Interactions between fibroblasts, macrophages, Th2 and Th17 immunity regulate fibrosis

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Abstract

Dysregulated wound healing leads to fibrosis, where fibroblasts synthesize excess extracellular matrix and scarring impairs proper organ function. Although fibrotic diseases arise from diverse causes and display heterogeneous features, fibrosis commonly associates with chronic inflammation. Recent discoveries reinforce the idea that communication between fibroblasts, macrophages, and CD4 T cells integrates the processes of wound healing and host defense. Signals between macrophages and fibroblasts can exacerbate, suppress, or reverse fibrosis. Fibroblasts and macrophages are activated by T cells, but their activation also engages negative feedback loops that reduce fibrosis by restraining the immune response, particularly when the Th2 cytokine IL-13 contributes to pathology. Thus, the interactions among fibroblasts, macrophages, and CD4 T cells likely play general and critical roles in initiating, perpetuating, and resolving fibrosis in both experimental and clinical conditions.

Introduction

Injuries activate and engage a range of cell types to carry out a wound healing response and repair damage, control infection, and return injured tissue to its full and normal function. Improperly regulated wound healing causes fibrosis, a scarring process often correlated with
repeated injury, where extracellular matrix (ECM) and fibroblasts replace parenchymal cells and
impair organ function. Fibrosis is a complicated, multi-stage, progressive process, often lacking
a defined cause, whose different forms display different characteristics. Fibrotic diseases are
therefore difficult to study and perplexing to treat. However, the consistent involvement of
fibroblasts, macrophages, and other immune cells suggests that communication among these
cell types generally regulates fibrotic diseases (15, 20, 43, 45-46). If so, then understanding
these intercellular interactions may simplify the explanation of a diverse array of disorders and
identify widely applicable therapeutic targets.

This mini-review sketches the orchestration of fibroblasts, macrophages, and the
immune response in fibrosis and emphasizes the regulatory roles of macrophages in wound
healing. Fibrosis is ultimately caused by activated fibroblasts building ECM, but these cells do
not operate autonomously. Fibroblasts intimately associate with macrophages and
lymphocytes at sites of injury and respond directly to immune-mediated signals (7-8, 10, 15, 20,
22, 37, 45). Macrophages integrate signals from the tissue microenvironment, the innate and
adaptive immune responses, and their associated fibroblasts, and have proven capable of
promoting, inhibiting, and reversing fibrosis in different situations (7-8, 14, 19, 22, 25, 36, 46).
When responding to damage and eliminating microbes, other immune cells, notably CD4+ T
lymphocytes, produce cytokines, growth factors, proteases, and other stimuli that alter the
phenotype and function of fibroblasts and macrophages (15, 20, 43, 45). The challenge posed
by fibrosis is to identify and correct defects in a prolonged process where the identities and
roles played by these different cell types are multiple and dynamic (7, 15, 20, 22, 25). The
ultimate goal of anti-fibrotic therapy is to restore homeostasis and recover normal tissue
architecture (9, 33).

Roles of fibroblasts

Activated fibroblasts are essential for wound healing and execute the final steps that
initiate and perpetuate fibrosis. The complex roles played by fibroblasts and the translational
research to target their actions are reviewed elsewhere (9-10, 15, 33, 43, 45). Fibroblasts can
originate from a variety of sources: resident tissue populations, recruited fibrocytes of hematopoietic origin, and converted epithelial cells. Quiescent fibroblasts are attuned to diverse activating signals, and thus able to respond to damage from unrelated causes. Reactive oxygen species and signs of stress, dying cells, the presence of microbes, and paracrine cues from neighboring cell types all stimulate fibroblasts. One way that macrophages and other immune cells govern fibrosis is by providing or altering these stimuli, such as by producing activated TGF-β and IL-13, eliminating infections, clearing apoptotic cells, or remodeling ECM. For example, hepatic stellate cells (HSCs) that sense LPS secrete chemokines that attract macrophages, increase intercellular adhesion molecules, and become more sensitive to TGF-β produced by the Kupffer cells they recruit; interfering with these steps reduces liver fibrosis (37). Once activated, fibroblasts proliferate, differentiate into myofibroblasts, add to and remodel ECM, contract wounds, and recruit other cell types. The loss of stimulated fibroblasts by death or deactivation correlates with reduced pathology and is likely required to stop or reverse fibrosis (6-7, 9, 20, 31).

The versatile HSCs demonstrate that fibroblasts may not passively acquiesce when interacting with immune cells. HSCs can present antigens to CD4+, CD8+, and Natural Killer T cells, as well as provoke Natural Killer cells, and each type of these leukocytes can affect liver fibrosis (10, 20, 44). HSCs also modulate these interactions with auxiliary signals, including B7.2, PD-L1, IL-6, IL-10, IL-12, IL-15, TGF-β and retinoic acid, all of which influence the proliferation, effector function, and differentiation of immune cells.

Roles of macrophages

Given their multi-functional capabilities and heterogeneous phenotypes, it is not surprising that macrophages can both enhance and limit fibrosis (7, 14-15, 20, 25, 35, 46). Both resident tissue macrophages and their circulating precursors home to sites of injury in response to MIP-1α, MIP-1β, RANTES, MCP-1, and other chemokines that correlate with fibrosis (20, 22, 35-36, 43, 45). Activated fibroblasts summon and stimulate macrophages by producing MCP-1,
other chemokines, and M-CSF (10, 37), and activated macrophages secrete cytokines that
attract fibroblasts and amplify inflammation (37, 45-46). However, the consequences of
depleting macrophages or blocking their recruitment appear to depend on the stage of the
wound healing response, as well as the selectivity with which macrophages are targeted and
their activation state (7, 14-15, 20, 22, 25, 35-36, 46). This point was elegantly demonstrated in
CCL4-induced liver fibrosis, where depleting CD11b+ macrophages with diphtheria toxin at the
time of injury reduces pathology, but depleting during the wound healing process makes
fibrosis worse (7).

When appropriately activated and positioned, macrophages supply a suite of factors
that promote fibrosis by stimulating fibroblasts (46). Macrophage-derived TGF-β causes
fibroblasts to produce interstitial fibrillar collagens and Tissue Inhibitor of Metalloproteinases
(TIMPs) to block ECM degradation (10, 22, 25). TGF-β has proved a key to liver fibrosis, in part
by triggering quiescent HSCs to differentiate into myofibroblasts, and reducing the number of
TGF-β-producing macrophages can slow disease progression (7, 10, 22, 35, 40). Macrophages
also stimulate fibroblast proliferation, survival and migration with Platelet-Derived Growth
Factor (PDGF), and alveolar macrophages recovered from Idiopathic Pulmonary Fibrosis (IPF)
patients spontaneously produce PDGF (27). Imatinib mesylate (Gleevec), which inhibits
tyrosine kinases in the TGF-β and PDGF (and other) signaling pathways, is now being tested as a
therapy for several fibrotic diseases (33). Macrophages from IPF patients and bleomycin-
treated mice produce IL-1β as another stimulus for fibroblast to secrete collagen and
chemokines, and signaling through MyD88 and the inflammasome, steps in the IL-1 pathway, is
essential for lung, and possibly liver, fibrosis (12, 37, 42). IL-1β also cooperates with and is
cross-regulated by IL-17A; together these cytokines critically contribute to neutrophil
recruitment and bleomycin-induced lung fibrosis, and IL-17A is also elevated in IPF patients
(42). Such signals that recruit, expand, and activate fibroblasts to produce collagen likely
explain how blocking macrophage migration into and within damaged tissue reduces fibrosis
(15, 22, 25, 35, 37, 46).
Macrophages do not produce collagen, but do produce Matrix Metalloproteinases (MMPs) and TIMPs that alter ECM turnover and composition (45-46). MMP-2, -9, and -13 enable macrophages to break down ECM and reverse scarring, and depleting macrophages at the resolution stage of wound healing reduces ECM degradation (7, 15). In liver fibrosis models, deleting MMP-13 impairs resolution (8), and augmenting MMP-13 production by Kupffer cells with GdCl₃ reduces ECM formation (19). Similarly, in bleomycin-induced lung fibrosis, over-expression of MMP-9 in macrophages attenuates pathology (3). Once cleaved, macrophages use Mfge8 and other molecules to take up extracellular collagen, which reduces fibrosis (1).

MMP production by macrophages leads to consequences beyond ECM consumption. Fibroblasts recognize their contact with ECM and, by changing in its composition and flexibility, MMPs and TIMPs alter fibroblast behavior (9-10, 15, 20). MMP-9 contributes to TGF-β activation and promotes IL-13-driven lung fibrosis (24). In response to schistosome eggs and IL-13, MMP-12 augments liver and lung fibrosis by limiting expression of collagen-degrading MMP-2, -9, and -13 (26). Since ECM degradation breaks down a potential barrier to cell migration, the combination of chemokines and MMPs produced by macrophages indirectly affect fibrosis by recruiting other leukocytes (3, 39). As with macrophages, these recruited cells can promote or repress fibrosis in different circumstances (15, 20, 43, 45). MMP-13 facilitates neutrophil infiltration and increases liver fibrosis during injury caused by bile duct ligation (39). In similar experiments, but modeling recovery, macrophage depletion reduces neutrophils but now enhances fibrosis (14). In IPF patients, elevated IL-8 synthesis by alveolar macrophages correlates with more neutrophils in the airway, lower oxygen pressure, and more severe disease (4). Activated macrophages also attract CCR2+ monocytes with MCP-1 (22, 46). Inflammatory monocytes can promote lung and liver fibrosis using the same mechanisms as macrophages, but their rapid influx could quickly scale up these processes (20, 22, 25).

The phagocytic role of macrophages plays an integral part in wound healing by removing microbes, debris and dead cells (1, 31, 45-46). Injury and infection may initially stimulate fibroblasts because ingesting dying cells prompts macrophages to produce TGF-β and IL-1β,
and recognizing microbes triggers inflammation (12, 46). However, clearing apoptotic cells may later help halt and resolve fibrosis by both enhancing macrophages’ MMPs activities and eliminating the causes of pro-fibrotic and pro-inflammatory signals (6, 31).

Finally, macrophages couple the adaptive immune response to the wound healing process (15, 34, 46). Macrophages sample their environment and present the antigens they encounter to T cells, which locally amplifies inflammatory responses and can produce IL-4 and IL-13 to stimulate collagen production by fibroblasts (45). These interactions also engage feedback loops, discussed in the next section, where activated T cells stimulate macrophages to produce immunoregulatory mediators such as Arginase-1 or inducible Nitric Oxide Synthase (iNOS), Relm-α, and chitinase family proteins.

Connections between Th2 immunity, macrophage activation, and fibrosis

CD4+ helper T cells adapt and amplify their responses to match different categories of infections and coordinate the many types of immune cells that can affect fibrosis (15, 20, 45). The heterogeneity of fibrotic diseases rules out a simple association between one type of T cell response and a pro- (or anti-) fibrotic effect of the immune system (43). However, extensive evidence links wound healing and fibrosis with T helper type 2 (Th2) differentiation, characterized by the cytokines IL-4 and IL-13 and protective against helminths (parasitic worms) (15, 20, 34, 45). The size and life cycle of helminths inflicts large injuries over a long time, implying that successful co-evolution evident between parasites and hosts required immune-mediated damage control mechanisms (21).

Th2 cells can directly promote fibrosis by stimulating fibroblasts to synthesize collagen with IL-4 and IL-13 (5, 40, 45). Th2 cytokines additionally generate alternatively-activated macrophages (AAMs) (13). Although implicated in the pathogenesis of fibrosis, AAMs also participate in wound healing and modulate immune responses to limit fibrosis (11, 13, 16-18, 28-30, 34, 45-46). IL-13 is the most important cause of liver and lung fibrosis in Schistosomiasis, with additive effects by IL-4, IL-5, IL-10 and IL-21 (but not TGF-β) (5, 40, 45). IL-13 is linked with
fibrosis in Hepatitis C- or gamma herpesvirus-infected liver and steatohepatitis (11, 40) and in lung with a diverse (though not inclusive) list of animal models and human diseases (43).

AAMs act by mechanisms that remain controversial and only partly explained (13). AAMs express Arginase-1, Relm-α and β, chitinase family members, and a set of other markers, some of which affect T cell responses (28, 34, 46). In mice where alternative activation is prevented, by deleting IL-4Rα in neutrophils and macrophages, schistosome infection causes abnormal intestinal pathology, elevated IFN-γ, and premature death from sepsis but liver fibrosis still begins to develop (16). In this setting, AAMs act as a necessary check on inflammation and maintain gut integrity but do not initiate liver fibrosis.

While IL-4 or IL-13-producing Th2 cells induce Arginase-1 in AAMs, IFN-γ-secreting Th1 cells produce classically activated macrophages (CAMs) with distinctly different features, including high iNOS and suppressed Arginase-1 expression (13, 18). Both enzymes act on the same substrate, L-Arginine, and the activity of each antagonizes the other (2). If the normal Th2 and fibrotic response to schistosome eggs is deviated to Th1 immunity, CAMs replace AAMs and Arginase-1 is exchanged for iNOS (18). This immune deviation reduces liver fibrosis, but not in iNOS-deficient mice, demonstrating that the iNOS activity of CAMs can block fibrosis by a still unexplained mechanism (17-18, 34, 45). The Arginase-1 pathway generates proline for collagen synthesis and polyamines for cell division, suggesting Arginase-1 would play a pro-fibrotic, reciprocal role to iNOS (18). Instead, deletion of Arginase-1 in macrophages increases the Th2 response to schistosome eggs and exaggerates fibrosis, but without the pathology and sepsis observed when macrophages cannot respond to IL-4 or IL-13 (16, 29). This outcome may be explained if AAMs minimize T cell proliferation because Arginase-1 activity depletes L-Arginine from the local granuloma environment.

AAMs express a subset of genes also induced by Th2 cytokines in other cell types, particularly epithelial cells. These include Relm-α, chitinases, and chitolectins which affect fibrotic, inflammatory, and allergic responses and participate in negative feedback loops (13, 38, 45-46). Relm-α is secreted by epithelial cells, macrophages, eosinophils, and other cell types in the lung, liver and gut during parasite infections, allergic reactions, and bleomycin-
induced fibrosis (28, 30, 34, 46). Although proposed to promote the survival and differentiation of fibroblasts (46), Relm-α-deficient mice develop stronger Th2 inflammatory responses and fibrosis that can be reversed with exogenous Relm-α (28, 30). This inhibition may be partly caused by Relm-α interfering with BTK signaling in T cells and reducing IL-4 and IL-13 production (28). Thus, in some situations, both Relm-α and Arginase-1 modulate immune-driven fibrosis by restraining the T cell response that drives their expression.

Like Relm-α, chitin-binding proteins are associated with Th2 inflammation and fibrosis and affect immune responses (32, 34, 38, 47). IL-13 promotes Acidic Mammalian Chitinase (AMCase) expression by epithelial cells and macrophages and its neutralization reduces Th2 inflammation, AAMs, and airway remodeling and reactivity in an asthma model (47). Similar results have been reported for the human chitin-binding protein YKL-40 and its mouse homologue, BRP-39 (23). However, in a contradictory study, AMCase’s chitin-digesting activity ameliorates these same processes (32). While the roles of chitin-binding proteins remain disputed, elevated expression of chitotriosidase in nonalcoholic steatohepatitis, IPF, and sarcoidosis, and of YKL-40 in liver fibrosis, presents a correlation between this family of molecules and fibrosis, tissue damage, and inflammation (38, 46).

Persistent immune responses engage negative feedback loops which normally limit pathology, and IL-10 and IL-13Rα2 play crucial roles in restraining inflammation and fibrosis (13, 15, 20, 34, 41, 43, 45). IL-10 from T cells and macrophages suppress immune responses in diverse and independent ways, and also shuts down TGFβ-induced collagen synthesis by fibroblasts. IL-13Rα2, abundantly produced in fibroblasts by Th2 and other inflammatory cytokines, acts as a high affinity decoy receptor for IL-13.

Challenges and goals

Progress in fibrosis research and therapy has produced notable achievements but also points towards more difficult challenges (9, 15, 33, 45). The key mediators of fibrosis cause pleiotropic effects, making it important to test the hierarchy of their actions. TGF-β, for
example, can promote collagen synthesis, suppress inflammation, sensitize cells to apoptosis, and alter differentiation; it is essential to discern which of these functions predominates in clinical settings (9, 22, 25, 33, 37, 46). Similarly, IL-13 can act directly on fibroblasts to stimulate collagen production (5), or indirectly by inducing and activating TGF-β (24), but it also alternatively activates macrophages and induces Arginase-1, Relm-α, and chitin-binding proteins that may modulate Th2 immunity and thus limit fibrosis (13, 28-30, 46). Further investigations into the downstream and indirect effects of TGF-β and IL-13 could predict how neutralization of these pathways may provide benefit or cause harm and identify specific strategies to improve their efficacy.

The dynamic and plastic identities of the cell types causing fibrosis present another challenge. The populations of fibroblasts and macrophages located in a fibrotic lesion can originate from different sources and their phenotypes change as disease progresses (7, 15, 20, 22, 25). Put simply, does the same population of cells adjust its function, or do different populations of cells proliferate or arrive in waves and play largely fixed roles? The responsiveness of macrophages, which generally enter reversible activation states rather than undergoing true differentiation, favors the former possibility and suggests that instructing macrophages may be crucial to reversing fibrosis (13). However, injury may recruit an influx of monocyte-derived macrophages whose role is limited to exacerbating fibrosis (22, 25). As suggested, together these possibilities may explain why blocking cell migration or depleting macrophages can cause opposite effects at different stages of fibrosis (7, 22, 25, 36, 46). One priority for future studies is to trace the lineages and distinguishing features of participating cell types from injury to resolution.

One vexing obstacle to studying fibrosis is that experimental variables, particularly deleted genes, often alter the early process of disease and confound subsequent observations. As a result we understand the initiation of wound healing better than the progression or resolution of fibrosis, though the latter are more clinically relevant since patients are often diagnosed from symptoms caused by advanced disease (9, 15). Genetic-based systems to inducibly switch genes on or off in specific cell types offer one potential solution. Another
promising technology is laser microdissection which, coupled with better discrimination between cellular lineages and phenotypes, could help integrate histology-based analyses with gene expression profiling (8).

Finally, the heterogeneity of fibrosis creates the challenge of identifying regulatory mechanisms that are conserved across diseases affecting different organs and arising from a variety of causes. One starting point is to characterize and promote the activity of cells producing MMPs that degrade scar-associated ECM. Control of MMP production and activity has proven tight and complicated, and problems with side effects, specificity, and delivery have hindered the development of therapeutic MMP inhibitors (26). Intervention at the cellular level, perhaps directed at AAMs, might manipulate MMP activity better than targeting these enzymes directly. From a broader perspective, the homology between the host defense and damage control mechanisms activated by parasite infections and the wound healing response suggests that the immune system holds the potential to regulate fibrosis (15, 20-21, 45). For example, if macrophages or T cells provide important survival signals to activated fibroblasts, blocking these signals might halt progressive fibrosis. If so, then dissecting and exploiting the communication between fibroblasts, macrophages, and the Th2 immune response will advance our understanding of the wound healing process, how its normal limits are violated, and create opportunities to discover new ideas for treating the millions of people affected by fibrotic diseases.

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References


**Figure legends**

**Figure 1**

A model of the initiation and perpetuation of fibrosis

Injuries and infections activate macrophages and fibroblasts, which move together, enabling macrophages to simulate fibroblasts with TGF-β, PDGF, IL-1β, and other factors. Macrophages also promote inflammation, by recruiting and activating monocytes and neutrophils, present antigens to CD4 T cells, and modulate T cell responses with costimulation and cytokines. Fibroblasts also influence T cells. CD4 T cells coordinate the immune response with cytokines, enhancing neutrophil recruitment with IL-17A, activating macrophages with IL-4 and IL-13 or IFNγ, and inducing collagen production by fibroblasts with IL-4, IL-13, and possibly TGF-β. The combination of activating signals from the inflammatory environment, macrophages, and CD4 T cells stimulate fibroblasts to proliferate and synthesize collagens, MMPs and TIMPs that construct and remodel extracellular matrix and lead to fibrosis.

**Figure 2**

A model of the resolution of fibrosis

The immune response eliminates infections, with phagocytosis by macrophages clearing dead cells, microbes, and debris that would otherwise activate myofibroblasts. Macrophages also reverse myofibroblast activation with IL-10, by stopping production of TGF-β, PDGF, and IL-1β, and by acting in negative feedback loops that reduce immune-mediated fibrosis. IL-4 and IL-13-stimulated alternatively activated macrophages restrain the CD4 T cell response with Arginase-1, RELM-α, and IL-10, while IFNγ-stimulated classically activated macrophages reduce fibrosis by producing nitric oxide. Fibroblasts produce IL-13Rα2, a decoy receptor for IL-13, and
contract to seal lesions. Fibrosis resolves as activated fibroblasts die or return to a quiescent state and cease adding to the extracellular matrix, and as macrophages degrade excess ECM with MMPs.
IL-17A

Neutrophils

Monocytes

Recruitment & activation
MCP-1

IL-8
IL-1β

IL-1β

Macrophages

Recruitment; Antigen presentation modulated by cytokines, costimulation
TGF-β, PDGF, IL-1β

IL-4 & IL-13, IFNγ

TGF-β, IL-4 & L-13

Activated and proliferating fibroblasts

Collagens, MMPs, TIMPs

Synthesis remodeling

Extracellular matrix

Dead cells, debris, microbes

Lesion

Apoptotic cells

Epi/endothelium

CD4 T cells

IL-17A

Chemotaxis

Activation

Recruitment & activation
Macrophages

Degraded ECM

MMPs

Immune modulation
Arginase-1
RELIMα
IL-10

Stop producing
TGF-β, PDGF, IL-1β

Nitric oxide

Phagocytosis

ECM remodeling

Quiescent fibroblasts

IL-4 & IL-13

IL-4 & IL-13, or IFNγ

IL-10 reduced

IL-13Rα2

Lesion sealed

Contraction

IL-10

Stop synthesis remodeling

IL-10

IL-10

IL-10