Published in final edited form as:
J Pathol. 2008 January ; 214(2): 199–210. doi:10.1002/path.2277.

Cellular and molecular mechanisms of fibrosis

TA Wynn*
Immunopathogenesis Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Abstract

Fibrosis is defined by the overgrowth, hardening, and/or scarring of various tissues and is attributed to excess deposition of extracellular matrix components including collagen. Fibrosis is the end result of chronic inflammatory reactions induced by a variety of stimuli including persistent infections, autoimmune reactions, allergic responses, chemical insults, radiation, and tissue injury. Although current treatments for fibrotic diseases such as idiopathic pulmonary fibrosis, liver cirrhosis, systemic sclerosis, progressive kidney disease, and cardiovascular fibrosis typically target the inflammatory response, there is accumulating evidence that the mechanisms driving fibrogenesis are distinct from those regulating inflammation. In fact, some studies have suggested that ongoing inflammation is needed to reverse established and progressive fibrosis. The key cellular mediator of fibrosis is the myofibroblast, which when activated serves as the primary collagen-producing cell. Myofibroblasts are generated from a variety of sources including resident mesenchymal cells, epithelial and endothelial cells in processes termed epithelial/endothelial-mesenchymal (EMT/EndMT) transition, as well as from circulating fibroblast-like cells called fibrocytes that are derived from bone-marrow stem cells. Myofibroblasts are activated by a variety of mechanisms, including paracrine signals derived from lymphocytes and macrophages, autocrine factors secreted by myofibroblasts, and pathogen-associated molecular patterns (PAMPS) produced by pathogenic organisms that interact with pattern recognition receptors (i.e. TLRs) on fibroblasts. Cytokines (IL-13, IL-21, TGF-β1), chemokines (MCP-1, MIP-1β), angiogenic factors (VEGF), growth factors (PDGF), peroxisome proliferator-activated receptors (PPARs), acute phase proteins (SAP), caspases, and components of the renin–angiotensin–aldosterone system (ANG II) have been identified as important regulators of fibrosis and are being investigated as potential targets of antifibrotic drugs. This review explores our current understanding of the cellular and molecular mechanisms of fibrogenesis.

Keywords

fibrosis; myofibroblast; collagen; wound healing; liver; lung

Introduction

In contrast to acute inflammatory reactions, which are characterized by rapidly resolving vascular changes, oedema and neutrophilic inflammation, fibrosis typically results from chronic inflammation — defined as an immune response that persists for several months and in which inflammation, tissue remodelling and repair processes occur simultaneously. Despite having distinct aetiological and clinical manifestations, most chronic fibrotic disorders have

*Correspondence to: TA Wynn, Immunopathogenesis, Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 50 South Drive, Room 6154, MSC 8003, Bethesda, MD, 20892, USA. E-mail: E-mail: twynn@niaid.nih.gov.

No conflicts of interest were declared.

in common a persistent irritant that sustains the production of growth factors, proteolytic enzymes, angiogenic factors and fibrogenic cytokines, which stimulate the deposition of connective tissue elements that progressively remodel and destroy normal tissue architecture [1–3].

Damage to tissues can result from various stimuli, including infections, autoimmune reactions, toxins, radiation and mechanical injury. The repair process typically involves two distinct phases: a regenerative phase, in which injured cells are replaced by cells of the same type, leaving no lasting evidence of damage; and a phase known as fibroplasia or fibrosis, in which connective tissues replaces normal parenchymal tissue. Although initially beneficial, the repair process becomes pathogenic when it is not controlled appropriately, resulting in substantial deposition of ECM components in which normal tissue is replaced with permanent scar tissue [4]. In some diseases, such as idiopathic pulmonary fibrosis, liver cirrhosis, cardiovascular fibrosis, systemic sclerosis and nephritis, extensive tissue remodelling and fibrosis can ultimately lead to organ failure and death (Table 1).

Wound healing versus fibrosis

When epithelial and/or endothelial cells are damaged, they release inflammatory mediators that initiate an anti-fibrinolytic coagulation cascade [5], which triggers blood-clot formation and formation of a provisional ECM. Platelets are exposed to ECM components, triggering aggregation, clot formation and haemostasis. Platelet degranulation also promotes vasodilation and increased blood vessel permeability, while myofibroblasts (activated collagen secreting, α-SMA$^+$ fibroblasts) and epithelial and/or endothelial cells produce MMPs, which disrupt the basement membrane, allowing inflammatory cells to be easily recruited to the site of injury. Growth factors, cytokines and chemokines are also produced, which stimulates the proliferation and recruitment of leukocytes across the provisional ECM. Some of the early responders include macrophages and neutrophils, which eliminate tissue debris, dead cells and any invading organisms. They also produce cytokines and chemokines, which are mitogenic and chemotactic for endothelial cells, which begin to surround the injured site. They also help form new blood vessels as epithelial/endothelial cells migrate towards the centre of the wound. During this period, lymphocytes and other cells become activated and begin secreting profibrotic cytokines and growth factors, such as TGFβ, IL-13 and PDGF [6–8], which further activate the macrophages and fibroblasts. Activated fibroblasts transform into α-SMA-expressing myofibroblasts as they migrate along the fibrin lattice into the wound. Following activation, the myofibroblasts promote wound contraction, the process in which the edges of the wound migrate towards the centre. Finally, epithelial and/or endothelial cells divide and migrate over the basal layers to regenerate the damaged tissue, which completes the wound-healing process. However, chronic inflammation and repair can trigger an excessive accumulation of ECM components, which leads to the formation of a permanent fibrotic scar. Collagen turnover and ECM remodelling is regulated by various MMPs and their inhibitors, which include the tissue inhibitors of metalloproteinases (TIMPs). Shifts in synthesis versus catabolism of the ECM regulate the net increase or decrease of collagen within the wound [9]. Fibrosis occurs when the synthesis of new collagen by myofibroblasts exceeds the rate at which it is degraded, such that the total amount of collagen increases over time.

The cellular origins of myofibroblasts

Local tissue myofibroblasts were originally believed to be the primary producers of ECM components following injury [5]; however, it is now thought that fibroblasts can be derived from multiple sources [10]. In addition to resident mesenchymal cells, myofibroblasts are derived from epithelial cells in a process termed epithelial–mesenchymal transition (EMT) [10–12]. More recently, it was suggested that a similar process occurs with endothelial cells,

termed endothelial–mesenchymal transition (EndMT) [13]. Bucala and colleagues also identified a unique circulating fibroblast-like cell derived from bone marrow stem cells [14]. These blood-borne mesenchymal stem cell progenitors have a fibroblast/myofibroblast-like phenotype (they express CD34, CD45 and type I collagen) and are now commonly called fibrocytes [15–18]. Finally, in some tissues, resident fibroblasts are not the only source of myofibroblasts. For example, in liver fibrosis the resident hepatic stellate cell (HSC) appears to be the primary source of myofibroblasts, although bone-marrow-derived cells can also contribute [18,19]. Because it is now thought that fibrocytes and EMT-derived myofibroblasts participate with resident mesenchymal cells in the reparative process, there has been growing interest in dissecting the role of the various myofibroblast subpopulations in fibroproliferative disease [20]. Because bone marrow-derived fibrocytes must find their way to sites of tissue injury to participate in wound healing and fibrosis, there has been a great deal of interest in understanding the role of chemokines and acute phase proteins, such as serum amyloid P (SAP), in the development and recruitment of myofibroblasts [20–22]. Because fibrocytes and EMT-derived myofibroblasts produce a variety of factors that are involved in the fibrotic process [10], interrupting their development, recruitment and/or activation could provide a unique therapeutic approach to treat a variety of fibrotic diseases.

Innate and adaptive immune mechanisms regulate myofibroblast activity

Many fibrotic disorders are thought to have an infectious aetiology, with bacteria, viruses, fungi and multicellular parasites driving chronic inflammation and the development of fibrosis. It was recently suggested that conserved pathogen-associated molecular patterns (PAMPs) found on these organisms help maintain myofibroblasts at a heightened state of activation [23]. Bacteria living in the gut can also contribute to the activation of myofibroblasts [24]. PAMPs are pathogen byproducts, such as lipoproteins, bacterial DNA and double-stranded RNA, which are recognized by pattern recognition receptors (PRRs) found on a wide variety of cells, including fibroblasts [25]. The interaction between PAMPs and PRRs serves as a first line of defence during infection and activates numerous proinflammatory cytokine and chemokine responses. In addition, because fibroblasts express a variety of PRRs, including Toll-like receptors (TLRs), Toll ligands can directly activate fibroblasts and promote their differentiation into collagen-producing myofibroblasts [23,24,26]. Thus, inhibiting TLR signalling might represent a novel approach to treat fibrotic disease.

Nevertheless, pathogenic organisms are not responsible for all fibrotic disorders. Therefore, additional mechanisms must also participate in the activation of myofibroblasts. For example, in the case of systemic sclerosis (SSc), fibroblasts obtained from lesional skin or fibrotic lungs have a constitutively activated myofibroblast-like phenotype, characterized by enhanced ECM synthesis, constitutive secretion of cytokines and chemokines and increased expression of cell surface receptors [27–29]. Because most of the characteristics of fibroblasts from patients with SSc are reproduced in normal human fibroblasts following stimulation with TGFβ, it is thought that the SSc fibroblast phenotype is maintained by an autocrine TGFβ signal. However, TGFβ/SMAD3-independent mechanisms have also been proposed [30,31], including a role for viruses such as CMV, which stimulate the production of auto-antibodies and connective tissue growth factor (CTGF), both of which are known to participate in the activation of myofibroblasts [28,32]. Epigenetic changes may also contribute to the persistent activation of myofibroblasts [33]. B cells have also been implicated, either by producing autoanti-bodies or by secreting IL-6, a well-known fibroblast growth factor [34]. Still other studies have argued that Th2-type cytokines derived from a variety of cellular sources are critically involved in the mechanism of fibrosis [35–38]. Therefore, paracrine signals derived from activated lymphocytes, autocrine factors produced by fibroblasts, as well as molecules derived from pathogenic organisms can cooperate to initiate and maintain myofibroblast activation.

Chemokines regulate fibrogenesis by controlling myofibroblast recruitment

Chemokines are leukocyte chemoattractants that cooperate with profibrotic cytokines in the development of fibrosis by recruiting myofibroblasts, macrophages and other key effector cells to sites of tissue injury. Although a large number of chemokine signalling pathways are involved in the mechanism of fibrogenesis, the CC- and CXC-chemokine receptor families have consistently exhibited important regulatory roles. Specifically, CCL3 (macrophage inflammatory protein 1α) and CC-chemokines such as CCL2 (monocyte chemoattractant protein-1), which are chemotactic for mononuclear phagocytes, were identified as profibrotic mediators. Macrophages and epithelial cells are believed to be the key sources of CCL3, and studies in the bleomycin model of pulmonary fibrosis showed that anti-CCL3 antibodies could significantly reduce the development of fibrosis [39,40]. Similar results were obtained when CCL2 was neutralized, suggesting that a variety of CC-chemokines are involved [41,42]. Subsequent studies with CC-chemokine receptor 1 (CCR1)- and CCR2-deficient mice produced similar results, confirming critical roles for CCL3/CCL2-mediated signalling pathways in fibrogenesis [43–47]. Interestingly, in several of these blocking studies, the absence of fibrosis was associated with decreased IL-4/IL-13 expression [44,48], suggesting a direct link between CC-chemokine activity and the production of profibrotic cytokines such as IL-13. IL-13 is a potent inducer of several CC-chemokines, including CCL3, CCL4 (MIP-1β), CCL20 (MIP-3α), CCL2, CCL11, CCL22 (macrophage-derived chemokine) and CCL6 (C10), among others, suggesting that a positive feedback mechanism exists between IL-13 and the CC-chemokine family [49,50]. As seen with anti-CCL3 and anti-CCL2 antibody treatment, antibodies to CCL6 significantly attenuated lung remodelling responses in IL-13-transgenic mice [50] as well as in mice challenged with bleomycin [49], indicating non-redundant roles for a variety of CC-chemokines in the pathogenesis of fibrosis. In mice, CXC chemokine receptor 4 (CXCR4), CC chemokine receptor 7 (CCR7) and CCR2 have also been shown to regulate the recruitment of fibrocytes to the lung [20,21]. Thus, interrupting specific chemokine signalling pathways could have a significant impact on the treatment of a variety of fibroproliferative diseases.

Th1 and Th2 cells differentially regulate organ fibrosis

Chronic inflammatory reactions are typically characterized by a large infiltrate of mononuclear cells, including macrophages, lymphocytes, eosinophils and plasma cells. Lymphocytes are mobilized to sites of injury and become activated following contact with various antigens, which stimulate the production of lymphokines that further activate macrophages and other local inflammatory cells. Thus, there is significant activation of the adaptive immune response in many chronic inflammatory diseases. Although inflammation typically precedes the development of fibrosis, results from a variety of experimental models suggest that fibrosis is not always characterized by persistent inflammation, implying that the mechanisms regulating fibrosis are to a certain extend distinct from those controlling inflammation. Findings from our own studies of schistosomiasis-induced liver fibrosis support this theory [35]. In this model, fibrosis develops progressively in response to schistosome eggs that are deposited in the liver, which induce a chronic granulomatous response. As in many other experimental models of fibrosis, $CD4^+$ T cells play a prominent role in the progression of the disease. Studies conducted with multiple cytokine-deficient mice have demonstrated that liver fibrosis is strongly linked with the development of a $CD4^+$ Th2 cell response (involving IL-4, IL-5, IL-13 and IL-21) [51–55].

Several experimental models of fibrosis in addition to our own have also documented potent antifibrotic activities for the Th1-associated cytokines IFNγ and IL-12. In schistosomiasis, while treatment with IFNγ or IL-12 has no effect on the establishment of infection, collagen deposition associated with chronic granuloma formation is substantially decreased [51].

Similar results have been obtained in models of pulmonary, liver and kidney fibrosis [56–59]. These findings suggest that it might be possible to develop an antifibrosis vaccine based on immune deviation [51,60], in which the profibrotic effects of the Th2 response are switched off in favour of an antifibrotic Th1 response. Indeed, similar approaches have been proposed for individuals suffering from allergic airway inflammation [61], which is also driven by Th2-type responses. Studies investigating the gene expression patterns of fibrotic tissues found that markedly different gene expression profiles are induced during Th1 and Th2 polarized responses [62,63]. As might be expected, a large number of IFNγ-induced genes are upregulated in the tissues of mice exhibiting Th1-polarized responses, with no evidence of significant activation of the fibrosis-associated genes in this setting [62–64]. Instead, two major groups of genes were identified in Th1-polarized mice: those associated with the acute-phase reaction and apoptosis (cell death), findings which may explain the extensive tissue damage that is commonly observed when Th1 responses continue unchecked [65]. By contrast, several genes known to be involved in the mechanisms of wound healing and fibrosis were upregulated in animals exhibiting Th2-polarized inflammation [62,63]. The regulation and function of a few of the genes, including procollagens I, III and VI, arginase-1 [66], lysyl oxidase [67,68], matrix metalloproteinase-2 (MMP-2) [69,70], MMP-9 [71,72] and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) [73,74], have been investigated in some detail. Several additional Th2-linked genes [62,63], including haem oxygenase, procollagen III, secreted phosphoprotein 1, procollagen V, reticulocalbin and fibrillin 1 have also been reported in the fibrotic lungs of bleomycin-treated mice [75] and in CCl_4-stimulated rat hepatic stellate cells (collagen-producing cells in the liver) [76], providing further evidence that fibrosis is often associated with the development of Th2-type responses.

Unique roles for the Th2 cytokines IL-4, IL-5, IL-13 and IL-21 in fibrosis

The Th2 cytokines IL-4, IL-5, IL-13 and IL-21 each have distinct roles in the regulation of tissue remodelling and fibrosis. IL-4 is found at increased levels in the bronchoalveolar lavage fluids of patients with idiopathic pulmonary fibrosis (IPF) [77], in the pulmonary interstitium of individuals with cryptogenic fibrosing alveolitis [78] and in peripheral blood mononuclear cells (PBMCs) of those suffering from periportal fibrosis [79]. Development of post-irradiation fibrosis is also associated with increased production of IL-4 [80]. Although the extent to which IL-4 participates in fibrosis varies in different diseases, it has long been considered a potent profibrotic mediator. In fact, studies have suggested that IL-4 is nearly twice as effective as TGFβ [81], another potent profibrotic cytokine that has been extensively studied [82]. Receptors for IL-4 are found on many mouse [83] and human fibroblast subtypes [84] and *in vitro* studies showed the synthesis of the extracellular matrix proteins, types I and III collagen and fibronectin, following IL-4 stimulation. One of the first *in vivo* reports to investigate the contribution of IL-4 was a study of schistosomiasis in mice, in which neutralizing antibodies to IL-4 were shown to significantly reduce the development of hepatic fibrosis [52]. Inhibitors of IL-4 were also found to reduce dermal fibrosis in a chronic skin graft rejection model and in a mouse model of scleroderma [85,86].

IL-13 shares many functional activities with IL-4 because both cytokines exploit the same IL-4Rα/Stat6 signalling pathways [87]. However, with the development of IL-13 transgenic and knockout mice [88,89], as well as IL-13 antagonists [53,90], unique and non-redundant roles for IL-13 and IL-4 have been revealed in numerous models. When IL-4 and IL-13 were inhibited independently, IL-13 was identified as the dominant effector cytokine of fibrosis in several experimental models of fibrosis [38,53,91–94]. In schistosomiasis, although the egg-induced inflammatory response was unaffected by IL-13 blockade, collagen deposition decreased by more than 85% [53,95], despite continued and undiminished production of IL-4 [53,96]. Related studies have also shown a dominant role for IL-13 in the pathogenesis of pulmonary fibrosis. Over-expression of IL-13 in the lung triggered significant subepithelial

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