

■ R E V I E W

Eicosanoids: mediators and therapeutic targets in fibrotic lung disease

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A B S T R A C T

Fibrosis is a common end-stage sequella of a number of acute and chronic lung diseases. Current concepts of pathogenesis implicate dysregulated interactions between epithelial cells and mesenchymal cells. Although investigative efforts have documented important roles for cytokines and growth factors in the pathogenesis of fibrotic lung diseases, these observations have not as yet been translated into efficacious therapies, and there is a pressing need for new pathogenetic insights and therapeutic approaches for these devastating disorders. Eicosanoids are lipid mediators derived from arachidonic acid, the most studied of which are the prostaglandins and leukotrienes. Although they are primarily known for their roles in asthma, pain, fever and vascular responses, present evidence indicates that eicosanoids exert relevant effects on immune/inflammatory, as well as structural, cells pertinent to fibrogenesis. In general, leukotrienes promote, whereas prostaglandin E₂ opposes, fibrogenic responses. An imbalance of eicosanoids also exists in pulmonary fibrosis, which favours the production of leukotrienes over prostaglandin E₂. This review highlights the role of this imbalance in the evolution of fibrotic lung disease, discusses the mechanisms by which it may arise and considers approaches for therapeutic targeting of eicosanoids in these conditions.

INTRODUCTION

Pulmonary fibrosis represents an end-stage sequella of a myriad of acute and chronic lung diseases, resulting in significant morbidity and mortality. Although many of these diseases involve chronic inflammation, the fact that anti-inflammatory and immunosuppressive drugs are largely ineffective in fibrotic lung diseases is consistent with the emerging view that their pathogenesis involves dysfunctional fibroproliferative responses to injury, rather than merely inflammation [1]. IPF (idiopathic pulmonary fibrosis) is the most common of the fibrotic lung diseases. As no effective treatment exists for this progressive disorder, there is a conspicuous need for pathogenetic insights with therapeutic potential. Although research

into the development of fibrotic lung disease has centred on the participation of polypeptide mediators, such as cytokines and growth factors, a growing body of evidence supports a potentially pivotal role for lipid mediators. Metabolism of AA (arachidonic acid) yields a family of bioactive lipids called eicosanoids, the best known of which are the LTs (leukotrienes) and PGs (prostaglandins). The generation of eicosanoids and their interactions with cellular receptors result in a host of responses in target cells and tissues which are central to normal homeostasis, as well as to the pathogenesis of many disease states [2]. Because of their long-recognized roles in pain, platelet aggregation, microvascular permeability, smooth muscle contraction and inflammation, eicosanoids have been traditionally linked with diseases

Key words: cyclo-oxygenase, fibrotic lung disease, leukotriene, 5-lipoxygenase, prostaglandin, pulmonary fibrosis.

Abbreviations: AA, arachidonic acid; AEC, alveolar epithelial cell; AM, alveolar macrophage; BAL, bronchoalveolar lavage; COX, cyclo-oxygenase; cysLT, cysteinyl leukotriene; EP receptor, E prostanoid receptor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; IPF, idiopathic pulmonary fibrosis; LO, lipoxygenase; LT, leukotriene; MCP, monocyte chemotactic protein; PG, prostaglandin; PGI₂, prostacyclin; PLA₂, phospholipase A₂; TGF, transforming growth factor; Th, T-helper; TNF, tumour necrosis factor.

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such as arthritis, ischaemic cardiovascular disease and asthma. A contemporary view recognizes a broader spectrum of eicosanoid actions, including effects on immune responses, cellular proliferation and apoptosis. These actions, in turn, reflect modulation of such fundamental cellular processes as signal transduction and gene expression. Data from patients with IPF, as well as from animal models, suggest that an imbalance favouring pro-fibrogenic LTs over anti-fibrogenic PGs promotes the development of pulmonary fibrosis. This review will focus on: (i) the actions of eicosanoids relevant to pulmonary fibrosis, (ii) the evidence that abnormalities in eicosanoid production are found in fibrotic lung disease, and (iii) the therapeutic potential for interventions targeting these mediators.

CLINICAL SIGNIFICANCE AND PATHOBIOLOGY OF PULMONARY FIBROSIS

Pulmonary fibrosis occurs in the setting of chronic systemic disease (e.g. rheumatoid arthritis and scleroderma), in response to toxin exposure (e.g. asbestosis, silicosis, amiodarone and cytotoxic drugs) and in the absence of identifiable causative agents (e.g. IPF and sarcoidosis). It is also a recognized consequence of acute respiratory distress syndrome. Although these disorders might vary in their histopathological patterns and development, they manifest common characteristics which include breathlessness, diffuse radiographic opacities, reduced lung compliance and impaired gas exchange. Not uncommonly, pulmonary fibrosis progresses to respiratory failure and death, or is an indication for lung transplantation. It is generally thought that fibrotic lung disease represents an abnormal response (Figure 1) to some inciting injurious event, with resultant distortion of lung architecture and compromised function. Considerable investigative efforts have been focused upon IPF due to its incidence, the fact that there is no known effective therapy and because fibrosis appears to progress despite a lack of significant inflammation or an obviously persistent injurious process.

The precise role and temporal sequence of the various component abnormalities involved in the pathogenesis of pulmonary fibrosis, in general, and IPF, in particular, are unclear. However, as determined from studies of both human disease and animal models, the key pathobiological elements (Figure 1) are thought to include the following [1,3,4]: (i) expansion of populations of inflammatory and immune effector cells (macrophages, lymphocytes, neutrophils and eosinophils); (ii) a Th2 (T-helper type 2) cellular immune response characterized by production of cytokines, such as IL-4 (interleukin-4), IL-5 and IL-13, in preference to Th1 cytokines IL-2, IL-12 and IFN- γ (interferon- γ); (iii) production by activated macrophages and other effector cells of mediators, in-

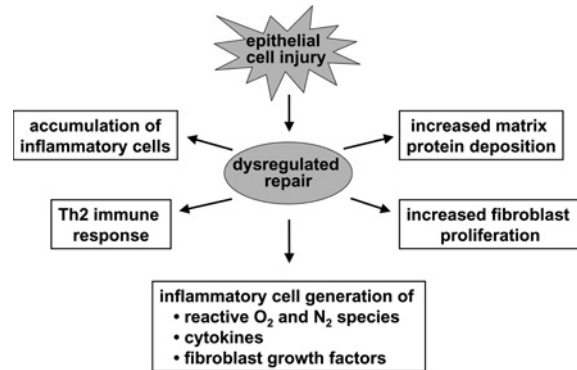


Figure 1 Key elements in the pathogenesis of fibrotic lung disease

Fibrotic lung diseases are the consequence of a variety of known and unknown inciting injurious events, resulting in epithelial cell injury and dysregulated repair. The key aspects of the dysregulated repair process are depicted within the open boxes.

cluding reactive oxygen intermediates, cytokines [such as TNF- α (tumour necrosis factor- α)], chemokines [such as IL-8 and MCP-1 (monocyte chemotactic protein 1)], and fibroblast growth and differentiation factors [such as platelet-derived growth factor, basic fibroblast growth factor and TGF- β (transforming growth factor- β)]; (iv) acquisition by fibroblasts of altered phenotypes characterized by dysregulated proliferation and differentiation to smooth muscle actin-positive myofibroblasts capable of excessive collagen synthesis; and (v) loss of the normally suppressive signals for fibroblasts produced by AECs (alveolar epithelial cells) due to epithelial cell loss or injury.

EICOSANOIDS AND THEIR SYNTHESIS

The initial step in the eicosanoid biosynthetic pathway [2] (Figure 2) involves activation of PLA₂ (phospholipase A₂) enzyme, which hydrolyses AA from membrane phospholipids. This is generally a consequence of an increase in intracellular calcium, which can be triggered by a number of endogenous and exogenous substances. Once liberated, free AA is converted into a variety of oxygenated metabolites by several parallel metabolic pathways, the most studied of which are the COX (cyclooxygenase) and 5-LO (5-lipoxygenase) pathways. The former gives rise to the prostanoids [PGs, PGI₂ (prostacyclin) and thromboxane A₂], whereas the latter yields the LTs and 5-hydroxyeicosatetraenoic acid. The COX enzyme exists in two specific isoforms, the constitutively expressed COX-1 isoform and the inducible COX-2 isoform. COX converts AA to an unstable intermediate, PGH₂, which is then metabolized into the various bioactive prostanoids by their corresponding terminal synthases. Alternatively, 5-LO, acting in concert with 5-LO-activating protein, converts AA into LTA₄. This

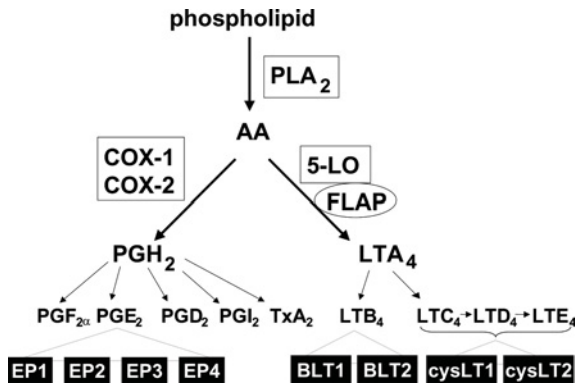


Figure 2 Biosynthetic pathways and receptors for LTs and prostanoids

The distal enzyme responsible for converting PGH_2 into a given prostanoid (not shown) is called the relevant 'prostanoid synthase'. The distal enzymes responsible for converting LTA_4 into LTB_4 and LTC_4 (not shown) are called 'LTA₄ hydrolase' and 'LTC₄ synthase' respectively. Each of the terminal eicosanoids acts by ligating one or more G-protein-coupled receptors; the receptors for PGE_2 , LTB_4 and cysLTs only are depicted in closed boxes. FLAP, 5-LO-activating protein; TxA_2 , thromboxane A_2 .

unstable intermediate can be hydrolysed by LTA_4 hydrolase to form LTB_4 , or conjugated with GSH to form LTC_4 by a glutathione transferase, LTC_4 synthase. Once secreted from a cell, LTC_4 can be metabolized into LTD_4 and then LTE_4 . Due to their similar structures and biological functions, LTC_4 , LTD_4 and LTE_4 are collectively named cysLTs (cysteinyl LTs).

Whereas both leucocytes and structural cells synthesize prostanoids, LT synthesis is confined primarily to leucocytes. PGE_2 is the major prostanoid product of epithelial cells, fibroblasts and smooth muscle cells, and PGD_2 is the major prostanoid product of mast cells. With respect to LTs, eosinophils and mast cells synthesize primarily LTC_4 , neutrophils synthesize primarily LTB_4 and macrophages synthesize both. The cellular capacity for eicosanoid synthesis can be regulated in a number of ways. Protein expression can be modulated at transcriptional or translational steps. Catalytic efficiency of enzyme molecules can be influenced by post-translational modifications, such as phosphorylation, or by alterations in the level of essential enzyme cofactors. Intracellular localization of key synthetic enzymes, such as PLA_2 and 5-LO, can also affect their function [5]. Whereas synthesis of protein mediators, such as cytokines, typically requires a lag phase of several hours or more, eicosanoid synthesis can proceed within minutes, because many of the requisite enzymes are expressed constitutively. However, the fact that eicosanoid synthesis can be further amplified by increases in expression of eicosanoid-forming enzymes also permits sustained generation or delayed bursts of synthesis. Eicosanoids are therefore unique among mediators in their ability to participate in both immediate and delayed phases of various responses.

EICOSANOID RECEPTORS AND SIGNAL TRANSDUCTION MECHANISMS

Eicosanoids mediate their biological actions through the binding to specific seven-transmembrane G-protein-coupled receptors on target cells (Figure 2). Receptors for most of the major eicosanoids were initially characterized using classic pharmacological techniques and many have now been cloned [6]. Ligation of these receptors evokes signal transduction events, including alterations in intracellular calcium and cAMP concentrations, in turn influencing the activation of a diverse range of protein kinases. Eicosanoid receptors are primarily distributed on the plasma membrane, although nuclear membrane localization has also been reported [7].

The receptors for a given eicosanoid can be encoded by multiple genes. For example, there are four distinct receptors for PGE_2 [termed the EP (E prostanoid) receptors], which are coupled to the generation of different second messengers [6]. Two receptors for cysLTs (cysLT1 and cysLT2) and LTB_4 (BLT1 and BLT2) have also been identified that vary in their ligand specificity and tissue distribution [8–11]. In general, the opposing actions of LTs and PGE_2 pertinent to fibrosis reflect their activation of opposing signal transduction pathways. The ligation of LT receptors results in increases in intracellular calcium (or decreases in cAMP), whereas ligation of relevant PGE_2 receptors (EP2 and EP4) results in increases in cAMP. It is likely that eicosanoid receptor diversity provides a means for functional and cellular specificity in the actions of these potent mediators. Furthermore, the profile of receptors expressed on a given cell can be regulated by inflammatory mediators and tissue injury, altering the effects of eicosanoids on that cell.

EFFECTS OF EICOSANOIDS ON THE PATHOBIOLOGICAL FEATURES OF FIBROTIC LUNG DISEASE (TABLE 1)

Effects on accumulation of inflammatory and immune effector cells

LTs increase the number of inflammatory cells in the lung [12,13] by acting on a variety of determinants of their accumulation. They stimulate granulocyte and macrophage colony formation in the bone marrow [14,15]. LTB_4 acts as a direct chemoattractant for neutrophils, eosinophils and lymphocytes [16]. CysLTs up-regulate endothelial cell expression of adhesion molecules that are necessary for leucocyte emigration into tissues [17]. Finally, LTs are potent inhibitors of neutrophil (LTB_4) [18] and eosinophil (cysLTs) [19] apoptosis, thereby prolonging the survival of cells that have been recruited. These mechanisms explain the ability of drugs that inhibit LT synthesis (zileuton) or block cysLT1 (zafirlukast,

Table 1 Effects of LTs and PGE₂ on the pathobiological features of pulmonary fibrosis

↑, increase; ↓, decrease; (↓), predominant decrease; ↔, unpredictable; ?, unknown.

Feature	Effect on pulmonary fibrosis	
	LTs	PGE ₂
Leucocyte accumulation		
Myelopoiesis	↑	↓
Chemotaxis	↑	↓
Adhesion-molecule expression	↑	↓
Leucocyte survival	↑	↔
Immune responses		
Dendritic cell function	↑	(↓)
Lymphocyte function	↑	↓
Th2 polarization	↑	↔
Leucocyte activation		
Reactive oxygen intermediates	↑	↓
Pro-inflammatory cytokines	↑	↓
Chemokines	↑	↓
Growth factors	↑	↓
Nuclear factor-κB activation	↑	↓
Fibroblast activation		
Chemotaxis	↑	↓
Growth-factor-receptor expression	?	↓
Proliferation	↑	↓
Collagen synthesis	↑	↓
Collagenase activity	?	↑
Myofibroblast differentiation	↑	↓

montelukast and pranlukast) to reduce leucocyte numbers in the lung of asthmatic patients [20]. PGE₂ has opposing effects on leucocyte accumulation. It inhibits myelopoiesis [21] and also inhibits leucocyte recruitment by suppressing adhesion-molecule expression [22], as well as chemotaxis [23].

Effects on immune responses

LTs have been shown to play a role in the generation of systemic immune responses [24]. For example, recent data indicate that cysLTs are required for the migration of antigen-presenting dendritic cells to lymph nodes [25], and that LTB₄ is required for T-lymphocyte recruitment to sites of immunological challenge [26]. There is also emerging evidence that cysLTs can promote a polarized Th2 response, both *in vitro* and *in vivo*. This reflects their capacity to selectively augment synthesis of IL-4 and IL-5 [27–29] without increasing IFN-γ synthesis. Indeed, a cysLT receptor antagonist has been shown to increase IFN-γ production by T-lymphocytes [28], and lung leucocytes from 5-LO knockout mice produced greater amounts of IFN-γ than cells from wild-type animals [30].

PGE₂ has long been recognized to inhibit both B- and T-cell responses [31]. However, it has been noted to have conflicting effects on dendritic cells, promoting their maturation [32], but inhibiting antigen presentation by mature cells [33]. The role of PGE₂ in the polarization of T-cell responses is equivocal. PGE₂ is known to selectively inhibit Th1 cytokine expression in lymphocytes *in vitro* [34] and is commonly thought to favour Th2 polarization. However, inhibition of PGE₂ synthesis *in vivo* during a period of antigen sensitization has been shown to enhance Th2 responses in the lung [35]. This discrepancy could suggest that responses *in vivo* involve cell types other than lymphocytes, or that differences in the profile of EP receptors exist on T-lymphocytes *in vitro* versus those within the context of an *in vivo* allergic response.

Effects on inflammatory mediators and leucocyte activation

5-LO metabolites activate AMs (alveolar macrophages) and other cells to produce a number of mediators implicated in the pathogenesis of fibrotic lung disease, including reactive oxygen intermediates [36], IL-6 [37], IL-8 [38], TNF-α [39], fibroblast-growth-factor activity [40], endothelin-1 [41] and matrix metalloproteinases [42]. By contrast, PGE₂ generally abrogates the production of a variety of inflammatory mediators, such as reactive oxygen intermediates [43], IL-8 [44], TNF-α [45], fibroblast growth factor [40], endothelin-1 [41] and LTs [46]. Most of these salutary effects appear to be explained by increases in intracellular cAMP, and are likely to be mediated by PGE₂ actions at the adenylate-cyclase-coupled receptors EP2 and EP4 [47]. Some of the anti-inflammatory activities of PGE₂ are likely exerted through its well-described ability to up-regulate expression of the suppressive cytokine IL-10 [48], also via EP2/EP4 [47].

It is appealing to explain the opposing actions of LTs and PGE₂ by virtue of their contrasting effects on centrally important signalling mechanisms that regulate inflammatory mediator generation. For example, LTs have been reported to stimulate and PGE₂ to inhibit activation of the transcription factor nuclear factor-κB [37,49–51], protein kinase C and mitogen-activated protein kinase [52–55].

Direct effects on fibroblasts

In contrast to the well-known effects of LTs on inflammatory cells, it is not generally recognized that LTs can exert direct stimulatory effects on fibroblasts. LTB₄ is chemotactic for fibroblasts [56], as it is for leucocytes; cysLTs stimulate proliferation, myofibroblast differentiation and collagen synthesis by fibroblasts [57–59]. The ability of PGE₂ to impede fibroblast function has been extensively investigated.

PGE₂ inhibits fibroblast chemotaxis [60,61]. It also potently suppresses fibroblast proliferation in response to a gamut of mitogens, including serum, TGF- β , platelet-derived growth factor, epidermal growth factor, insulin and fibronectin [62,63]. This growth-inhibitory effect may be mediated by reduced cellular uptake of nucleotides and amino acids [64], or by reduced surface expression of growth factor receptors [65]. PGE₂ inhibits differentiation of fibroblasts to myofibroblasts [66], and selectively inhibits collagen synthesis [67] and promotes its degradation [68], all of which result in a marked attenuation of collagen accumulation. Once again, most of these suppressive effects of PGE₂ on fibroblasts are mediated by the cAMP-coupled EP2 and EP4 receptors [61,69,70]. Interestingly, some limited data suggest that PGE₂ inhibition of lung fibroblast activation is diminished in patients with IPF as compared with normal subjects [71].

ABNORMALITIES IN EICOSANOID SYNTHESIS IN PULMONARY FIBROSIS

Overproduction of pro-fibrogenic LTs

There is considerable evidence to suggest that imbalances in eicosanoid synthesis occur and may be relevant to the pathogenesis of pulmonary fibrosis. LTB₄ levels in BAL (bronchoalveolar lavage) fluid have been reported to be greater in patients with IPF than in normal volunteers [72,73]. Lung homogenates from IPF patients contained 15-fold more LTB₄ and 5-fold more cysLTs than did those from non-fibrotic lung [74]. Furthermore, homogenate LT levels correlated significantly with the histological extent of fibrosis, suggesting a possible causal relationship between LTs and the fibrotic phase of this disease. AMs appear to be mainly responsible for LT overproduction in IPF. Asbestosis and systemic sclerosis share similar pathobiological features with IPF, and elevated levels of LTB₄ have also been demonstrated in BAL fluid and AM-conditioned medium from patients with asbestosis [75]. Likewise, elevated levels of LTB₄ and LTE₄ were identified in BAL fluid obtained from patients with systemic sclerosis, and those who received 6 months of standard therapy with cyclophosphamide and prednisone then demonstrated a reduction in BAL LTB₄ [76]. Increases in lung LT levels have also been demonstrated in hamsters [77] and mice [30], following the intra-tracheal administration of bleomycin, the most studied animal model of pulmonary fibrosis.

The *in situ* stimulus for LT synthesis in the lungs of patients with pulmonary fibrosis is unknown, but a number of possible candidates exist that are known to be both elevated in fibrotic lung and capable of activating the 5-LO pathway *in vitro*. These substances include immune complexes [78], IL-8 [79], IL-4 [80], IL-5 [81], endothelin-1 [82], MCP-1 [83] and TGF- β [84,85].

Cytokines are capable not only of stimulating LT synthesis, but also of increasing LT receptor expression [86].

Underproduction of anti-fibrogenic PGE₂

Reduced levels of PGE₂ in BAL fluid [87] and AM-conditioned medium [72] in patients with IPF have also been described. Lung fibroblasts isolated from patients with IPF have a reduced capacity for PGE₂ synthesis both basally [88] and in response to stimuli, such as lipopolysaccharide, IL-1, TNF- α and TGF- β [88–90]. This defect is associated with impaired induction of COX-2 mRNA and protein. As fibroblast PGE₂ production appears to be an important ‘autocrine brake’ on fibroblast function [89,91], an inability to augment PGE₂ production in response to growth factors, pro-inflammatory cytokines and LTs during lung injury might be partially responsible for the pathologically activated fibroblast phenotype observed in these patients. Fibroblast PGE₂ also increases generation of HGF (hepatocyte growth factor), an anti-apoptotic factor for AECs [92].

It is becoming increasingly recognized that fibrotic lung diseases are characterized by dysfunctional interactions between AECs and pulmonary fibroblasts [1]. Normally, AECs help to suppress fibroblast proliferation [93], in part through the production of anti-fibrotic prostanoids, such as PGE₂. Indeed, the ability of AEC-conditioned medium to suppress fibroblast proliferation was eliminated when AECs were treated with the COX inhibitor indomethacin [93] or obtained from COX-2-deficient mice [94]. AEC damage and loss are widely observed in lung injury. In animal models, a failure of re-epithelialization of the alveolar surface is a central determinant of fibrotic, rather than reparative, responses to injury [95,96]. Although the capacity for injured AECs to produce prostanoids is not known, it is plausible that a relative lack of AEC PGE₂ production, through either an absolute loss of AECs or alterations in PGE₂ biosynthesis, might encourage the development of pulmonary fibrosis.

Underproduction of PGE₂ by AECs as well as other cell types, such as fibroblasts and AMs, might reflect alterations in the injured lung in the levels of various substances that can modulate the expression or activity of PG-synthesizing enzymes. For example, PGE synthase is dependent on GSH [97], and IPF is characterized by local deficiency of GSH, reflecting oxidant stress [98]. Additionally, diminished granulocyte/macrophage colony-stimulating factor expression has been reported in bleomycin-treated lung [99]. As this substance regulates PGE₂ synthesis in AMs, AECs and fibroblasts [100, 101], it was not surprising that granulocyte/macrophage colony-stimulating factor-knockout mice developed exaggerated fibrosis in response to bleomycin in association with reduced PGE₂ levels in lung homogenates [101]. Injured and fibrotic lungs also contain mediators, such as IL-4 and IL-13, which have been shown to inhibit

COX-2 induction *in vitro* [102]. Finally, the protection of CC-chemokine-receptor-2-deficient mice from experimental fibrosis may be explained by the fact that MCP-1, a key CC chemokine receptor 2 ligand induced in fibrotic lung, inhibits PGE₂ synthesis when added directly to normal AECs [103]. We speculate that AECs from mice deficient in this receptor overproduce PGE₂, because they are protected from this inhibitory effect of MCP-1. It is apparent that multiple mechanisms are likely to contribute to the imbalance favouring production of LTs over PGs in fibrotic lung.

EFFECT OF EICOSANOID MODULATION ON EXPERIMENTAL PULMONARY FIBROSIS

LTs

Although the demonstration of LT overproduction in fibrotic lung disease in humans and animal models is intriguing, such data fail to establish a pathogenic role for LTs in pulmonary fibrosis. Nearly 20 years ago, the first-generation LO inhibitor nordihydroguaiaretic acid was shown to markedly attenuate bleomycin-induced fibrosis and, concomitantly, the release of AM-derived fibroblast growth factor activity [104]. However, this agent inhibits not just 5-LO, but all LO pathways, and it also possesses generalized antioxidant activity. Dietary γ -linolenic acid has also been reported to suppress bleomycin-induced fibrosis in parallel with lung LTB₄ content [77]. Similar to nordihydroguaiaretic acid, however, such fatty acids may exert anti-inflammatory actions independently of their effects on 5-LO-derived eicosanoids [105].

To more conclusively determine whether products of the 5-LO pathway play a causative role in the pathogenesis of pulmonary fibrosis, bleomycin-induced fibrosis was investigated in 5-LO knockout mice [30]. These LT-deficient mice exhibited significantly less fibrosis in response to bleomycin than wild-type mice, both histologically and biochemically. Several potential mechanisms for this protection were identified. The knockout mice demonstrated a marked reduction in the pulmonary accumulation of all leucocyte subsets following bleomycin. Additionally, lung mononuclear cells from 5-LO knockout animals produced increased amounts of the anti-fibrotic cytokines IFN- γ and IL-10, and the LT-deficient mice synthesized increased levels of the anti-fibrotic eicosanoid PGE₂. These data unequivocally suggest that 5-LO metabolites can play an important causal role in the pathogenesis of pulmonary fibrosis.

PGs

Modulation of prostanoid synthesis in animal models of pulmonary fibrosis has produced conflicting outcomes. Continuous administration of the COX inhibitor indo-

methacin protected against bleomycin-induced fibrosis [106,107]. However, a significant worsening of fibrosis was recently observed when indomethacin was administered exclusively during the post-inflammatory fibrotic phase following bleomycin administration [101]. Likewise, the effects of indomethacin on pulmonary fibrosis induced by butylated hydroxytoluene depended upon the timing of its administration [108]. The particular effects of indomethacin on pulmonary fibrosis in various experimental systems are likely to depend upon which prostanoids are present at a given moment, i.e. anti-fibrotic PGE₂ or PGI₂ versus PGD₂ or thromboxane A₂, which might promote pro-fibrotic Th2 immune responses. Moreover, differences in the ambient profile of EP receptors on target cells may also contribute to variable outcomes. Because indomethacin can act in a COX-independent manner [109], targeted gene deletion may represent a more specific approach to elucidating the role of COX metabolites in pulmonary fibrosis. Indeed, it has recently been reported that COX-2 heterozygous mice which manifest impaired pulmonary synthesis of PGE₂ developed an exaggerated fibrotic response to bleomycin [110]. Needless to say, a full understanding of the contribution to fibrogenesis of specific prostanoids and their receptors will require further efforts to explore pharmacological or genetic manipulations that are much more selective than those studied to date.

Possible implications for airway remodelling in asthma

Like parenchymal pulmonary fibrosis, airway remodelling complicating chronic asthma is characterized by subepithelial fibrosis and mesenchymal cell proliferation in the context of a Th2-type inflammatory process with dysregulated epithelial cell–mesenchymal cell interactions [111]. It is of interest, therefore, that the continuous administration of a cysLT1 receptor antagonist markedly attenuated subepithelial fibrosis in a mouse model of chronic antigen-induced asthma [112]. Moreover, impaired capacity for PGE₂ synthesis by bronchial fibroblasts from severe asthmatics has been reported [113]. These data indicate that an imbalance between LTs and PGs may characterize airway remodelling in asthmatics, as it does parenchymal remodelling in fibrotic lung disease.

EFFECTS OF COMMONLY USED THERAPEUTIC AGENTS FOR PULMONARY FIBROSIS ON EICOSANOID SYNTHESIS

Corticosteroids

Corticosteroids have long been the mainstay of therapy for IPF and other fibrotic lung diseases. No randomized placebo-controlled trials have ever been conducted evaluating the efficacy of corticosteroids in IPF. However,

the existing data in humans are disappointing [114], as they are in animal models of pulmonary fibrosis [115,116]. Such negative data may reflect the indiscriminate inhibition by corticosteroids of both pro-fibrotic (e.g. TNF- α) and anti-fibrotic (IFN- γ , IL-10 and HGF) mediators [117]. With respect to eicosanoids, corticosteroids are potent inhibitors of the induction of COX-2 [118] and the inducible form of PGE synthase [119], and therefore of PGE₂ synthesis. Since PGE₂ is an important inhibitor of fibroblast activation, treatment with corticosteroids could theoretically potentiate fibrogenesis. As for the 5-LO pathway, there is a growing body of evidence indicating that corticosteroids generally fail to inhibit *in vivo* LT production in patients with asthma [120]. This may reflect a release of the usual brake on LT synthesis provided by PGE₂, or the fact that, *in vitro*, corticosteroids have been shown to enhance gene expression of 5-LO and/or 5-LO-activating protein in monocytes [121]. By these mechanisms, corticosteroids might exaggerate the imbalance between LTs and PGE₂ that already exists in pulmonary fibrosis, thereby possibly limiting any potential therapeutic benefit.

Immunosuppressive and cytotoxic drugs

Immunosuppressive and cytotoxic agents are also commonly used in the treatment of fibrotic lung disorders. As with corticosteroids, the evidence for the efficacy of these drugs is limited. There is scant information available regarding the effects of these agents on eicosanoid synthesis, and little of this is based on studies of lung cells. Azathioprine has been shown to inhibit prostanoid production by peritoneal macrophages [122]. Methotrexate has been reported to have similar activity in both peritoneal macrophages [123] and synoviocytes [124]. Cyclophosphamide has been reported to stimulate LTB₄ generation by lung fibroblasts [60] and to amplify peritoneal macrophage synthesis of cysLTs and PGE₂ [125]. If applicable to relevant lung cells, these stimulatory effects on LTs and inhibitory effects on prostanoids might, as suggested above for corticosteroids, exaggerate the eicosanoid imbalance that already exists, thereby potentiating fibrogenesis.

IFN γ -1b

An agent that has recently received considerable attention is IFN γ -1b, a cytokine with anti-fibrotic actions. A randomized, double-blind, placebo-controlled clinical trial evaluating the efficacy of IFN γ -1b in patients with IPF was recently performed [126]. The drug did not affect progression-free survival, pulmonary function or quality of life. Nevertheless, the data did suggest that there may be a subset of patients who do benefit, and a second study to identify such patients is ongoing. As previously mentioned, some evidence suggests that LTs may inhibit production of IFN- γ [28,30]. The impact of IFN- γ on LTs is poorly understood, but there is evidence that

IFN- γ up-regulates expression of both LT synthetic enzymes [127] and receptors [128]. The limited data available addressing effects of IFN- γ on PG production are equivocal.

N-Acetylcysteine

Fibrotic lung diseases are characterized by oxidant stress and a deficiency of GSH [98]. Animal data [129] and a small clinical trial [130] have suggested that *N*-acetylcysteine, the precursor of GSH, may be efficacious for pulmonary fibrosis. Interestingly, *in vitro* administration of extracellular GSH has been shown to suppress human lung fibroblast proliferation by an undetermined mechanism [131]. Since PGE synthase, the terminal enzyme responsible for synthesis of PGE₂, is GSH-dependent [98], it is appealing to postulate that GSH deficiency may contribute to the relative deficiency of PGE₂ in pulmonary fibrosis, and that the *in vitro* and *in vivo* benefits of *N*-acetylcysteine in this condition might relate to augmented synthesis of this anti-fibrotic prostanoid.

ROLE OF EICOSANOIDS IN THE PATHOGENESIS OF PULMONARY FIBROSIS: AN INTEGRATED MODEL

Although our understanding of the pathobiology of pulmonary fibrosis is rudimentary at best, it is clear that it represents an aberrant response to injury involving dysregulated cell-cell interactions. Although many types of molecules and mediators have been the focus of interest in investigations of fibrotic lung disease, eicosanoids have received comparatively little attention. We will briefly discuss here a proposed model (Figure 3) of the pathogenesis of pulmonary fibrotic disease that incorporates eicosanoids and derangements in their production by AECs, AMs and fibroblasts.

Under normal homeostatic conditions, AMs and fibroblasts remain unactivated, and a quiescent state is fostered by constitutive PGE₂ production by AECs, AMs and fibroblasts. An injurious event results in dysfunction or loss of AECs and a concomitant activation of AMs. An important consequence of AEC dysfunction/loss is diminished capacity for PGE₂ secretion, and this has a permissive effect on AM activation, promoting the generation of pro-inflammatory and pro-fibrotic substances, such as TNF- α , MCP-1, IL-8, TGF- β and LTs, as well as reducing AM synthesis of PGE₂. Injured AECs may themselves now secrete pro-inflammatory/pro-fibrotic mediators, including MCP-1, IL-8 and TGF- β . A milieu characterized by the lack of PGE₂, along with the presence of potential activation signals, results in fibroblast activation. If reconstitution of the epithelium (with a recovery of PGE₂ synthetic capacity), control of AM activation and appropriate fibroblast induction of COX-2 (with resultant increased endogenous capacity for PGE₂

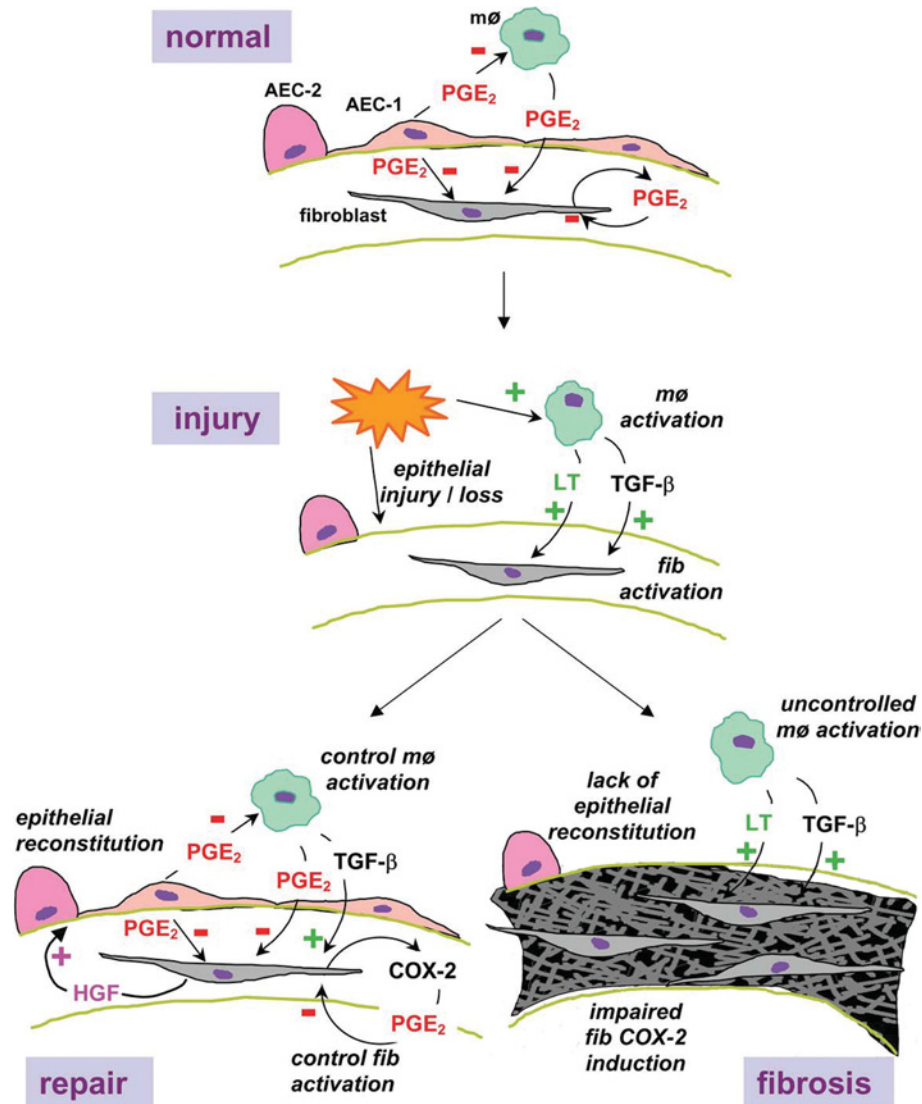


Figure 3 Model for the role of eicosanoids in the pathogenesis of pulmonary fibrosis

Under normal conditions, PGE₂ production by type I (AEC-1) and type II (AEC-2) AECs, macrophages (mΦ), and fibroblasts (fib) contribute to the maintenance of lung suppression of fibrogenesis. Lung injury results in epithelial cell loss or damage, with impaired capacity for PGE₂ generation, as well as macrophage activation to produce LTs and growth factors; these events promote fibroblast activation. If the epithelium can be reconstituted and if fibroblast COX-2 can be appropriately induced, PGE₂ elaboration is sufficient to control macrophage and fibroblast activation, and repair can take place. HGF generation by fibroblasts also promotes AEC survival. If these critical responses are impaired, macrophage and fibroblast activation are unchecked and fibrosis develops. —, inhibition; + stimulation.

synthesis) occur, repair can take place. Fibroblast synthesis of PGE₂ also promotes elaboration of HGF, a pro-survival factor for AECs. However, if there is an inadequate degree of epithelial recovery, AM deactivation or fibroblast COX-2 induction, then fibrosis ensues.

THERAPEUTIC STRATEGIES TO ALTER EICOSANOID SYNTHESIS OR ACTIONS

In view of the available data implicating eicosanoids in the pathobiology of fibrotic lung disease, modulation of their

tissue concentrations or actions represent appropriate therapeutic targets in these disorders. Three generic strategies will be considered: the inhibition of potentially pro-fibrotic LTs, antagonism of LT receptors and augmentation of anti-fibrotic prostanoids. The synthesis or actions of LTs could be limited by inhibition of the 5-LO enzyme or by appropriate LT receptor antagonists. In asthma, cysLTs appear to be the prime 5-LO metabolite relevant to airway remodelling, and cysLT1 receptor antagonists (e.g. montelukast, zafirlukast and pranlukast) have been shown to abrogate this process. There are three theoretical reasons for favouring a LT biosynthesis

inhibitor (e.g. zileuton) over a cysLT receptor antagonist, as a therapeutic approach for the treatment of pulmonary fibrosis. As already mentioned, human IPF is characterized by a substantially greater production of LTB₄ than cysLTs [74]; in this important regard, the human disease differs from mouse models [30]. Also, there are two known receptors for both LTB₄ and cysLTs, and the relative contribution of each to pulmonary fibrosis is not known; however, recent data suggest a key role for cysLT2 in murine bleomycin-induced fibrosis [132]. Finally, *in vivo* inhibition of LT biosynthesis from AA might result in shunting of AA to COX-derived products, thereby increasing the generation of anti-fibrotic prostanoids.

Another approach for inhibiting the synthesis of pro-fibrotic LTs could be by blocking the initial release of AA from membrane phospholipids, either by the inhibition of PLA₂-mediated deacylation or by reducing the AA content of phospholipids via dietary intake of fish-oil-derived *n*-3 fatty acids. Although both of these approaches have been reported to ameliorate bleomycin-induced fibrosis [77,133], this strategy seems less predictable, since both pro-fibrotic LTs and anti-fibrotic prostanoids would be inhibited.

The final potential therapeutic strategy involves the augmentation of anti-fibrotic prostanoids, with PGE₂ being the obvious candidate, given its suppressive actions on multiple aspects of fibrogenesis. Because systemic administration has adverse effects on vasoregulation, targeted delivery to the lung would be preferable. The feasibility of this approach is supported by documentation that inhalation of exogenous PGE₂ has been shown to increase the BAL levels of this prostanoid in animals and humans [87], and that chronic inhalation of the PGI₂ analogue iloprost is a well-established approach for the treatment of pulmonary hypertension [134]. As discussed above, selective EP2 and/or EP4 agonists might confer more specificity of response than would PGE₂ itself, since these receptors are coupled only to increases in cAMP and not to potentially opposing signals, such as increased intracellular calcium (EP1) or decreased cAMP (EP3); this might obviate the bothersome cough that has been noted with inhalation of PGE₂ in the past. Furthermore, the desired increase in cAMP could be further amplified by the co-administration of an inhibitor of phosphodiesterase, the enzyme responsible for cAMP degradation, an approach which has been shown to be additive with PGI₂ in an animal model of pulmonary hypertension [135].

CONCLUSION

Although the role of eicosanoids in the pathogenesis of numerous lung diseases, such as asthma and pulmonary hypertension, is widely known, their potential significance in the pathogenesis of pulmonary fibrosis is less well

appreciated. The growing body of evidence presented in this review suggests that an imbalance of eicosanoid synthesis (overproduction of LTs and underproduction of prostanoids) exists in pulmonary fibrosis that may contribute to the dysfunctional cell–cell interactions responsible for fibrogenesis. Of particular significance is the fact that LTs promote and prostanoids inhibit so many of the key pathobiological elements of fibrosis, through both their direct actions on fibroblasts and their interactions with other fibrotic mediators. New therapeutic strategies are desperately needed for fibrotic lung diseases, and there is a strong rationale for targeting the eicosanoid imbalance in such disorders. The continued development of clinical approaches to this should permit this potential to be tested in the clinic.

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