-α has been reported to exert antifibrotic effects and to accelerate resolution of established pulmonary fibrosis by decreasing M2 macrophages, potentially due to CCR2 down-regulation and/or increased susceptibility of M2 macrophages to TNF-α–induced apoptosis (100). On the other hand, arginase-1, expressed by M2 macrophages, showed potent antifibrotic activity during Th2-driven inflammatory responses through depleting L-arginine, an amino acid essential for $CD4^+$ T cell and myofibroblast proliferation (101). In other models, conditional depletion of the M2-associated arginase-1 from macrophages did not affect Th2-mediated lung inflammation (102). Depletion of macrophages/monocytes in an animal model of pulmonary fibrosis reduced ECM deposition and, conversely, adoptive transfer exacerbated fibrosis (103). The profibrotic roles of macrophages are mainly associated with recruitment and activation of fibroblasts through TGF-β1 and PDGF secretion (87, 89, 104). Depending on the cellular and environmental context, macrophages are also able to produce TIMPs, thereby inhibiting degradation of ECM (89, 104). Antifibrotic roles of macrophages are believed to be mediated by a variety of mechanisms, including scavenging proinflammatory

cellular debris, digesting ECM components by activation of collagen-degrading MMPs, and by secreting mediators that induce myofibroblast apoptosis (89, 105–107). In summary, several *in vitro* and *in vivo* studies have involved macrophages and their products in pulmonary fibrosis, yet the distinct beneficial versus harmful roles of specific M1/M2 phenotypes remain unclear and controversial.

Neutrophils

Like macrophages, neutrophils are not just antibacterial effectors, but also shape their tissue environment by releasing proteases, oxidants, cytokines, and chemokines (108). Neutrophils were found to be increased in BAL fluid from patients with IPF, and were associated with early mortality (109). Consistently, levels of IL-8/CXCL8, a key chemotactic factor for neutrophils, were increased in human IPF (110), and neutrophil counts in IPF BAL fluid correlated with levels of granulocyte colony–stimulating factor (G-CSF), a key growth factor for neutrophils (111). Cytokeratin 19, a potential marker for alveolar epithelial injury, correlated with the number of neutrophils in BAL fluid of patients with IPF (112). Airway neutrophils in IPF seem to be activated, as reflected by their main proteolytic product, neutrophil elastase (NE), which was increased in airway fluids from patients with IPF (113). NE breaks down a variety of ECM proteins, including collagens (types I–IV), laminin, entactin, fibronectin, and elastin, and thereby orchestrates the outcome of pulmonary fibrosis (114, 115). NE-deficient mice showed attenuation of pulmonary fibrosis through impaired TGF-β activation (115). Likewise, Sivelestat, an NE inhibitor, ameliorated pulmonary fibrosis through abrogation of TGF-β activation and inflammatory cell recruitment to the lung (116). Recently, Gregory and colleagues (117) extended these findings by demonstrating a significant reduction of fibroblast and myofibroblast accumulation in $NE^{-/-}$ mice, which were protected from asbestos-induced pulmonary fibrosis. Further studies showed that NE promoted fibroblast proliferation and enhanced myofibroblast differentiation (117). Besides serine proteases, neutrophils are also a substantial source of MMPs, such as MMP-2, MMP-8 (collagenase 2), and MMP-9 (gelatinase B), which are involved in pulmonary fibrosis (32, 118). The balance between MMPs and their antiproteases (TIMPs) plays a critical role in accumulation or degradation of ECM in pulmonary fibrosis (32, 119). The depletion of neutrophils has been associated with an MMP-9/TIMP-1 imbalance, but did not alter the susceptibility to bleomycin-induced pulmonary fibrosis (120).

Fibrocytes

Traditionally, fibroblasts are regarded as mesenchymal tissue originating/resident cells, but recent studies have established the concept that circulating myeloid-derived cells, termed fibrocytes, can migrate into tissues and differentiate into fibroblasts and myofibroblasts (121). Furthermore, fibrocytes secrete paracrine factors, which activate resident fibroblasts to promote lung fibrosis (122). Fibrocytes express myeloid markers, such as CD45 and CD34, the chemokine receptor, CXCR4, and collagen-1 (123). Fibrocytes produce ECM components (collagen I, collagen III, fibronectin, and vimentin), cross-linking enzymes (lysyl oxidase family), cytokines (TNF-α, IL-6, IL-8, and IL-10), chemokines (macrophage inflammatory protein 1-1α/β, monocyte chemoattractant protein-1, and GROα), growth factors (VEGF, PDGF, granulocyte-macrophage colony–stimulating factor [GM-CSF], and others), and various MMPs, such as MMP-9 (124–126). Moeller and colleagues (127) demonstrated that circulating fibrocytes were elevated in patients with IPF and represented a prognostic marker and an independent predictor of early mortality. CCL12 and CXCL12 were found to be involved in attracting circulatory fibrocytes to the site of pulmonary injury (128, 129). The neuronal guidance protein, slit guidance ligand 2, secreted by fibroblasts, was found to inhibit fibrocyte differentiation and reduce bleomycin-induced pulmonary fibrosis in mice (130). Recently, novel immunoregulatory properties of fibrocytes have been established by demonstrating that fibrocytes with myeloid-derived suppressor cell (MDSC) characteristics accumulate in patients with metastatic cancer (131). MDSCs are basically referred to as monocytic or granulocytic/neutrophilic innate immune cells, characterized by their potential to suppress T cells (132). The novel proposed subtype of MDSCs, fibrocytic MDSC, was found to differentiate from umbilical cord blood precursors upon culture with GM-CSF/G-CSF (133). Further studies demonstrated that distinct factors, including $CD4^+$ T cells, IL-2, IL-4, IFN-γ, TNF-α, GM-CSF/G-CSF, Kruppel-like factor 4, and fibroblast-specific protein 1, transdifferentiated $Gr1^+$ MDSC-like cells into ECM (collagen type I)–producing fibrocytes (133–135). Fibrocytic MDSCs were also found to expand Tregs (133).

Cytokines in Pulmonary Fibrosis

TGF-β

TGF-β is probably the best-studied cytokine in fibrosis, and is regarded as a prototypical "profibrotic" mediator (24). Among three isoforms, TGF-β1 has been described to be mainly involved in pulmonary fibrosis (136). After dissociation from latency-associated protein, TGF-β1 increases the transcription of downstream target genes, including procollagen I and III, via transmembrane receptor serine/threonine kinases and the cytoplasmic SMAD-2/3 signaling pathways (137). In particular, SMAD-3 deficiency has been shown to ameliorate bleomycin-induced pulmonary fibrosis (138). Moreover, extracellular signal–regulated kinase (ERK), mitogen-activated protein kinase, the phosphatidyl inositol 3-kinase/Akt pathway, and Rho-like GTPase pathways have also been shown to be involved in TGF-β1–induced fibrosis (139–141). Mechanistically, TGF-β1 promotes ECM accumulation, especially collagen and fibronectin, and drives phenotypic changes of fibroblasts (43, 142). TGF-β1 differentiates fibroblasts into myofibroblasts by inducing expression of α-SMA (143). However, it has recently been shown that α-SMA–expressing myofibroblasts may not represent the only source of pathologic collagen deposition in fibrotic settings (144). Recently, another study showed that TGF-β1 increases vascular cell adhesion molecule 1 and promotes fibroblast proliferation in patients with IPF (145). Furthermore, TGF-β1 enhances fibroblast proliferation and promotes pulmonary fibrosis via breast cancer susceptibility gene 1-associated really interesting new gene domain 1 pathway (146). Galectin-3 is also involved in TGF-β1–induced pulmonary fibrosis by increasing EMT, myofibroblast activation, and collagen production (147). Glycogen synthase kinase-3 regulates TGF-β1–induced fibroblast-to-myofibroblast

differentiation via a cAMP response element–binding protein–dependent mechanism (148). Furthermore, chitinase-1 has been observed to be involved in TGF-β1–induced pulmonary fibrosis by increasing TGF-β1 receptor expression (149). Recently, Oruqaj and colleagues (150) showed that peroxisomes are involved in TGF-β–induced myofibroblast differentiation and collagen production in IPF.

PDGF

Aside from TGF-β, PDGF represents another potent fibrogenic cytokine/growth factor that promotes pulmonary fibrosis through fibroblast activation (151). PDGF expression was found to be increased in epithelial cells and macrophages in the lungs of patients with IPF (152). *In vivo*, pulmonary PDGF overexpression induced severe pulmonary fibrosis (153). PDGF acts through inositol triphosphate-gated channels and increases Ca^{2+} release to modulate ECM gene expression in human pulmonary fibroblasts (154). PDGF is a potent mitogen and chemoattractant for lung fibroblasts, and acts through the PDGF receptor α (151). IL-13 was found to increase PDGF gene expression in lung fibroblast through STAT1 and STAT6 (155). Tregs promote pulmonary fibrotic responses by stimulating fibroblasts through the secretion of PDGF in silica-induced pulmonary fibrosis (156). Imatinib, a PDGF tyrosine kinase inhibitor, showed strong antifibrotic effects in bleomycin-induced pulmonary fibrosis via inhibiting mesenchymal cell proliferation (157).

IL-1β

IL-1β, the primary cytokine product of the inflammasome, is mainly produced by activated macrophages, dendritic cells, neutrophils, and epithelial cells, and has been shown to contribute to the progression of pulmonary fibrosis (158). Expression of *IL-1β* mRNA was found to be up-regulated in bleomycin-induced pulmonary fibrosis (159), and overexpression of IL-1β in rat lungs promoted lung fibrosis characterized by the presence of myofibroblasts, fibroblast foci, and ECM accumulation (158). Bleomycin-induced pulmonary fibrosis was attenuated in IL-1 receptor (IL-1R)– or myeloid differentiation primary response gene 88–deficient mice, and exogenous recombinant IL-1β protein resembled bleomycin-induced lung pathology, corroborating a key role for IL-1 β in fibrogenesis *in vivo* (160). In BAL fluid and serum of patients with IPF, the ratio of IL-1R antagonist (IL-1Ra) and IL-1β was decreased (161). Several studies further involved the NACHT, LRR and PYD domains-containing protein 3 inflammasome in silica- and asbestos-induced pulmonary fibrosis (162, 163). Extracellular ATP, an activator of the NACHT, LRR and PYD domains-containing protein 3 inflammasome, was increased in BAL fluid of patients with IPF and in bleomycin-induced pulmonary fibrosis (164). Like ATP, the NACHT, LRR and PYD domains-containing protein 3 inflammasome activator, uric acid, has been involved in bleomycin-induced pulmonary fibrosis (165). Wilson and colleagues (166) further showed that IL-1β–induced pulmonary fibrosis is IL-17 dependent. The WNT/β-catenin signaling pathway was found to induce IL-1β expression by alveolar epithelial cells in pulmonary fibrosis (167).

IL-13

The Th2 cytokine, IL-13, was found to be increased in the blood and BAL fluid of patients with IPF and correlated with disease severity (168). IL-13 promoted pulmonary fibrosis in fluorescein isothiocyanate– and radiation-induced lung fibrosis models (169, 170), whereas IL-13 inhibition decreased fibrotic changes in pulmonary fibrosis model *in vivo* (171). IL-13–induced pulmonary fibrosis was reported as either TGF-β dependent or independent (172, 173). Mechanistically, IL-13 differentiates human lung fibroblast to myofibroblast through a c-Jun N-terminal kinases-dependent pathway (174). Downstream IL-13 effects were mediated through a complex receptor system that includes IL-4Rα, IL-13Rα1, and/or the IL-13Rα2 (175). IL-13–induced fibrosis was exaggerated when IL-13Rα2 was low or absent in target cells, such as fibroblasts (176). The transcription factor, Yin Yang 1, has been shown to directly regulate collagen and α-SMA expression in fibroblasts (177). IL-13, in turn, was found to stimulate fibroblasts and increase α-SMA through AKT-mediated Yin Yang 1 activation (178).

IL-17

Previous studies linked IL-17 to profibrotic effects, such as EMT and collagen production, through interactions with TGF-β signaling (55, 166). IL-17 inhibition attenuated pulmonary fibrosis via autophagic degradation of collagen and increased survival in bleomycin-induced lung fibrosis (55). Neutralizing IL-17 ameliorated progression of silica-induced lung fibrosis associated with delayed neutrophil recruitment, decreased Th17 cells, decreased IL-6/IL-1β production, and increased Tregs (75). Neutrophils and monocytes/macrophages, rather than Th17 lymphocytes, were identified as the cellular source of IL-17, and promoted pulmonary fibrosis in experimental hypersensitivity pneumonitis (179). It has been recently shown that B cell activating factor was increased in BAL fluid of patients with IPF, enhanced IL-17 release from Th17 cells, and was involved in IL-17–induced pulmonary fibrosis (180). IL-27 attenuated pulmonary fibrosis by suppressing the secretion of IL-17 and the Janus kinase/signal transducer and activator of transcription and TGF-β1/SMA/MAD homology signaling pathways (181). IL-17 production by γδ T cells in response to epithelial cell injury was mediated via IL-23 in pulmonary fibrosis (182).

Chemokines in Pulmonary Fibrosis

CCL2

CCL2 (monocyte chemoattractant protein-1), is produced by monocytes/macrophages, fibroblasts, and epithelial cells, and acts via CCR2 (183). CCL2 was found to be increased in BAL fluid and serum of patients with IPF (184), and murine pulmonary fibrosis studies showed that ECM deposition is attenuated in CCR2 knockout mice (183), and that this effect is linked to a reduction in macrophage infiltration and macrophage-derived MMP-2 and MMP-9 production (99). Moreover, CCL2 increased fibrocyte recruitment to the alveolar space and promoted differentiation into fibroblasts, resulting in excessive collagen deposition (185). Proteinase-activated receptor-1 increased CCL2 release (186). The CCL2/CCR2 axis was further found to be involved in IL-10–induced macrophage and fibrocyte recruitment, as well as M2 activation in pulmonary fibrosis (90). CCL2 stimulated IL-6 production by human lung fibroblasts through ERK1/2 signaling pathway and

enhanced fibroblast survival by inhibiting apoptosis through IL-6/STAT3 in pulmonary fibrosis (187).

CCL17

Thymus and activation-regulated chemokine (CCL17) is constitutively expressed in the thymus, and is inducible in peripheral blood mononuclear cells, macrophages, bronchial epithelial cells, endothelial cells, and dendritic cells. CCL17 binds to CCR4 for its biological effects (188). CCL17 was found to be increased in both animal models of pulmonary fibrosis and human patients with IPF, and promoted fibrosis through the recruitment of CCR4$^+$ Th2 cells and alveolar macrophages (189–191). Neutralizing CCL17 could significantly ameliorate fibrosis progression *in vivo* (191). CCR4 was found to be highly expressed on T lymphocytes in the BAL fluid of patients with IPF (192).

CCL18

The profibrotic chemokine, CCL18, previously known as pulmonary and activation-regulated chemokine, is produced by macrophages, dendritic cells, peripheral blood monocytes, eosinophils, and neutrophils. CCL18 levels have been observed to be increased in serum, BAL fluid, and sputum of patients with IPF (96, 193, 194). Patients with IPF with a CCL18 serum cutoff level higher than 150 ng/ml showed an increased risk of mortality (193). Mechanistically, CCL18 increased collagen production in lung fibroblasts through different pathways, including ERK1/2, protein kinase Cα, and specificity protein 1/SMAD3 (195–197). After adenoviral gene transfer, CCL18 promoted T cell infiltration and collagen accumulation in a mouse model of pulmonary fibrosis *in vivo* (198).

CXCL12

The CXCL12/CXCR4 axis has been reported to be involved in bleomycin-induced pulmonary fibrosis, as neutralizing CXCL12 dampened fibrocyte recruitment and pulmonary collagen deposition (129). Likewise, pharmacological CXCR4 antagonists alleviated bleomycin- and radiation-induced pulmonary fibrosis (199, 200). Bone marrow–derived lung CXCR4$^+$ cells were found to migrate in response to CXCL12 and differentiated to collagen-producing lung fibroblasts (201). In both familial and sporadic pulmonary fibrosis, gene expression of *CXCL12* was increased (202). Recently, Lin and colleagues (203) showed that the CXCL12/CXCR4 axis activated the Ras-related C3 botulinum toxin substrate 1/ERK and c-Jun N-terminal kinases signaling pathways to induce activator protein-1 activation and CTGF expression in human lung fibroblasts. CTGF, in turn, mediated CXCL12-induced α-SMA expression and fibroblast differentiation to myofibroblasts (203).

Therapeutic Consequences

Anti–TGF-β1

TGF-β1 is potentially one of the main targets for treatment of pulmonary fibrosis (24, 204), as TGF-β1 inhibition showed antioxidant, antiinflammatory, and antifibrotic properties both in *in vitro* and *in vivo* models of pulmonary fibrosis (205, 206). Studies have demonstrated that targeting TGF-β1 by monoclonal antibodies reduced pulmonary fibrosis in a murine model of bleomycin-induced pulmonary fibrosis (207). Targeting αvβ6-integrin, a key activator of TGF-β, also attenuated pulmonary fibrosis (208). Paclitaxel, an antitumor drug that stabilizes cellular microtubules, decreased TGF-β1/SMAD3 via up-regulating microRNA-140 and ameliorated pulmonary fibrosis (209). Targeting the activin receptor–like kinase 5, a type I receptor of TGF-β that phosphorylates and activates SMADs, was further shown to inhibit pulmonary fibrosis (210). A TGF-β1 peptide inhibitor was found to alleviate pulmonary fibrosis in a murine model of bleomycin-induced pulmonary fibrosis through inhibition of fibroblast differentiation into myofibroblasts (211).

Anti–IL-13

Immunoneutralization of IL-13 attenuated pulmonary fibrosis in bleomycin-induced pulmonary fibrosis (212). Jakubzick and colleagues (213) further demonstrated that an IL-13 immunotoxin chimeric molecule attenuated bleomycin-induced pulmonary fibrosis by reducing the number of IL-13– and IL-4–responsive cells. Recently, it has been further demonstrated that tralokinumab, a human IL-13–neutralizing monoclonal antibody, dampened pulmonary fibrosis and promoted lung repair in a humanized severe combined immunodeficiency IPF model (214).

New Approaches

Pirfenidone. Pirfenidone (5-methyl-1-phenyl-2-[1H]-pyridone) was approved for the treatment of IPF in Japan in 2008, and later in Europe, India, Canada, and, recently, in the United States (215). Although, to date, the exact mechanism of action of pirfenidone is poorly understood, pirfenidone shows evidence to attenuate lung fibrosis via inhibition of collagen synthesis and heat shock protein 47 expression in lung fibroblasts (216), inhibition of profibrotic and proinflammatory cytokines, including TGF-β1, IL-1β, IL-6, and FGF (217), and inhibition of fibrocyte migration via the attenuation of CCL2 and CCL12 production (218). Moreover, pirfenidone decreased human lung fibroblast proliferation and differentiation into myofibroblasts by inhibiting TGF-β–induced phosphorylation of SMAD3 (219), whereas it has been shown to be ineffective in reducing collagen secretion in primary human lung fibroblasts in another study (220). Clinical Studies Assessing Pirfenidone in Idiopathic Pulmonary Fibrosis: Research of Efficacy and Safety Outcomes (CAPACITY)-2, Assessment of Pirfenidone to Confirm Efficacy and Safety in Idiopathic Pulmonary Fibrosis (ASCEND), and a clinical trial performed in Japan demonstrated that pirfenidone slowed lung function decline and improved patient survival (221, 222). In those trials, pirfenidone was more effective in patients with mild-to-moderate IPF, highlighting the importance of early diagnosis and treatment in pulmonary fibrosis.

Nintedanib. Nintedanib (BIBF 1120), a triple-tyrosine kinase inhibitor, was approved by the U.S. Food and Drug Administration on the same day as pirfenidone for IPF (223, 224). Nintedanib ameliorated progression of pulmonary fibrosis in murine models of silica- or bleomycin-induced pulmonary fibrosis (225, 226). Mechanistically, it has been shown that nintedanib inhibits FGF-, PDGF-, and VEGF-induced profibrotic effects, attenuates TGF-β–induced collagen deposition, reduces infiltration of inflammatory cells into the lungs, and prevents TGF-β–induced human lung

fibroblast differentiation to myofibroblast (220, 225–227). Furthermore, nintedanib potently blocked FGF receptors 1–3, PDGFR as well as VEGF receptor kinase activity (223) and modulated the protease/antiprotease balance (pro–MMP-2 and TIMP-2) (226). The To Improve Pulmonary Fibrosis with BIBF 1120 (TOMORROW) and INPULSIS studies showed that nintedanib slows lung function decline, decreases the frequency of short-term exacerbations and mortality, and retains quality of life of patients with mild-to-moderate IPF (224, 228).

Conclusions

Pulmonary fibrosis is a progressive, irreversible, and usually lethal lung disease. Alveolar epithelial cell microinjuries are thought to initiate the disease, followed by expansion of myofibroblasts and excessive deposition of ECM components that finally remodel and destroy the lung architecture. Immune mechanisms contribute to fibrogenesis at several cellular and noncellular levels. In adaptive immunity, most published evidence exists for T cells, the role of which seems to be complex and subset dependent. Although Th1, Th22, and γδ-T cells have been proposed to attenuate pulmonary fibrosis, Th2 and Th17 cells were found to promote fibrotic disease. Tregs and Th9 subsets have been shown to exert both anti- and profibrotic effects. Among innate immune cells, M2 macrophages and neutrophils were particularly suggested to enhance pulmonary fibrosis, whereas M1 macrophages were assigned a protective role, but contradictory effects have also been described, and future studies are required to clearly define their roles *in vivo*. Fibrocytes represent bone marrow–derived immune cells that migrate to the lung and promote fibrosis. TGF-β, PDGF, IL-13, IL-17, and IL-1β are the major cytokines and CCL2, CCL17, CCL18, and CXCL12 the main chemokines involved in the immunopathogenesis of pulmonary fibrosis. Targeting specific profibrotic immune cell subsets (such as Th2/M2 cells) or profibrotic cytokines/chemokines (such as TGF-β, IL-13, CCL2, or CCL18) by monoclonal antibodies or small molecules, or by expanding/activating antifibrotic cell types (such as Th1/M1 cells) may pave the way for novel immunopharmacological interventions for treating pulmonary fibrosis. Despite these intriguing insights, further studies are warranted to better understand the functional role of immune cell subtypes and their microenvironmental and contextual interactions with epithelial cells, (myo)fibroblasts, and ECM components in the pathogenesis of pulmonary fibrosis. ■

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