Fibrosis is regulated by Th2 and Th17 responses and by dynamic interactions between fibroblasts and macrophages

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Barron L, Wynn TA. Fibrosis is regulated by Th2 and Th17 responses and by dynamic interactions between fibroblasts and macrophages. Am J Physiol Gastrointest Liver Physiol 300: G723–G728, 2011. First published February 3, 2011; doi:10.1152/ajpgi.00414.2010.—Dysregulated wound healing leads to fibrosis, whereby 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.

inflammation; wound healing

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). If so, then understanding these intercellular interactions may simplify the explanation of a diverse array of disorders and identify widely applicable therapeutic targets.

This minireview 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 (Fig. 1). 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 in which the identities and roles played by these different cell types are multiple and dynamic (7, 15, 20, 22, 25). The ultimate goal of antifibrotic therapy is to restore homeostasis and recover normal tissue architecture (9, 33) (Fig. 2).

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

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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+ and CD8+ T cells, as well as provoke natural killer T cells, and each type of cell can influence the fibroblast response. CD4 T cells coordinate the immune response with cytokines, enhancing neutrophil recruitment with IL-4 and IL-13 or IFN-γ, 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 stimulates fibroblasts to proliferate and synthesize collagens, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs) that construct and remodel extracellular matrix and lead to 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, whereas IFN-γ-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 extracellular matrix (ECM) with MMPs.
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 multifunctional 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, 25, 35, 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 inhibitors 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 (3). 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, overexpression 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 profibrotic and proinflammatory 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 inflict large injuries over a long time, implying that successful coevolution 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.

Whereas 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 polymers for cell division, suggesting that arginase-1 would play a profibrotic, 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 chitooligomers, 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 airflow remodeling and activity 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). Although 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 that normally limit pathology, and IL-10 and IL-13Rsβ2 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 TGF-β-induced collagen synthesis by fibroblasts. IL-13Rsβ2, 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 toward 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 monocye-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 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).

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DISCLOSURES

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