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Pulmonary fibrosis: pathogenesis, etiology and regulation

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Pulmonary fibrosis and architectural remodeling of tissues can severely disrupt lung function, often with fatal consequences. The etiology of pulmonary fibrotic diseases is varied, with an array of triggers including allergens, chemicals, radiation and environmental particles. However, the cause of one of the most common pulmonary fibrotic conditions, idiopathic pulmonary fibrosis (IPF), is still unclear. This review examines common mechanisms of pulmonary wound-healing responses following lung injury, and highlights the pathogenesis of some of the most widespread pulmonary fibrotic diseases. A three phase model of wound repair is reviewed that includes; (1) injury; (2) inflammation; and (3) repair. In most pulmonary fibrotic conditions dysregulation at one or more of these phases has been reported. Chronic inflammation can lead to an imbalance in the production of chemokines, cytokines, growth factors, and disrupt cellular recruitment. These changes coupled with excessive pro-fibrotic IL-13 and/or TGFβ1 production can turn a well-controlled healing response into a pathogenic fibrotic response. Endogenous regulatory mechanisms are discussed including novel areas of therapeutic intervention. Restoring homeostasis to these dysregulated healing responses, or simply neutralizing the key pro-fibrotic mediators may prevent or slow the progression of pulmonary fibrosis.

INTRODUCTION

Following injury it is paramount that tissue architecture is restored to regain normal organ function. Acute inflammatory responses that result from infection or injury can disrupt epithelial and endothelial integrity leading to edema, the recruitment of leukocytes and angiogenesis. The resolution of inflammation through apoptotic and phagocytic pathways often leaves minimal damage and restores normal tissue architecture. However, common to most fibrotic conditions is the presence of a persistent irritant, which can be known agents, such as allergens, toxic chemicals, radiation, or other persistent irritants or unknown factors that trigger idiopathic pulmonary fibrosis (IPF). Indeed, a dysregulated healing response can gradually evolve into a pathogenic fibrotic response when important checkpoints are missed and inflammation becomes unrelenting. These processes can result in a local milieu rich in chemokines, pro-inflammatory, angiogenic, and fibrogenic cytokines, growth factors and tissue destructive enzymes.[1–3] This mélange of dysregulated processes can result in an increased accumulation of extracellular matrix (ECM) components and fibrotic lesions. Concurrent inflammation, tissue destruction and tissue regeneration can present a "perfect storm" of damage and regeneration.

A tightly regulated repair response following tissue injury is therefore critical. A well-coordinated influx of cells replace resident tissue cells, supply essential nutrients, and reform the tissue during a regenerative period. In some cases, this is followed by a period of fibroplasia, with too much extracellular matrix deposition and connective tissue formation. These events are often associated with vascular diseases and can give rise to many clinical conditions such as atherosclerosis, cirrhosis, scleroderma, asthma, and various types of pulmonary fibrosis. The regenerative process following tissue damage, despite having common mechanisms, can lead to various organ-specific disorders. This review will focus on pulmonary fibrotic conditions and, if known, present common regulatory mechanisms across diseases.

The prevalence and incidence of pulmonary fibrotic diseases are hard to estimate, given the vast array of clinical conditions. IPF affecting 30 in 100 000[4] with 34 000 new cases annually[5] and allergic asthma, affecting one in five in the United States; (http://www.cdc.gov/nchs/fastats/asthma.htm) although not always leading to airway remodeling and fibrosis, which are two of the most common pulmonary fibrotic diseases. In addition, there are many other fibrotic diseases of the lung including

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Received 14 November 2008; accepted 2 December 2008; published online 7 January 2009. doi:10.1038/mi.2008.85

cystic lung disease, scleroderma, radiation and chemotherapy-induced fibrosis, granulomatous lung disease, sarcoidosis and environmental, and smoking-associated COPD. These fibrotic conditions are frequently fatal, with a median survival time following diagnosis of 3–5 years in the case of IPF.[6]

MECHANISMS OF WOUND HEALING AND FIBROSIS

A wound-healing response is often described as having three distinct phases—injury, inflammation and repair (**Figure 1**). Although not all pulmonary fibrotic conditions follow this simple paradigm, it has been a useful model to elucidate the common and divergent mechanisms of pulmonary fibrosis.

Phase I: injury

Injury caused by autoimmune or allergic reactions, environmental particulates, infection or mechanical damage often results in the disruption of normal tissue architecture, initiating a healing response. Inflammation following insult, can also contribute to cellular damage and tissue destruction. Damaged epithelial and endothelial cells must be replaced to maintain barrier function and integrity and prevent blood loss, respectively. Acute damage to endothelial cells leads to the release of inflammatory mediators and initiation of an anti-fibrinolytic coagulation cascade,[7] temporarily plugging the damaged vessel with a platelet and fibrin-rich clot. Lung homogenates, epithelial cells or BAL fluid[8] from IPF patients express greater levels of the platelet-differentiating factor, X-box-binding protein-1, compared with COPD and control patients,[9] suggesting that clot-forming responses are continuously activated. In addition, thrombin (a serine protease required to convert fibrinogen into fibrin) is also readily detected within the lung and intra-alveolar spaces of several pulmonary fibrotic conditions,[10–12] further confirming the

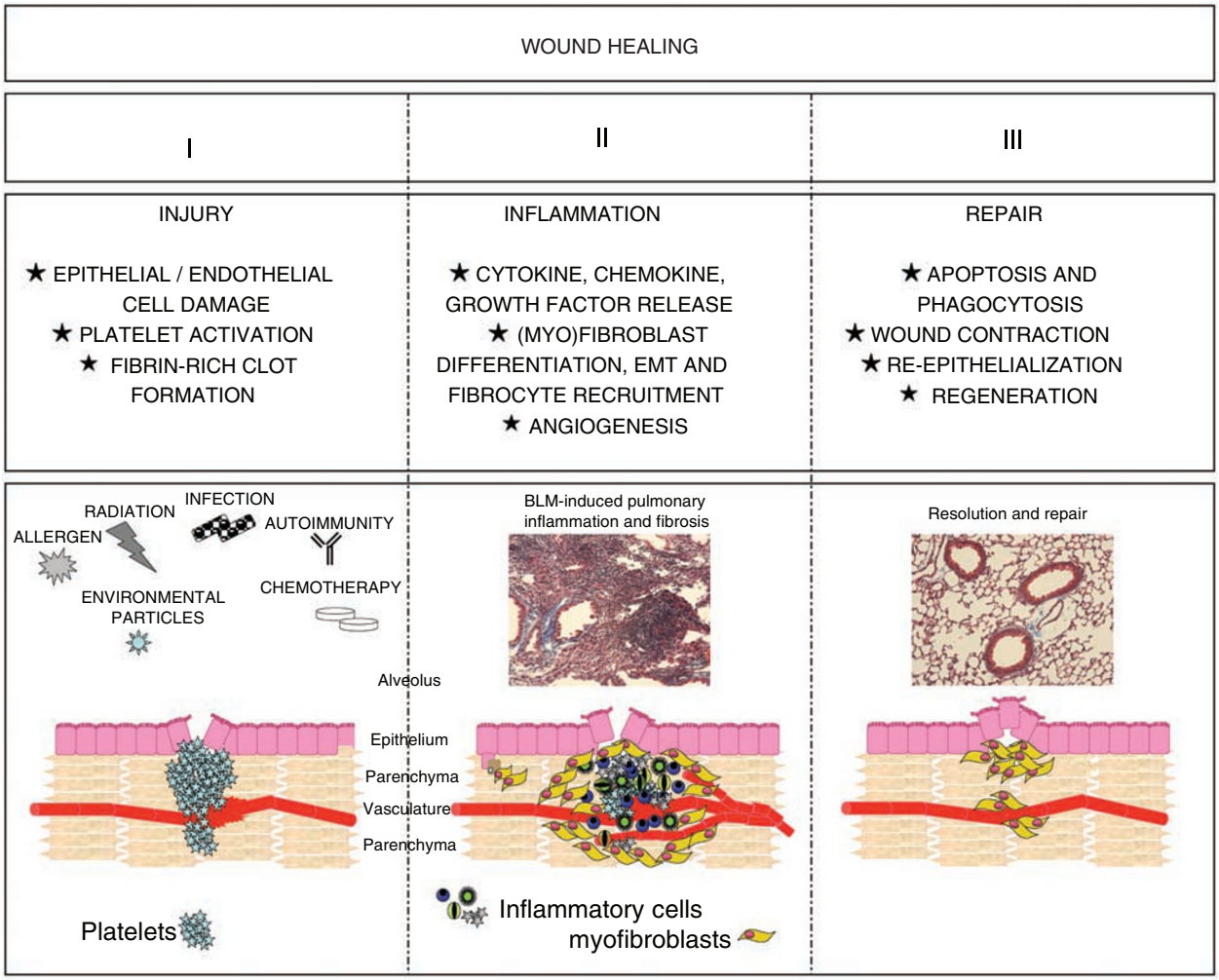

Figure 1 Phases of wound healing. A three-phase injury and wound-healing model describes distinct phases of a successful response. (1) Injury; many agents can cause pulmonary injury, including environmental particles, allergens, infectious agents, chemotherapy and radiation. Disruption of epithelial and endothelial cells initiate an anti-fibrinolytic cascade, temporarily plugging the affected tissue. (2) Inflammation; circulating inflammatory cells and fibrocytes are recruited to the injured site through chemokine gradients, supplying fibroblast-activating cytokines and growth factors. Neovascularization provides access to damaged areas and a steady stream of inflammatory, anti-inflammatory, and phagocytic cells. (3) Fibroblasts contract and decrease the size of the wound. Inflammatory cells and α-SMA+ myofibroblasts undergo apoptosis, terminating collagen deposition, and are cleared by phagocytic cells. Epithelial and endothelial cells are replaced and tissue architecture is restored.

activation of the clotting pathway. Thrombin can also directly activate fibroblasts,[13] increasing proliferation and promoting fibroblast differentiation into collagen-producing myofibroblasts.[14,15] Damage to the airway epithelium, specifically alveolar pneumocytes[16] can evoke a similar anti-fibrinolytic cascade and lead to interstitial edema, areas of acute inflammation and separation of the epithelium from the basement membrane.[17]

Platelet recruitment, degranulation and clot formation rapidly progress into a phase of vasodilation with increased permeability,[18] allowing the extravasation and direct recruitment of leukocytes to the injured site. The basement membrane, which forms the ECM underlying the epithelium and endothelium of parenchymal tissue, precludes direct access to the damaged tissue. To disrupt this physical barrier, zinc-dependent endopeptidases, also called matrix metalloproteinases (MMPs), cleave one or more ECM constituents allowing extravasation of cells into, and out of, damaged sites. Specifically, MMP-2 (gelatinase A, Type N collagenase) and MMP-9 (Gelatinase B, Type IV collagenase) cleave type N collagens and gelatin, two important constituents of the basement membrane.[19–21] In the majority of studies, but not all,[22] MMP-2 and MMP-9 are upregulated[23–26] highlighting the tissue destructive and regenerative processes common in fibrotic conditions.

The precise function of MMP-2 and MMP-9 was elegantly demonstrated in a model of allergic airway inflammation and remodeling with MMP-2$^{-/-}$, MMP-9$^{-/-}$ and MMP-2$^{-/-}$ MMP-9$^{-/-}$ double knockout mice.[27,28] In these studies, the authors demonstrated that MMP-2, and more importantly MMP-9, were required for successful egression and clearance of inflammatory cells out of the inflamed tissue and into the airspaces. In the absence of these MMPs, cells were trapped within the parenchyma of the lung and were not able to move into the airspaces, which resulted in fatal asphyxiation.

The activities of MMPs are controlled by several mechanisms including transcriptional regulation, proenzyme regulation, and specific tissue inhibitors of MMPs. The balance between MMPs and the various inhibitory mechanisms can regulate inflammation and determine the net amount of collagen deposited during the healing response.[4]

Phase II: inflammation

Once access to the site of tissue damage has been achieved, chemokine gradients recruit inflammatory cells. Neutrophils, eosinophils,[29] lymphocytes, and macrophages are observed at sites of acute injury with cell debris and areas of necrosis cleared by phagocytes. The influence of specific inflammatory cells on downstream fibrosis, particularly in IPF, is controversial ([30–32] and was recently reviewed[33]). One school of thought stems from the observation that anti-inflammatory agents have little efficacy in the treatment of IPF[34–36] and usual interstitial pneumonia patients. Based on these observations, many investigators have suggested that inflammation *per se* may not be a contributing factor in fibrosis. However, we believe the controversy reflects our limited knowledge and insight into the causative agent(s) and mechanisms involved in IPF. The timing of inflammatory events may determine the role played by the inflammatory process. Early inflammation that is diminished at the later stages of disease may promote wound healing and may contribute to fibrosis. For example the early recruitment of eosinophils, neutrophils, lymphocytes, and macrophages providing inflammatory cytokines and chemokines can contribute to local TGFβ and IL-13.[37–41] However, following the initial insult and wave of inflammatory cells, a late-stage recruitment of inflammatory cells may assist in phagocytosis, clear cell debris, and control excessive cellular proliferation, which together may contribute to normal healing. Thus late-stage inflammation may in fact serve an anti-fibrotic role and could be required for successful resolution of wound-healing responses. For example a late-phase inflammatory profile rich in phagocytic macrophages,[42] assisting in fibroblast clearance, in addition to IL-10-secreting regulatory T cells, suppressing local chemokine production and TGFβ,[43] may prevent excessive fibroblast activation. Thus, the absence of inflammation observed in IPF patients,[36] and interpretation that inflammation is not involved, may simply be a matter of timing. Indeed, corticosteroids that inhibit endogenous suppressive and phagocytic pathways may even be detrimental. However, It should not be forgotten that the mechanisms leading to pulmonary fibrosis are diverse, with immeasurable genetic, environmental and immunological interactions regulating the entire process.

The nature of the insult or causative agent often dictates the character of the ensuing inflammatory response. For example, exogenous stimuli like pathogen-associated molecular patterns (PAMPs) are recognized by pathogen recognition receptors, such as toll-like receptors and NOD-like receptors, and influence the response of innate cells to invading pathogens.[44] Endogenous danger signals[45] can also influence local innate cells and orchestrate the inflammatory cascade. For immunologists, classifying the type of immune response into Type-1 (Th1 cells, IFNγ, TNFα, and IgG2 antibody responses, generally considered pro-inflammatory) Type 2 (Th2 cells, IL-4, IL-5, IL-13, and IgE, generally considered as a wound-healing response) and type 17 (Th17 cells, recently associated with pro-inflammatory conditions) based upon the T helper cell-dominant cytokine responses, although often oversimplifying, allows for easier discussion.

The nature of the inflammatory response dramatically influences resident tissue cells and the ensuing inflammatory cells. Inflammatory cells themselves also propagate further inflammation through the secretion of chemokines, cytokines, and growth factors. Many cytokines are involved throughout a wound-healing and fibrotic response, with specific groups of genes activated in various conditions. For example, chronic allergic airway disease in asthmatics is commonly associated with elevated type-2 cytokine profiles (IL-4, IL-5, IL-13, IL-9, IL-3[46]) whereas IPF patients more frequently present pro-inflammatory cytokine profiles (IL-1α, IL-1β, TNFα, TGFβ, and platelet-derived growth factors (PDGF)[47]). Among many cytokines in various pulmonary fibrotic conditions, IL-4, IL-13, and TGF-β have received significant attention. Each of these cytokines can exhibit significant pro-fibrotic activity,[48–51] acting through the recruitment, activation and proliferation of fibroblasts, macrophages, and myofibroblasts.[2]

Type-2 inflammatory responses: pro-fibrotic IL-4 and IL-13. IL-4, the archetypal type-2 cytokine, has been firmly established as a pro-fibrotic cytokine and is elevated in IPF,[52] cryptogenic fibrosing alveolitis,[53] radiation-induced pneumonitis and pulmonary fibrosis[54] as well as liver fibrosis following infection with *Schistomsoma mansoni*.[55] IL-4 receptors are present on lung fibroblasts[49] with IL-4 signaling increasing extra cellular matrix proteins and collagen deposition. Surprisingly, some studies have suggested that IL-4 is superior to TGF-β1 at inducing collagen synthesis from fibroblasts.[49] Indirect mechanisms of IL-4 include its ability to promote the alternative activation of macrophages (AA-Mac), identified by the expression of arginase,[56] Fizz-1,[57] Ym-1,[58] and mannose receptors.[59] Macrophages in general have long been associated with pulmonary fibrosis. However, the precise mechanisms and functions of AA-Macs in pulmonary fibrosis are only now being dissected. AA-Macs can produce TGF-β, PDGF[60] and, through arginase upregulation, modulate polyamine and proline biosynthesis, cell growth, and collagen formation.[61] AA-Macs have been isolated and cultured from the bronchoalveolar lavage (BAL) of IPF patients,[62] with culture supernatants from these AA-Macs significantly increasing collagen production by normal human fibroblasts in a CCL18-dependent manner. Animal studies have also identified the involvement of AA-Macs in several models of fibrosis, including mice overexpressing human TGFβ in the lung,[63] in human and animal studies of dystrophic muscle fibrosis[64] and in multiple organ fibrosis following infection of IFNγR$^{-/-}$ mice with γ herpes virus.[65] Although not identified as AA-Macs, macrophages in general have long been appreciated in human[66] and animal models of pulmonary fibrosis.[67–69] Together these data suggested that direct secretion of TGFβ, PDGF and proline by AA-Macs are just a few of the many ways in which AA-Macs influence the progression of pulmonary fibrosis.

Finally, one of the most renowned properties of IL-4 is its ability to promote the differentiation of T cells into Th2 cells, providing a source of many type-2 cytokines in this inflammatory axis (IL-5, IL-9, IL-13, and IL-21). The Th2 cytokines interact in dramatic ways propagating wound healing and potentially pro-fibrotic responses. For example, IL-5 mobilizes, matures, and recruits eosinophils,[70] with IL-4 promoting TGF-β production from eosinophils.[37] IL-5 can also augment IL-13 production and increase IL-13-dependent fibrosis.[71] IL-9 can selectively recruit and activate mast cells,[72] with mast-cell-derived chymase increasing TGFβ activity and contributing to pulmonary fibrosis.[73] Mast cells can also promote fibroblast proliferation, collagen, and MMP production,[74] and may be involved in subepithelial fibrosis following allergen challenge.[75] IL-21 can also amplify Th2 pulmonary responses and IL-13-associated fibrosis by upregulating IL-4/IL-13 receptor expression. Mice deficient in the IL-21R showed reduced IL-13-dependent fibrosis following *S. mansoni* infection[76] and reduced IL-13-mediated AHR in a murine model of asthma, suggesting it may be an important regulator of Th2-driven remodeling in the lung.[77]

IL-13 shares many properties with IL-4, due to common receptor subunits (IL-4Rα), signal transduction pathways and transcription factors (STAT-6). However, recent animal studies have identified IL-4Rα-[78] and STAT-6[79]-independent IL-13-associated responses, which may involve IL-13 signaling through IL-13Rα2.[80–82] Despite the common properties between IL-4 and IL-13, IL-13 has been identified as a key fibrogenic cytokine in many fibrotic conditions ([83], reviewed in [51]) and can function independently of TGF-β.[84] IL-13 can trigger the differentiation of fibroblasts into α-smooth muscle actin (α-SMA) expressing myofibroblasts and PDGF-producing cells[85] with significant mitogenic properties. Interestingly, IL-13-mediated differentiation of fibroblasts into myofibroblasts is refractory to steroid inhibition, which may explain why steroids are not effective at inhibiting fibrosis.

Pro-fibrotic IL-13 has been widely studied in animal models, where gain of function experiments using a novel transgenic approach (overexpressing IL-13), led to subepithelial fibrosis accompanied by eosinophilic inflammation and mucus production,[86] comparable to allergen-induced airway responses. Similarly, loss of function studies, blocking or germ line deletion of IL-13 but not IL-4, reduced collagen deposition following exposure to aspergillus,[87] OVA,[88] bleomycin[89,90] and FITC.[91] It is important to note that the pro-fibrotic properties of IL-13 are not restricted to the lung, because hepatic fibrosis, following infection with *Schistomsoma mansoni* is also significantly decreased following IL-13 blockade.[91]

In vitro culture of normal human fibroblasts with normal human epithelial cells, which were pre-treated with IL-13, produced significantly more TGF-β, soluble and fibrillar collagen,[92] supporting the notion that IL-13 can both directly and indirectly promote collagen production by fibroblasts. Indeed, fibroblasts isolated from IPF patients[93] and allergic asthmatics[94] demonstrate a hyper-responsiveness to IL-13, as well as TGFβ and CCL-2, with significant interplay between these three mediators.[93] Several animal studies also propose a model where IL-13, through various receptor subunits[80,81] can induce plasminogen activator and MMP-9, enhancing the release of active TGFβ[95] and subsequent fibrosis. Together these human and animal studies indicate a coordinated and potentially combined effect of IL-13 and TGFβ on fibroblast activation and collagen deposition.[40]

Involvement of TGFβ in pulmonary fibrosis. TGFβ is derived from most cell lineages derived from the bone marrow[96] including T cells, macrophages,[97] eosinophils, and neutrophils[98] and is one of the most widely studied pro-fibrotic cytokines. The potent activity of TGFβ is regulated at the post-transcriptional level by a latency-associated protein (LAP), which keeps TGFβ in an inactive state. Dissociation of TGFβ from LAP is achieved by many agents commonly found in fibrotic conditions, including cathepsins, plasmin,[99] calpain,[100] thromombospondin,[96] integrin αvβ6,[101] and MMPs.[102] These agents trigger the release of biologically active TGFβ. Once active, TGFβ is incredibly pleiotropic with growth and chemotactic properties, stimulating fibroblast proliferation and the synthesis of extracellular matrix proteins,[50] recruiting inflammatory cells through MCP-1 (CCL2)[103] and suppressing T-cell responses. The various and often opposing functions of TGFβ are likely explained by its various sources and cellular targets.[104] The inhibitory and

suppressive properties of TGFβ were reviewed elsewhere,[105–107] whereas this review focuses on the pro-fibrotic properties of TGFβ.

Similar to the approach employed to study IL-13-mediated fibrosis in the lung,[86] active TGFβ has also been overexpressed in the lungs of mice, with the development of severe interstitial and pleural fibrosis, consisting of excess collagen deposition, extracellular matrix proteins, fibronectin, elastin, and the presence of myofibroblasts.[108] Interestingly, unlike IL-13 overexpression, TGFβ did not recruit inflammatory cells or enhance mucus secretion in the lung, suggesting that TGFβ can directly induce fibrosis in the absence of significant inflammation. Inhibiting TGFβ activity, by interfering with SMAD-mediated signaling,[109] significantly reduced dermal,[110,111] renal,[112–114] ocular,[115] and pulmonary fibrosis.[116,117] As mentioned above, TGFβ-independent[84,118,119] as well as TGFβ- and IL-13-combined mechanisms can contribute to wound healing and fibrosis. Knowledge of the precise interactions and non-redundant compensatory pathways in addition to disease-specific dominance of IL-13 and/or TGFβ could significantly improve therapeutic options.

Chemokine cocktails in the fibrotic lung. Cytokine-producing cells are efficiently recruited to sites of injury through chemokine gradients. Many chemokine gradients develop during wound-healing responses, each recruiting specific chemokine receptor-bearing cells; however, the CC and CXC chemokine families have received considerable attention in fibrotic responses. For example, eosinophils bearing CCR3 and following CCL11 (Eotaxin) gradients and neutrophils, macrophages and monocytes, bearing CCR2 and following IL-8 (KC in mice), IL-17, CCL2 (MCP-1) and CCL3 (MIP1α) gradients[120] have all been implicated in pulmonary fibrosis. CCL2, CCL3, and CCL11 are themselves upregulated in pulmonary fibrotic conditions,[121–127] with gene-deficient animal models confirming their importance.[124,128,129] However, a previously underappreciated circulating cell, the fibrocyte, expressing CCR2,[130] CCR3,[131] CCR5,[132] and CCR7,[131] as well as CXCR4,[133] represents a significant population of collagen-producing cells.[134] The discovery of a rapid, ready, and plentiful supply of collagen-producing fibrocytes from the bone marrow adds a new dimension to pulmonary wound repair and fibrosis.[135,136] Currently, there are three potential origins of α-SMA$^+$ myofibroblasts in lung fibrosis; (1) resident interstitial fibroblasts differentiating into collagen-secreting and extracellular matrix producing cells; (2) a process of epithelial to mesenchymal transformation (EMT) where local epithelial cells adopt fibroblast-like properties and (3) the extravasation of circulating fibrocytes, originating from the bone marrow and differentiating in the tissue into myofibroblasts.[137]

During chronic injury, endothelial cells enter a process of vasculogenesis (*de-novo* blood vessel formation) and angiogenesis (budding of new capillary branches from existing blood vessels),[138] laying down dense vascular beds permeating fibrotic and regenerative tissue. Angiogenesis can be controlled by several angiogenic factors including vascular endothelial growth factor (VEGF), fibroblast growth factor, TGFβ, PDGF, angiopoietin 1 (Ang1) and a vast array of cytokines[139] and chemokines.[137]

In particular, CXC chemokines identified as angiogenic or angiostatic by their amino terminus, 3-aa sequence (Glu-Leu-Arg), known as the ELR motif, regulate the degree of neo-vascularization and remodeling. In general ELR$^+$CXC chemokines (CXCL1, 2, 3, 5, and 8), which bind to CXCR2, are angiogenic and ELR$^-$CXC chemokines (CXCL4, 9, 10, and 11), which bind to CXCR3, are angiostatic. BAL fluid from IPF patients is rich in ELR$^+$CXC chemokines with a relative downregulation of ELR$^-$CXC chemokines.[139–141] Imbalanced ELR$^+$ and ELR$^-$ CXC chemokine levels have also been observed in animal models of pulmonary fibrosis,[142–144] confirming observations made in patients.

In summary, inflammation and the recruitment of circulating granulocytes, lymphocytes, monocytes, macrophages, and fibrocytes, presents a continuous supply of pro- and anti-fibrotic players, vital for efficient wound repair but potentially deadly when not adequately controlled. Every step of this pathway requires negative feedback loops that evoke significant control over the entire process. An imbalance in chemokine production coupled with dysregulated cellular recruitment can result in an excess of pro-fibrotic IL-13 or TGFβ, a surplus of myofibroblasts, and can convert a normal wound-healing response into a pathological fibrotic reaction.

Phase III: tissue repair and contraction

The closing phase of wound healing consists of an orchestrated cellular re-organization guided by a fibrin-rich scaffold formation, wound contraction, closure and re-epithelialization. The vast majority of studies elucidating the processes involved in this phase of wound repair have come from dermal wound studies and *in vitro* systems. For this reason, we will extrapolate these studies to the lung, where possible.

Myofibroblast-derived collagens and α-SMA form the provisional extracellular matrix, with macrophage, platelet, and fibroblast-derived fibronectin[145,146] forming a fibrin scaffold. Collectively, these structures are commonly referred to as granulation tissues. Primary fibroblasts or alveolar macrophages[147] isolated from IPF patients produce significantly more fibronectin and α-SMA than control fibroblasts,[148] indicative of a state of heightened fibroblast activation. Interestingly, IPF patients undergoing steroid treatment had similar elevated levels of macrophage-derived fibronectin as IPF patients without treatment. Thus, similar to steroid resistant IL-13-mediated myofibroblast differentiation,[85] macrophage-derived fibronectin release[147] also appears to be resistant to steroid treatment, providing another reason why steroid treatment may be ineffective. From animal models, fibronectin[149,150] appears to be required for the development of pulmonary fibrosis, as mice with a specific deletion of extra type III domain of fibronectin (EDA) developed significantly less fibrosis following bleomycin administration[148] compared with their wild-type counterparts.

In addition to fibronectin, the provisional extracellular matrix consists of glycoproteins (such as PDGF[151]), glycosaminoglycans (such as Hyaluronic acid[152]), proteoglycans,[153] and elastin.[154,155]

Growth factor and TGFβ-activated fibroblasts migrate along the extracellular matrix network and repair the wound. Within skin wounds, TGFβ also induces a contractile response, regulating the orientation of collagen fibers.[156] Fibroblast to myofibroblast differentiation, as discussed above, also creates stress fibers and the neo-expression of α-SMA,[157] both of which confer the high contractile activity[158] within myofibroblasts. The attachment of myofibroblasts to the extracellular matrix at specialized sites called the "fibronexus" or "super mature focal adhesions" pull the wound together, reducing the size of the lesion during the contraction phase.[159] The degree of extracellular matrix laid down and, the quantity of activated myofibroblasts[160] determines the amount of collagen deposition. To this end, the balance of MMPs to TIMPs[161–163] and collagens to collagenases vary throughout the response, shifting from pro-synthesis and increased collagen deposition, towards a controlled balance, with no net increase in collagen. For successful wound healing, this balance often occurs when fibroblasts undergo apoptosis, inflammation begins to subside, and granulation tissue recedes, leaving a collagen-rich lesion. The removal of inflammatory cells and especially α-SMA$^+$ myofibroblasts is essential to terminate collagen deposition.[164] Interestingly, in IPF patients, the removal of fibroblasts can be delayed, with cells resistant to apoptotic signals,[165–167] despite the observation of elevated levels of the apoptosis inductor[9] and FAS-signaling molecules.[164] This relative resistance to apoptosis may potentially underlie this fibrotic disease.[160,168] However, it is important to note that several studies have also observed increased rates of collagen-secreting fibroblast and epithelial cell[169] apoptosis in IPF,[170] suggesting that yet another balance requires monitoring—that of fibroblast apoptosis and fibroblast proliferation. The signals which initiate fibroblast apoptosis in IPF, are not very well understood with several factors postulated, such as cytokine imbalances, genetic causes, and constitutive anti-apoptotic pathways[160,165,170,171] similar to some cancerous cells.

From skin studies, re-epithelialization of the wound site re-establishes barrier function and allows encapsulated cellular re-organization. Several *in vitro* and *in vivo*[172] models, using human[173] or rat[174] epithelial cells grown over a collagen matrix, or tracheal wounds *in vivo*, have identified significant stages of cell migration, proliferation,[175] and cell spreading. Rapid and dynamic motility and proliferation, with epithelial restitution from the edges of the denuded area[172,173,176] occur within hours of the initial wound. In addition, sliding sheets of epithelial cells can migrate over the injured area[177,178] assisting wound coverage. Several factors can regulate re-epithelialization with serum-derived TGFα[174] or MMP-7[179,180] (which itself is regulated by TIMP-1[181]) appearing to play significant roles.

Collectively, the degree of inflammation, angiogenesis, and amount of extracellular matrix deposition all contribute to the net collagen deposition and ultimately whether a fibrotic lesion develops. Therapeutic intervention, interfering with fibroblast activation, proliferation or apoptosis requires a thorough understanding and appreciation of all of the phases of wound repair. Although these three phases are often presented sequentially, during chronic or repeated injury these processes function in parallel, placing significant demands on regulatory mechanisms.

ETIOLOGY AND PATHOGENESIS OF COMMON PULMONARY FIBROTIC DISEASES

Alleviating symptoms is the primary concern of patients presenting pulmonary fibrosis. Understanding the etiology of pulmonary fibrosis can provide long-term symptomatic relief and possible reversal of the disease. To this end, there are currently several well-known risk factors associated with pulmonary fibrosis that will be described below. In many cases, animal models have obvious advantages in studying the regulatory mechanisms in pulmonary fibrosis and airway remodeling.

Cystic fibrosis and cystic lung disease

With regard to etiology, cystic fibrosis (CF) is unique among pulmonary fibrotic conditions and can be attributed to a single gene mutation making it the most common monogenic disease of Caucasians, affecting 1 in 2,500–4,000.[182]

CF transmembrane conductance regulator (CFTR),[183] is the genetic "Achilles heal" responsible for the disease. The CFTR protein product is a chloride channel protein found in the membrane of cells lining the lungs, as well as the liver, pancreas, intestines, reproductive tract, and skin.[184–186] However, the leading cause of mortality in humans with CF is lung disease.[184] In addition to direct effects of CFTR mutations, resulting in deficient cAMP-mediated chloride secretion across epithelia and dysfunctional mucus regulation, CF patients are prone to progressive pulmonary damage, submucosal inflammation, and increased susceptibility to bacterial infection.[187] Long-term aerosolized antibiotics may limit bacterial colonization;[188–190] however, a consequence of chronic infection is recurring lung injury, chronic inflammation,[191,192] airway remodeling,[193] and fibrosis. The chronic inflammatory response, in particular the neutrophilic response, is a significant feature driving pathology in CF. Gaggar *et al.*[194] recently identified that neutrophil elastase, an enzyme that is significantly elevated in BAL fluid from CF patients, can promote pro-MMP9 and inhibit TIMP-1, thereby disrupting the protease/anti-protease balance.[194–196] In addition, epithelial cell regeneration and repair may also be disrupted, accounting for altered lung physiology in CF.[197] Several *cftr* $^{-/-}$ mice have been developed with varying degrees of lung disease, identifying CF-modifying genes within different founding lines.[198–200] The monumental task of developing a mouse that spontaneously develops lung disease was achieved, allowing the pathophysiological dissection of murine CF. *cftr* $^{-/-}$ mice develop parenchymal interstitial thickening and fibrosis with granulocyte influx, fibroblast infiltration and the deposition of matrix proteins.[199] The development of a mouse model of CF has allowed interesting studies addressing anti-inflammatory responses,[201] the involvement of modifier genes,[202,203] the impact of bacterial infection[204,205] and multi-organ complications.[206]

Radiation and chemotherapy-induced lung injury

Thoracic radiation therapy (RT) is used to treat lung, eosophageal, breast, and lymphoid cancers. However, a common

dose-limiting complication of RT is the development of pulmonary interstitial injury and inflammation, often referred to as radiation pneumonitis and emergence of fibrotic foci.[207–209] Multiple mechanisms have been identified in RT-induced fibrosis, including alveolar damage,[210] increased reactive oxygen species (ROS) and the toxic effects of ROS on parenchymal cells,[211,212] disruption of proliferation-associated transcription factors,[213] and the influx of inflammatory cells, such as macrophages and lymphocytes.[214,215] Furthermore, dysregulated pro-inflammatory and pro-fibrotic cytokines, TGFβ, IL-6, MMPs,[216–220] and chemokines,[221] in addition to reduced anti-inflammatory cytokines following radiation[222] can further exacerbate the inflammatory and wound-healing response. Animal models have revealed genetic determinants of RT-induced fibrosis[213,223] corresponding with similar genotype-related associations in humans.[224] Collectively, RT of the thoracic region can cause significant damage to radiation-sensitive alveolar regions of the lung invoking a dysregulated inflammatory cascade, rich in pro-inflammatory and pro-fibrotic mediators. Dysregulated chemokines, transcription factors, and anti-inflammatory pathways can further compound this uncontrolled response, leading to pulmonary fibrosis.

Similar to radiation therapy, chemotherapy can cause lung injury with variable consequences depending on dose rate, duration, pre-existing lung disease, and concomitant steroid use.[225,226] The *Streptomyces verticullatus*-derived antibiotic, bleomycin (BLM),[227] is effective against squamous cell carcinomas and skin tumors;[228] however, like RT, an unfavorable side effect involves inflammatory and fibrotic responses in the lung. BLM-induced inflammation occurs in up to 46% of patients treated[229] with complications in the lung and skin due to a lack of the endogenous bleomycin-inactivating enzyme, bleomycin hydrolase, in these tissues.[230]

Our understanding of BLM-induced fibrosis has been assisted by the development of animal models, which reproduce many, but not all, of the characteristics of the human disease.[230] BLM can directly cause cell death[231] and reduce O_2 into free radicals, causing DNA breakage.[232] Depending upon the route of administration, epithelial and endothelial cells are some of the earliest cells affected,[233] causing a leukocyte-rich inflammatory response. Blockade of this inflammatory response in animal models, with anti-CD11 Ab-inhibiting cellular extravasation, dramatically reduced pulmonary collagen and fibrosis, demonstrating the significant contribution of inflammatory cells on the resulting fibrotic response.[234] The inflammatory cytokines, TNFα,[235] IL-1β,[236] IL-6[237] and pro-fibrotic TGFβ[238,239] are accompanied by FAS-L-expressing cells, leading to more apoptosis.[16,240] Blockade of TNFα, IL-1, FAS-Ligand or TGFβ can reduce the inflammatory and resultant fibrotic response following BLM administration.[235,240–242] Thus, TNFα, IL-1, IL-6, and TGFβ are some of the possibly many mediators involved in BLM-induced fibrosis. The BLM model has been used to dissect the involvement of many cytokines in the pulmonary fibrotic response. The involvement of type-2 cytokines is less clear, with IL-4 and IL-5 playing no significant role,[243–245] whereas IL-13, either directly[90] or indirectly through TGFβ,[80,81] contributes to the fibrotic response. There is also evidence that Type-1 cytokines are involved,[246] with fewer inflammatory cells, lung hydroxyproline content, weight loss, and mortality observed in IFNγ$^{-/-}$ mice.[247] Blocking the IFNγ-promoting cytokine IL-12 or germ line deletion of IL-12[248] yielded similar results.[248] BLM, although invoking a significant inflammatory response, can also promote fibroblast proliferation[249] and TGFβ production from endothelial cells[250] directly. Thus, BLM appears to have multiple properties, directly causing cell death and apoptosis, invoking an inflammatory response and promoting fibroblast proliferation and TGFβ production. For these reasons, the mouse model of BLM-induced fibrosis provides a great tool to dissect the relative contribution of the many pathways, cells, and mediators involved in drug-induced fibrosis.

Asthma and allergic airway inflammation

The number of individuals suffering from allergic airway inflammation and asthma has seen an unprecedented growth over the past 30 years, particularly within the urban areas of both developed and developing countries.[251] Allergic asthma is a polygenic disease,[252] characterized by allergen-specific IgE and IgG1, airway and interstitial eosinophilia, mucus secretion and airway hyper-reactivity.[253] Chronic asthma with repeated bouts of allergen exposure and dysregulated inflammation at mucosal surfaces can lead to goblet cell hyperplasia, smooth muscle hypertrophy and hyperplasia, angiogenesis and ultimately subepithelial fibrosis.[254–257]

CD4$^+$ Th2 cells orchestrate many aspects of the allergic inflammation, driven by dendritic cell or basophil-derived IL-4 and IL-25.[258–263] Activation and egression of cytokine-secreting Th2 cells into the interstitium and mucosal surfaces of the lung propagate local cellular influx. More specifically, Th2-derived cytokines, IL-5 and IL-9, mobilize, mature and recruit eosinophils and mast cells[70,264,265] into the tissue and airspaces, and these cells are typically found in biopsies of asthmatic individuals. TGFβ is also significantly elevated in human asthmatics[41,266–270] with the degree of subepithelial fibrosis correlating with a loss of forced expiratory volume (FEV_1). These observations of increased eosinophils, TGFβ and subepithelial fibrosis led Flood-page *et al.*[271] to study the specific cellular source of TGFβ. Indeed, 86% of TGFβ mRNA$^+$ cells in the bronchial mucosa of asthmatics were eosinophils, distinguishing eosinophils as a significant source of pro-fibrotic TGFβ in the allergic lung.[39] Furthermore, several studies have identified correlated collagen deposition with increased numbers of tissue eosinophils and myofibroblasts[19,38] as well as the expression of submucosal MMP9 and MMP12.[272]

These observations have led to several clinical trials and treatment regimens using anti-IL-5 antibodies to block tissue eosinophilia with few successes. Treatment of allergic asthmatic patients, as well as atopic dermatitis patients,[273] with anti-IL-5 antibodies (mepolizumab) led to significant reductions in tissue eosinophilia,[271,274] despite no change in late-phase cutaneous allergic reactions. Most striking was a reduced thickness and density of the extracellular matrix (tenascin, lumican, and procollagen III (COL3A)) following anti-IL-5 treatment, suggesting

that IL-5-mediated tissue eosinophilia was indeed responsible for ECM deposition. However, despite these encouraging results, the precise role and involvement of eosinophils in human asthma is debated, with many clinical trials of anti-IL-5 mAb reporting little to no clinical improvement.[275,276]

Animal studies, using either IL-5-deficient mice[277] or eosinophil-ablated mice[278,279] have supported a significant role for eosinophils, with reduced airway remodeling, including peribronchial fibrosis and smooth muscle thickness, in addition to several other features of allergic asthma following chronic airway exposure. Similarly, blocking TGFβ[280] or interfering with TGFβ signaling[281] could also significantly attenuate airway remodeling following chronic allergen exposure.

Taken together, animal models have demonstrated a clear role for eosinophils and eosinophil-derived TGFβ in airway damage and remodeling. Human studies, however, have produced a spectrum of results and require additional studies, with well-defined end points to address the role of IL-5 and eosinophils in the progression and resolution of subepithelial fibrosis in asthmatic airways.

IL-13 may also be a damaging cytokine in allergic individuals. Many of the pathological conditions identified in allergic asthmatics can be traced to IL-13. For example, IL-13 can mediate goblet cell hyperplasia in local epithelia[282] and increase mucus production[92] that can block the small airways.[283,284] IL-13 can also promote epithelial repair,[285,286] fibroblast growth,[85,287] EMT,[288] and collagen deposition.[92] Beyond the airway epithelium, IL-13 also causes smooth muscle hyperplasia[289] and subepithelial fibrosis.[88] Similar to mechanisms proposed using the bleomycin model, IL-13 can synergize with and promote profibrotic TGFβ[290,291] eotaxin production,[40] and TIMP expression.[292] Thus, within the context of allergic asthma, eosinophils, TGFβ, and IL-13 may all contribute to airway remodeling and pulmonary fibrosis.

Less common pulmonary fibrotic conditions with known etiologies

Environmental particulates from smoking or occupational exposure can have toxic effects on the mucosal surfaces of the lung. For example, jobs that involve mining or that expose workers to asbestos, metal dusts, or silica dust can cause pulmonary fibrosis.[293] Agricultural workers can also be affected,[293] with exposure to organic and inorganic substances,[294,295] fumes,[296] or moldy hay[297] causing allergic inflammation and fibrosis, often referred to as Farmer's Lung.[298–300] Granulomatous lung disease and sarcoidosis is less common, with a global incidence of 16.5–19/100,000.[301] These diseases are significantly influenced by genetic and environmental factors. To date, the causative agents have not been identified.[302] An alveolar macrophage gene-transcript profile[303] that is similar to *Mycobacterium tuberculosis* infection has led to the hypothesis that bacteria may be involved. However, to date, bacteria have not been isolated from sarcoidosis patients. Chronic inflammation and the development of inflammatory cell-rich pulmonary granulomas,[304,305] rich in type-1 cytokines and chemokines[122,306–309] and T cells[310] can dramatically disrupt parenchymal architecture, endothelial cells and the alveolar spaces of sarcoidosis patients. Immunohistochemical analysis of human and animal lung biopsies and post-mortem histological sections have identified elevated collagen and fibronectin in granulomas of sarcoidosis patients.[305] Furthermore, co-expression of pro-fibrotic TGFβ within the granulomas was also observed in sarcoidosis granulomas.[311–313] Despite these varying etiologies, recurring lung injury and inflammation[314] is common to many of these fibrotic conditions, and may broadly underlie the pathogenesis of pulmonary fibrosis.

Idiopathic pulmonary fibrosis

When all known causes of interstitial lung disease and fibrosis have been ruled out, the condition is referred to as "idiopathic" (of unknown origin) pulmonary fibrosis (IPF). Despite an unknown etiology, there are a number of conditions and risk factors associated with the disease including; smoking,[315] farming, and occupational hazards[316,317] and viral, and bacterial infections.[318–320] Furthermore, in one study, IPF patients had a greater propensity to develop primary lung cancer, compared with non-IPF patients with chronic lung disease or patients without lung disease.[321] Reports of familial aggregation of IPF also suggest that there may be a genetic component to IPF.[322,323] As mentioned above, the incidence of IPF in the United States is 30/100 000[4] and 34 000 new cases annually,[5] with a similar increasing incidence of IPF in the United Kingdom.[324]

IPF is characterized by usual interstitial pneumonitis[325] and progressive interstitial fibrosis caused by excessive extracellular matrix deposition. Regions of fibroblast and myofibroblast accumulation, specifically between the vascular endothelium and alveolar epithelium disrupt the architecture of the lung, giving a "honeycomb" appearance.[321] The pathogenesis of IPF has been debated for many years with two different schools of thought. One group suggests an inflammatory stimulus is involved, with recurring inflammation leading to immunopathology, tissue destruction and the propagation of a wound-healing response.[32,36,326,327] Others suggest a slightly different pathogenic mechanism in which an initial or absent inflammatory stage is quickly followed by an uncontrolled wound-healing response.[30,328] Central to the argument negating the dominant role of inflammation is the inefficiency of corticosteroids and other anti-inflammatory agents to control IPF[34,35] despite some reports of enhanced survival.[329] Furthermore, the ability of epithelial cell-derived TGFβ[330,331] to invoke a fibrotic cascade with increased interstitial collagen and fibroblast proliferation in the absence of inflammation further support these views. We believe the controversy reflects our limited knowledge and insight into the causative agent(s) and pathogenesis of IPF. A common, and accepted view is the early role of inflammatory events, initiating a wound-healing response. Whether the dysregulated wound-healing response continues in the absence of subsequent inflammation or not has yet to be clarified. Continuous chemokine and cytokine production[47] in diagnosed IPF patients indicates that damage and subsequent inflammation may be ongoing.

The cytokine profile from biopsy or BAL-derived cells or BAL fluid of IPF patients is rich in pro-inflammatory cytokines; IL-1β,[332,333] IL-8,[122] IL-18,[334] TNFα,[335] MCP-1[122,336]as well as Type-2 cytokines, and their receptors.[337,338] The mixed cytokine profile, derived primarily from inflammatory cells[67] and leukocytes,[33,339] can have significant effects on all aspects of wound healing including vascular remodeling, myofibroblast differentiation, EMT, TGFβ, and IL-13 production. In addition to the direct fibroblast-activating properties of TGFβ and IL-13, co-expression of these two cytokines in IPF has been observed.[93] Fibroblast hyperplasia[170] and the reduced expression of apoptotic mechanisms (bcl-2 and membrane FAS-L)[340] in IPF can further augment the fibrotic response. Collectively, a cascade of failed regulatory mechanisms and hyper-secretion of cytokines, chemokines and growth factors,[47] culminates in an out-of-control fibroblast-mediated wound-healing response. Physiologically, IPF can dramatically compromise oxygen diffusion, lung function[341] and is typically a fatal disease.

REGULATION OF PULMONARY FIBROSIS

It is becoming clear that an imbalance of stimulatory cytokines, chemokines, and growth factors likely over-activate resident parenchymal and circulating cells and may underlie the over exuberant wound-healing responses that lead to fibrosis. In a normally controlled cellular response, negative feed back loops, anti-inflammatory molecules, inhibitory receptors, and apoptotic pathways operate to fine tune and terminate responses once a desired outcome is achieved. Common to many pulmonary fibrotic conditions with both known and unknown etiologies may be a break down in these regulatory mechanisms, resulting in an excessive inflammatory cascade, neo-vascularization, uncontrolled fibroblast activation, and fibrosis. In this section of the review we will highlight some of these endogenous regulatory mechanisms that either operate endogenously or can be exploited therapeutically to counter balance the uncontrolled responses.

Regulation of inflammatory responses: Tregs and IL-10

T cells with the primary function of attenuating immune cell activation and proliferation are frequently referred to as regulatory T cells (Treg). Although the specific details of antigen specificity, precise mechanisms of suppression, and distinguishing features continue to grow, their role in fibrotic responses have been under studied. However, given their ability to dampen inflammatory responses, the ability of Tregs to interfere with upstream events and slow the progression of fibrosis has been implied. In particular Treg-derived IL-10, and other surface molecules[342] although not exclusively derived from Tregs, can function as a general immunosuppressant[343] and control fibrosis.[342] Polymorphisms in the signal sequence of the IL-10 gene have been identified in IPF patients, corresponding to reduced IL-10 production, suggesting that endogenous anti-inflammatory mechanisms may be impaired in this condition. Supporting this notion, loss of function studies using LPS-induced lung injury and fibrosis in IL-10-deficient mice led to significantly stronger inflammatory responses with greater subepithelial thickening and extracelular matrix protein content.[344] In gain of function studies, induction of IL-10 significantly reduced collagen deposition following bleomycin administration in murine models.[345] Following IL-10 gene delivery BAL fluid TNFα and neutrophil-derived MPO levels were significantly reduced, with another similar study observing reduced macrophage-derived TGFβ,[43] suggesting that IL-10 inhibits inflammatory cell recruitment.[345] Corroborating these findings, Dosanjh *et al*.[346] reported higher levels of IL-8, with reduced IL-10 levels in the BAL fluid of cystic fibrosis patients. Thus, IL-10 can attenuate the inflammatory events upstream of the fibrotic pathway. In addition to suppressing inflammatory events, IL-10 can act directly on fibroblasts, reducing TGFβ-induced collagen production.[345] Following lung injury in a rat model of radiation-induced fibrosis, pneumocytes in the epithelial layer of the lung had reduced expression of IL-10, compared to control lungs, which may permit greater local inflammation.[222] Thus, IL-10 immunotherapy, with a sound understanding of timing and when to dampen inflammatory events may hold promise for pulmonary fibrotic conditions.[347] Beyond the lung, IL-10 has been shown to regulate kidney[348] and liver[349] inflammation, and fibrosis.[350] Therapeutically manipulating IL-10, in particular endogenous IL-10-producing cells which may be present but in too low frequencies to significantly halt the inflammatory onslaught, may be a useful avenue to pursue. This has been demonstrated successfully in models of allergic airway inflammation, where a reduction in airway and tissue inflammation, mucus production, and airway hyper-responsiveness was observed.[351]

IL-13Rα2 and LAP: endogenous attenuators of fibrosis

As discussed throughout this review, TGFβ and IL-13[51,84,85,352,353] are dominant pro-fibrotic cytokines, activating fibroblasts, and promoting differentiation into α-SMA-producing myofibroblasts and collagen production. Thus, tight regulation and fine-tuning of these two potent molecules is essential. Two molecules that can serve this very purpose are the IL-13Rα2, an endogenous decoy receptor that attenuates IL-13 activity and, as discussed earlier, LAP, a latency-associated protein, which keeps TGFβ in an inactive state. To our knowledge there are no studies to date reporting the specific induction of endogenous LAP to attenuate TGFβ bioactivity; however, introduction of exogenous recombinant LAP could theoretically be used to attenuate TGFβ activity[354] (similar to anti-TGFβ antibody[280]). Upregulation of a TGFβ-binding protein, endoglin, however has been observed in animal models of renal fibrosis.[355] Exploiting this pathway to attenuate TGFβ may be another option. Although the exact mechanism is unknown, the introduction of taurine and niacin into the diet of small rodents attenuates BLM-induced fibrosis, apparently by reducing TGFβ production,[356] suggesting that dietary supplements may be useful therapeutics. Disrupting TGFβ-associated ROS[357] or other downstream TGFβ-signaling pathways[358] also hold promise.

IL-13Rα2 is expressed predominantly by non-hematopoietic cells (unpublished observations) and attenuates IL-13 activity

in vivo.[359] Given that IL-13 can act at multiple stages of the inflammatory and wound-healing response,[51,92,284,288,358,360] it comes as little surprise that attenuation of IL-13 can have profound effects on the degree of pulmonary inflammation and fibrosis in many pulmonary disease models.[88,90,361] Several methods of IL-13 attenuation have been described, including neutralizing Abs,[87] treatment with sIL-13Rα2,[359,362–364] or targeting the IL-13Rα2-expressing cells. The conclusions from all of these studies indicate that targeting the IL-13 pathway holds great promise for the treatment of fibrosis.

Resetting the imbalance

An imbalance of cytokines, chemokines or cells can disrupt many downstream processes (**Figure 2**). For example, an imbalance between collagen-catabolizing MMPs and their specific inhibitors, TIMPs, can result in excessive collagen breakdown. However, they can also promote TGFβ activation in peripheral cells.[365–367] Increased TGFβ can further feed back to induce more MMPs[368] and promote EMT.[368] Thus, a breakdown in one process (MMP production) can quickly catalyze and disrupt other regulatory mechanisms (TGFβ responses). Within mammalian systems, a refined balance between "on" and "off" signals is critical to maintain homeostasis. In a dysregulated wound-healing response several key mechanisms appear to be off balance (**Figure 2**).

(1) *Inflammation* "*vs.*" *Immunosuppression*. Excessive or recurring inflammatory events can cause excessive wound-healing responses that lead to the development of fibrosis. Either eliminating the causative agent, such as allergen avoidance, or treatments with anti-inflammatory agents such as corticosteroids may help restore the balance.

(2) *MMP* "*vs.*" *TIMP*. MMPs can disrupt the basement membrane and allow the influx of inflammatory cells. Inhibiting MMP activity could be detrimental in immunity and in the process of re-epithelialization;[369] however, in pathological fibrotic responses, neutralization of specific MMPs either with small molecules,[157] inhibitors[157] or by influencing TIMP expression may help restore this imbalance.

(3) *Fibroblast apoptosis* "*vs.*" *proliferation*. The late-stage apoptosis of fibroblasts is required for successful wound healing

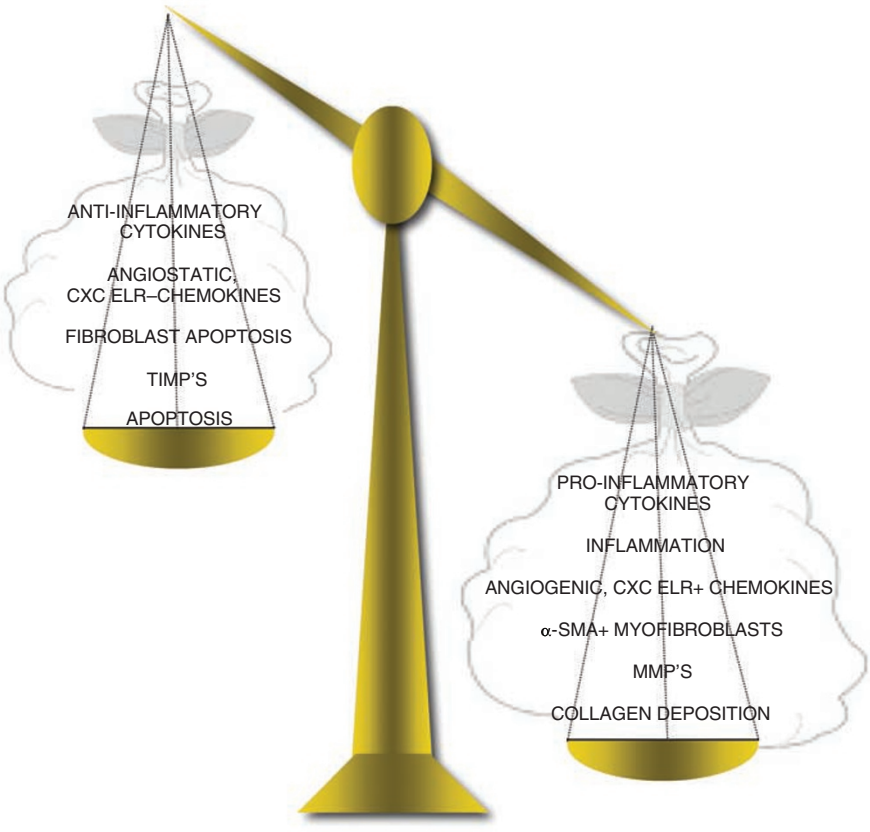

Figure 2 Imbalanced wound-healing response. For successful wound healing, a regulated response is maintained through negative feedback loops and a balance of catabolising and regenerative processes. Several imbalances may develop and lead a normal healing response into a fibrotic cascade. Excessive inflammation and the production of inflammatory and fibroblast-activating cytokines, through a breakdown in anti-inflammatory mechanisms can develop. Over-production of angiogenic CXC ELR$^+$ chemokines, the recruitment of fibrocytes and increased frequency of α-SMA+ cells in the injury site can result in too much collagen deposition. Resetting the balance with targeted therapeutics (i.e., cytokine-blocking antibodies) may help slow the progression of fibrosis.

and termination of collagen deposition. As mentioned above, resistance to apoptosis has been observed in fibroblasts from IPF patients.[165–167] Restoring fibroblast apoptotic pathways or selectively depleting fibroblasts at the appropriate time may help slow the progression of fibrosis. Modulating local cytokine and growth factor levels could also influence fibroblast proliferation and activation indirectly.

(4) *ELR$^+$ "vs." ELR$^-$ CXC chemokines.* The prolonged induction ELR$^+$ chemokines, due to inflammatory signals can lead to excessive vascularization. Anti-angiogenic therapy,[370] an area actively pursued in cancer therapy was recently investigated in fibrotic conditions.[371] Inhibiting VEGF or promoting endostatin and anastellin (endogenous inhibitors of angiogenesis) may limit inflammation and the recruitment of myofibroblasts. Neutralizing angiogenic ELR$^+$ CXC chemokines or enhancing angiostatic ELR$^-$ CXC chemokines,[372–374] in combination with other therapeutic interventions, may also dramatically halt the inflammatory cascade and avoid the requirements for angiogenesis.

CONCLUSION

Pulmonary wound repair is an extremely dynamic process intersecting immunology, structural biology, and airway physiology. For successful repair a collaborative effort between these systems is essential. Dysregulation in one response can have ripple effects on others and progressively turn a well-choreographed healing response into a fibrotic lesion. Vascular damage must be quickly repaired with a fibrin-rich clot. This is followed by an influx of inflammatory cells. Chronic or recurring inflammation requires rapid resolution to avert immunopathology while providing the necessary cellular participants. Parenchymal cells that are responsive to inflammatory cues must proliferate and migrate into the damaged area, restore tissue architecture, with the inflammatory cells ultimately undergoing apoptosis to prevent excessive collagen deposition.

In pulmonary fibrotic disease states, the development and progression of the healing response has slipped out of control, disrupting many delicate balances. As discussed in this review, there are a number of compensatory and redundant processes, which all contribute to proficient healing and remodeling. With this in mind, and despite significant advances in our understanding of these pathways and balances, there is a lack of therapeutic intervention and new therapies for pulmonary fibrosis. Further studies are required to elucidate the roles of the many mediators (cytokines, chemokines and growth factors) observed in both human and animal models of pulmonary fibrosis. Greater still, pre-clinical and clinical investigations with chemokine receptor antagonists, angiogenesis inhibitors and Abs to the pro-fibrotic molecules IL-13 and TGFβ are required. A combined effort by clinicians and lab researchers across platforms and disciplines could make this a climbable mountain.

DISCLOSURE

The author declared no conflict of interest.

ACKNOWLEDGMENTS

This review was improved by peer-review and funded by the Intramural Research Program at the NIH/NIAID. Owing to space and word limitations, we apologize to the many researchers whose work we have not mentioned in this review, but who have significantly contributed to our current understanding of pulmonary fibrosis. We also thank Dr Allen Cheever for helpful comments.

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