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Pathogenetic mechanisms in usual interstitial pneumonia/ idiopathic pulmonary fibrosis

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a progressive, usually fatal, form of interstitial lung disease characterized by failure of alveolar re-epithelialization, persistence of fibroblasts/myofibroblasts, deposition of extracellular matrix, and distortion of lung architecture which ultimately results in respiratory failure. Clinical IPF is associated with a histopathological pattern of usual interstitial pneumonia (UIP) on surgical lung biopsy. Therapy for this disease with glucocorticoids and other immunomodulatory agents is largely ineffective and recent trials of newer anti-fibrotic agents have been disappointing. While the inciting event(s) leading to the initiation of scar formation in UIP remain unknown, recent advances in our understanding of the mechanisms underlying both normal and aberrant wound healing have shed some light on pathogenetic mechanisms that may play significant roles in this disease. Unlike other fibrotic diseases of the lung, such as those associated with collagen vascular disease, occupational exposure, or chemotherapeutic agents, UIP is not associated with a significant inflammatory response; rather, dysregulated epithelial–mesenchymal interactions predominate. Identification of pathways crucial to fibrogenesis might offer potentially novel therapeutic targets to slow or halt the progression of IPF. This review focuses on evolving concepts of cellular and molecular mechanisms in the pathogenesis of UIP/IPF.

Keywords

pulmonary fibrosis; myofibroblast; extracellular matrix; alveolar epithelial cell; fibrogenesis

Introduction

Pulmonary fibrosis is the end result of a variety of insults to the lung. Antecedent injuries to the lung may be recognized, as with chemotherapy [1], collagen vascular disease [2], or inhalational injury [3]. However, in idiopathic pulmonary fibrosis (IPF) [also termed cryptogenic fibrosing alveolitis (CFA)], the inciting insult remains unidentified. In 1969, Liebow and Carrington first classified interstitial pneumonias into five distinct categories, based on histological features [4] (Table 1). Subsequently, giant cell interstitial pneumonia (GIP) was excluded from the category of IPF because GIP is the histological manifestation of hard-metal pneumoconiosis [5]. The classification scheme of interstitial lung diseases has undergone numerous revisions and currently, seven distinct subtypes of idiopathic interstitial pneumonia (IIP) have been proposed by the American Thoracic Society/European Respiratory Society (ATS/ERS): usual interstitial pneumonia (UIP), desquamative interstitial pneumonia (DIP), respiratory bronchiolitis interstitial lung disease (RB–ILD), acute interstitial pneumonia

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(AIP), non-specific interstitial pneumonia (NSIP), cryptogenic organizing pneumonia (COP), and lymphoid interstitial pneumonia (LIP) (Table 2) [6]. Of these histopathological subtypes, UIP is the histological pattern that characterizes patients with the clinical entity of IPF [6]. Based on the high proportion of UIP/IPF among the IIPs, its overall poor prognosis, and its unresponsiveness to currently available therapy, the remainder of this review will focus on mechanisms of fibrosis associated with UIP/IPF.

Epidemiology and clinical features of IPF

Epidemiology

IPF is a relatively rare disease, although the true prevalence is unknown. Prevalence rates of three to six cases per 100 000 population are often cited [7], but a recent study in Finland demonstrated a nationwide prevalence of 16–18 per 100 000 [8]. Subgroup analyses demonstrate a higher prevalence of IPF among older individuals. In one study, the prevalence of IPF was 2.7 per 100 000 population among adults between age 35 and 44 years but rose to 175 per 100 000 population in individuals older than 75 years [9]. The mean age at diagnosis of IPF is 66 years [7,10]. IPF is more common in males than in females and some studies indicate that an environmental or occupational exposure to dusts, organic solvents, or urban pollution increases the risk of developing IPF [7,11,12].

Recently, data have emerged linking both genetic and environmental factors to the development of UIP/IPF. A familial form of UIP has been reported in 0.5–2.2% of cases [13] and a number of candidate genes encoding both cytokines and structural proteins have been identified in large kindreds [14,15]. Using linkage analysis, Thomas *et al* demonstrated a mutation in the pro-surfactant protein-C (proSP-C) gene encoding a leucine → glutamine substitution in the C-terminus [14]. This mutation results in improper intracellular trafficking of proSP-C in type II alveolar epithelial cells, as evidenced by electron microscopy showing aberrant subcellular localization of the protein and abnormal lamellar bodies [14]. These findings suggest that in this kindred, improper cellular processing of proSP-C may contribute to pulmonary fibrosis.

Pantelidis *et al* evaluated single nucleotide polymorphisms (SNPs) in four pro-inflammatory genes of 74 unrelated patients with clinical or biopsy-proven CFA [15]. Candidate genes included tumour necrosis factor-*α* (TNF-*α)*, lymphotoxin-*α* (LT*α)*, high affinity TNF-*α*/LT*α* receptor 2 (TNF-RII), and interleukin-6 (IL-6). Although no difference existed between patients and controls with respect to genotype, allele, or haplotype frequencies, a strong association between carriage of the IL-6 (intron 4G) allele and the TNF-RII (1690C) allele was observed in patients, but not in controls [15]. Additionally, these investigators identified a strong linkage between the IL-6 (intron 4GG) genotype and disease progression as measured by diffusion capacity for carbon monoxide (DL_{CO}), suggesting that progression of disease may be associated with decreased production of IL-6 [15].

Data implicating the major histocompatibility (HLA) system are sparse. In one study, Fulmer *et al* found no difference when comparing 35 separate antigens of the HLA-A and HLA-B loci in 33 patients with IPF with 329 healthy controls [16]. However, Libby *et al* identified an increase in HLA-DR2 in patients with IPF compared with controls [17]. It should be noted that both of these studies were performed at a time when the term ‘IPF’ encompassed many different histopathological entities and may not be representative of UIP/IPF.

Environmental factors have also been thought to contribute to the development of pulmonary fibrosis. In 1977, Millar reported a case of infectious mononucleosis in a woman with pulmonary fibrosis [18]. Since that time, numerous investigators have evaluated a possible link between viral infection and pulmonary fibrosis. Egan *et al* first demonstrated evidence of

Epstein-Barr virus (EBV) replication in lower respiratory tract epithelial cells of patients with CFA [19]. Yonemaru *et al* subsequently showed increases in both cytomegalovirus (CMV) and EBV viral capsid antigen IgG and complement fixation titres in patients with IPF and connective tissue disease-related pulmonary fibrosis (CTD-PF) compared with controls or with patients with sarcoidosis or emphysema [20]. However, Wangoo *et al* evaluated the presence of EBV RNA and DNA in lung biopsy specimens from 12 patients with CFA compared with biopsy specimens from three normal controls and 12 patients with other diffuse lung diseases [21]. These investigators reported no difference in EBV antibody staining between patients and controls, no evidence for EBV RNA by *in situ* hybridization in any patient, and no difference in EBV DNA by polymerase chain reaction (PCR) [21]. This supports their conclusion that EBV infection is not associated with CFA. In contrast, Stewart *et al* observed that lung biopsy specimens from 12 of 27 (44%) patients with IPF stained positively for EBV compared with 3 of 28 (10%) controls ($p = 0.005$) [22]. Additionally, 13 IPF patients (48%) but only four controls (14%) were EBV-positive by PCR ($p = 0.007$) [22]. These investigators subsequently showed that a rearrangement of EBV DNA termed EBV WZhet was associated with active EBV replication and was found in the peripheral blood of 16/27 (59%) IPF patients compared with 0/26 (0%) lung transplant recipients and 1/24 (4%) normal blood donors [23]. This suggested an association between this DNA rearrangement pattern and IPF.

Together, these data suggest a potential link between genetic or environmental factors that may predispose to the development of UIP/IPF in a subset of patients.

Clinical features

Patients with IPF typically complain of a dry, non-productive cough and dyspnoea. Dyspnoea is often associated with exertion early in the disease course, but usually progresses to shortness of breath at rest [24]. On physical examination, bi-basilar, end-expiratory rales are appreciated in greater than 80% of patients [25]. Clubbing is noted in up to half of all patients [7]. Late in the course of the disease, cyanosis of the lips and fingers as well as signs of pulmonary hypertension may be seen [26]. Laboratory evaluation of patients with suspected UIP is primarily to rule out alternative causes of interstitial lung disease, such as sarcoidosis or CTD-PF. There are no laboratory tests specific for the diagnosis of UIP.

Restrictive pulmonary physiology is the classic finding on pulmonary function testing in IPF [27]. When a patient has concurrent emphysema, lung volumes may be relatively preserved [28]; however, the DL_{CO} is disproportionately reduced [29]. Severe derangements in DL_{CO} (<45%) and VC (<50%) are associated with increased mortality [30].

Greater than 90% of patients with UIP will have abnormal chest radiographs at the time of diagnosis [7]. The characteristic pattern is diffuse bilateral interstitial or reticulonodular infiltrates, most common in the basilar and subpleural regions of the lung [31]. Pleural disease and lymphadenopathy are rare and suggest an alternative diagnosis. Similar radiographic findings may be seen in secondary causes of pulmonary fibrosis including pneumoconioses and CTDPF [32].

High-resolution CT (HRCT) scanning has revolutionized the diagnostic evaluation of patients with suspected UIP. HRCT uses thin sections (1–2 mm slices) with special reconstruction of images that allows for enhanced visualization of lung parenchyma. Patterns typically seen include coarse reticular or linear opacities (intralobular and interlobular septal thickening) with a predilection for the periphery and lower lobes of the lungs, honeycomb cysts, and traction bronchiectasis [33]. Ground glass opacities (ill-defined hazy zones representing active alveolitis or fibrosis of the intralobular and alveolar septae) can be present; if they are the predominant pattern, it may indicate another subtype of IIP [34,35]. Extensive honeycombing, septal thickening, and a lack of ground glass opacities reflect a poor prognosis.

Diagnostic certainty of IPF is improved with a surgical lung biopsy showing a UIP pattern. Transbronchial lung biopsies usually do not allow for distinction among the various idiopathic interstitial pneumonias because of the limitations of the small biopsy size. Video-assisted thoracoscopic surgical (VATS) biopsy is the preferred method of obtaining lung tissue, as this procedure offers a similar yield to open thoracotomy [36]; VATS can be performed with relative ease and decreased morbidity [37].

Histopathology

UIP typically demonstrates a heterogeneous appearance, on low-power magnification, of normal-appearing lung alternating with areas of peripheral fibrosis, interstitial inflammation, and honeycomb changes [25], the so-called ‘temporal heterogeneity’ that is a hallmark of the disease (Figure 1). The inflammatory component is typically mild and consists primarily of lymphocytes and plasma cells. Other inflamma-tory cells such as neutrophils and eosinophils may be present, but are not abundant. Dense, relatively acellular, collagen bundles with smooth muscle meta-plasia can be seen on higher-power magnification. At the border between fibrotic and normal lung are collections of fibroblasts/myofibroblasts, termed fibroblastic foci, that are thought to represent the active lesion of UIP (Figure 2) [38]. Alveolar epithelial injury with hyperplastic type II pneumocytes is often seen at areas of active fibrosis [39]. Honeycomb changes are depicted by enlarged, cystic airspaces lined by hyper-plastic type II pneumocytes.

The presence of fibroblastic foci may be an important prognostic factor in IPF/UIP. Nicholson *et al* devised a semi-quantitative scoring system which grades four separate histological features (on a scale from 0 to 6): extent of fibroblastic foci, extent of interstitial mononuclear infiltrates, extent of established fibrosis, and extent of intra-alveolar macrophage accumulation [40]. These investigators showed that extent of fibroblastic foci strongly correlated with decreased Forced Vital Capacity (FVC) and DL_{CO} as well as mortality [40]. These data are in agreement with those of King *et al*, who devised a separate semi-quantitative scoring system to evaluate surgical lung biopsies of patients with UIP [41]. Biopsy scores were obtained for 14 histopathological features, which culminated in the derivation of four factor scores: the ‘fibrosis factor’, the ‘cellularity factor’, the ‘alveolar space cellularity factor’, and the ‘granulation and young connective tissue factor’ [41]. Controlling for age, gender, and smoking status, these researchers demonstrated that the granulation and young connective tissue score was a strong predictor of survival [41]. Together, these two studies suggest that the number of fibroblastic foci and the extent of granulation tissue and young connective tissue seen on surgical lung biopsies of patients with UIP/IPF may assist in predicting survival.

Mechanisms of fibrogenesis

Alveolar epithelial cells (AECs): targets of early injury

The normal alveolar basement membrane is lined with alveolar epithelial cells (AECs), which can be subdivided into type I and type II pneumocytes. Ninety-five per cent of the alveolar surface is covered with type I pneumocytes. These cells are metabolically active and harbour cell surface receptors for a variety of substances, including extracellular matrix (ECM) proteins, growth factors, and cytokines. The remaining 5% of alveolar lining cells consist of cuboidal epithelia (type II pneumocytes). Type II pneumocytes secrete surfactant, facilitate transepithelial movement of water, function as antigen presentation cells, and represent a reservoir of progenitor cells that regenerate the alveolar epithelium following lung injury [42].

It has been postulated that UIP may be the result of abnormal ‘wound healing’ of the alveolar epithelium after an injury [24,38]. In surgical lung biopsies of patients with UIP, morphological changes including hyperplastic type II cells, elongated type II cells, and bronchiolar cells lining

areas of honeycomb lesions can be seen [43]. In addition, the apposition of denuded basement membrane and the obliteration of the airspace may result from lack of alveolar epithelial cell proliferation and differentiation [44]. Evidence for an altered AEC phenotype is also supported by data demonstrating that AECs from patients with UIP synthesize a different set of cytokeratins than AECs from normal lung [45]. Epithelial cell function is often determined by the type of cytokeratin expression; thus, AECs in UIP may have both different form and function when compared with alveolar epithelial cells in normal lung.

Biopsy specimens of patients with UIP often demonstrate areas of denuded alveolar basement membrane. This implies either an increase in AEC death (necrosis or apoptosis), a lack of proliferative capacity of AECs, or a combination of both processes. Uhal *et al* demonstrated that myofibroblasts from patients with fibrotic lung disease secrete soluble factor(s) that induce apoptosis of human AECs [46]. A follow-up study from this same group of investigators showed that angiotensin II accounted for the majority of this effect; the addition of angiotensin II antibodies or receptor antagonists prevented fibrotic-lung fibroblasts from inducing apoptosis in AECs [47]. Other evidence for AEC apoptosis in the pathogenesis of UIP is provided by Maeyama *et al*, who demonstrated an up-regulation of the pro-apoptotic Fas–Fas ligand system in AECs from patients with pulmonary fibrosis [48].

Classically, local expansion of type II AECs following lung injury was thought to re-populate denuded alveolar basement membranes in response to growth factors such as hepatocyte growth factor and keratinocyte growth factor [43]. Recent data from animal models of lung injury also suggest that bone marrow-derived progenitor cells can differentiate into type I AECs [49,50], suggesting an additional mechanism for re-epithelialization of damaged alveoli. However, this mechanism has not yet been demonstrated in humans and further studies are needed to determine whether human bone marrow-derived cells possess the capacity to transdifferentiate into alveolar epithelium.

In the normal alveolus, an intact AEC lining may exert a homeostatic effect on local fibroblasts/mesenchymal cells. A discontinuous and/or damaged layer of AECs, as in patients with UIP, may induce the secretion of stimulatory molecules or result in diminished synthesis of inhibitory factors. Prostaglandin E2 (PGE_2) is a potent inhibitor of fibroblast collagen synthesis and proliferation [51,52]. In broncho-alveolar lavage (BAL) fluid from patients with UIP, PGE_2 levels have been shown to be approximately half those of control patients [53], suggesting that loss of AECs or the diminished capacity of AECs to synthesize PGE_2 may contribute to the pro-fibrotic milieu in the alveolar space.

In addition to prostanoids, AECs also synthesize numerous growth factors and cytokines that activate fibroblasts/mesenchymal cells. AECs are the primary source for transforming growth factor-beta (TGF-β) [54], a critical cytokine in the transdifferentiation of fibroblasts into the activated myofibroblast phenotype. Additionally, AECs produce platelet-derived growth factor (PDGF) [55], tumour necrosis factor alpha (TNF-α) [56], and endothelin-1 [57]. PDGF is a potent mitogen and chemoattractant for fibroblasts; PDGF mRNA and protein have been shown to be up-regulated in epithelial cells of patients with IPF [55]. TNF-α is secreted by hyperplastic type II AECs in pulmonary fibrosis [56,58] and promotes DNA synthesis and proliferation of fibroblasts [59]. Endothelin-1 has also been shown to stimulate fibroblast DNA synthesis and proliferation as well as to induce transdifferentiation of fibroblasts to myofibroblasts [60].

AECs also likely contribute to the pathogenesis of UIP by regulating the plasminogen activation system. The plasminogen activation system is critical to normal wound healing [61]. Plasminogen, activated by tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA) to plasmin, is the primary fibrinolytic enzyme responsible for degrading fibrin clots and allowing for wound reepithelialization. Plasminogen, and thus

plasmin activity, is negatively regulated by plasminogen activator inhibitors (PAIs). Animal models suggest that over-expression of PAIs (which inhibits plasmin activity) promotes fibrosis, whereas lack of PAIs (allowing greater plasmin activity) prevents the formation of significant fibrosis [62]. Additionally, overexpression of uPA was shown to decrease fibrosis in a murine model [63]. In UIP, the plasminogen activation system has been implicated in regulating fibrin turnover and ECM degradation [42]. It is known that AECs synthesize both PAI-1 and urokinase [64,65] as well as the urokinase receptor, uPAR [66]. Kotani *et al* showed that BAL fluid from patients with IPF contains significantly greater amounts of tissue factor and PAIs than normals, whereas uPA levels were similar between the two groups [67]. These studies suggest that the alveolar microenvironment in IPF favours a pro-coagulant, anti-fibrinolytic state that favours ECM accumulation and retards alveolar re-epithelialization.

Fibroblasts/myofibroblasts: key effector cells in fibrogenesis

Fibroblasts are the most versatile of the connective-tissue cell family and possess a remarkable capacity to undergo various phenotypic conversions between distinct but related cell types. This phenotypic plasticity is an important feature of the responses to many types of tissue injury [61]. Fibroblasts participate in repair and regenerative processes in almost every human tissue and organ. Their primary function is to secrete ECM proteins that provide a tissue scaffold for normal repair events such as epithelial cell migration. Eventual dissolution of this scaffold and apoptosis of fibroblasts/myofibroblasts are critical for restoration of normal tissue architecture [68,69].

Fibroblasts with an activated myofibroblast phenotype have been described in the fibroblastic foci that characterize UIP [39,70]. Gabbiani *et al* first described the transient appearance and disappearance of these so-called myofibroblasts in the granulation tissue of healing cutaneous wounds [71]. Myofibroblasts possess ultrastructural features intermediate between fibroblasts and smooth muscle cells and have been defined by their ability to express contractile proteins [72]. This contractile function is important in the re-epithelialization process by bringing wound margins closer together. In addition, myofibroblasts represent an ‘activated’ fibroblast phenotype with high synthetic capacity for ECM proteins [73,74], growth factors/cytokines [75], growth factor receptors [76], integrins [77], and oxidants [78,79]. Persistence of myofibroblasts in areas of active fibrosis appears to be a consistent finding in the pathology of human fibrotic diseases involving diverse organ systems [38,80].

Several studies have attempted to characterize the phenotype of fibroblasts in UIP/IPF. These studies have sometimes produced conflicting results, which may be related to inherent tissue fibroblast heterogeneity and changes in cellular microenvironment, including *in vitro* culture conditions. Fibroblasts derived from fibrotic tissue have been reported to demonstrate both high and low proliferative capacities [81–83]; lower rates appear to be associated with more advanced fibrosis [81]. Fibrotic lung fibrob-lasts demonstrate anchorage-independent growth in soft agar, whereas normal fibroblasts do not [84]. *In vivo* apoptotic rates of fibroblasts/myofibroblasts from UIP appear to be lower than those found in the fibromyxoid connective tissue of bronchiolitis obliterans organizing pneumonia [85]; paradoxically higher rates have been observed in *in vitro* culture of UIP fibroblasts [83]. The discrepancies in growth, apoptosis, and myofibroblast pheno-type most likely reflect differences in culture technique, passage number, and inter-patient variability. Newer techniques, such as laser capture microdis-section (LCM), have been proposed to address these inherent problems related to tissue heterogeneity [86]. Coupled with gene expression and protein expression arrays, LCM of fibroblastic foci fibrob-lasts will allow investigators to define the pheno-type of the active lesion in UIP fully and more accurately.

UIP fibroblasts possess a highly synthetic phenotype and produce a number of ECM proteins and integrin molecules [39,70,83,87,88]. This is accompanied by imbalances in the production

of matrix metalloproteinases (MMPs) and tissue inhibitors of metal-loproteinases (TIMPs) [83,89]. In particular, TIMP-2 expression by UIP fibroblasts/myofibroblasts appears to contribute to the irreversible structural remodelling in this disease [90–92]. Myofibroblasts in UIP have been shown to secrete angiotensin peptides that may induce apoptosis of adjacent alveolar epithelial cells [46,47,93]. Other phenotypic characteristics described in UIP fibroblasts include enhanced migratory capacity [94], increased fibroblast contractility [95], and diminished cyclooxygenase-2 expression/prostaglandin E2 synthesis [96]. Moreover, fibroblasts themselves express surface receptors such as CD40 typically associated with immune cells and are capable of producing a number of chemokines and cytokines [97–99]. Thus, fibroblasts and myofibroblasts, with their variegated phenotypes and multiple roles, appear to be key effector cells in the pathogenesis of fibrosis.

Neutrophils and other inflammatory cells: effector cell or bystander?

The role of neutrophils and other inflammatory cells such as eosinophils, mast cells, and lymphocytes in the pathogenesis of IPF is largely unknown. Neutrophils have been more extensively studied in pulmonary fibrosis than other inflammatory cells, but data demonstrating an active role for these cells in the late stages of UIP/IPF are scant. A number of studies have demonstrated an association between the presence of inflammatory cells and disease [100–107]; however, the precise cause–effect relationship is unclear.

Neutrophils circulate in blood as quiescent cells and express small quantities of surface proteins that serve as adhesion molecules [108]. Once recruited to sites of injury, neutrophils undergo a variety of functional changes affecting cellular adhesion, trans-migration, and the release of toxic products. Neutrophils contain a large number of hydrolytic enzymes and other toxic molecules in their granules, including neutrophil elastase, lysozyme, myeloperoxidase, and members of the MMP family [109]. In addition, these cells can generate various oxidant species, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl) [110,111]. Extracellular release of these toxic substances can result in lung parenchymal and stromal cell injury, as well as degradation of key ECM components of lung tissue. In addition to elastin, neutrophil elastase can degrade collagen types III and IV, laminin, fibronectin, and core proteins of proteoglycans [112]. Furthermore, elastase can cleave pro-enzyme forms of MMPs into the fully active form of these enzymes [113]. Elastase burden was found to be increased in BAL fluid from patients with IPF [107], suggesting a potential role for this protease in the pathogenesis of IPF.

Of the enzymes found in neutrophil granules, MMPs (notably MMP-2 and MMP-9) have been most closely linked with degradation of ECM components in UIP [89,91]. Exuberant expression of neutrophil-derived serine proteinases and MMPs has also been observed in the airspace and lung interstitium of patients with IPF [91,114]. These proteases may propagate abnormal tissue remodelling in IPF by damage to alveolar basement membranes. An alternative hypothesis suggests that enhanced protease expression may be overly compensated for by even greater expression of TIMPs and other anti-proteases in the lung interstitium of IPF patients, which would have the net effect of diminished proteolytic activity and progressive ECM accumulation. In support of this concept, Selman *et al* observed that IPF lungs display increased TIMP expression compared with MMP expression, suggesting an imbalance between MMPs and TIMPs [89].

In addition to proteolytic enzymes, the neutrophil is an important source of reactive oxygen species [111]. Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase is the predominant enzyme responsible for oxidant production. Upon neutrophil activation, NADPH-oxidase generates $O_2^{\bullet -}$, which dismutates to H_2O_2. Neutrophil-derived myeloperoxidase then catalyses the conversion of H_2O_2, a relatively weak oxidant, into the more potent oxidant HOCl [115]. HOCl and other oxidants may directly convert pro-metalloproteinases into active

enzymes [116], further altering the protease–anti-protease balance. Superoxide anion generated by activated neutrophils can also perpetuate tissue injury by reacting with nitric oxide released by neutrophils, macrophages, endothelial cells, and fibroblasts to form the highly reactive species peroxynitrite and peroxynitrous acid [117]. These metabolites can induce lipid peroxidation *in vitro*; enhanced peroxynitrite activity has been observed in the lungs of patients with IPF [118]. Furthermore, oxidants produced by neutrophils and myofibroblasts may mediate cross-linking of ECM proteins that contribute to the remodelling process [119]. Finally, reactive oxygen species can further amplify neutrophilic inflammation and injury by activating key cellular transcription factors, including NF-κB, AP-1, and JAK-STAT, critical for the activation of proinflammatory and pro-fibrotic genes [120,121].

It remains possible that neutrophils do not play an active role in the pathogenesis of IPF, but might instead be 'bystander' cells recruited in response to mediators released during the fibrotic response. Notably, a family of chemotactic cytokines, referred to as CXC chemokines, exerts potent neutrophil chemoattractant activity and are critical mediators of angiogenesis [122]. In human pulmonary fibrosis, the CXC chemokine family, which consists of both angiogenic and angiostatic molecules, is thought to be important in influencing angiogenesis [123,124]. It has been shown that the expression of the neutrophil chemotactic CXC chemokines interleukin-8 (IL-8/CXCL8) and epithelial neutrophil-activating peptide (ENA)-78/CXCL5 is markedly increased in the BAL fluid and cells isolated from patients with UIP, compared with patients with sarcoidosis or normal volunteers [125]. IL-8/CXCL8 mRNA expression positively correlated with the degree of BAL neutrophilia. However, there was a distinct lack of neutrophilic infiltration in areas of lung where ENA-78/CXCL5 was expressed, suggesting a role for ENA-78/CXCL5 in UIP that is distinct and separate from neutrophil chemotactic effects [124]. Similarly, in a murine model of bleomycin-induced pulmonary fibrosis, these investigators demonstrated that macrophage inflammatory protein (MIP)-2, the murine functional homologue of IL-8 [126], mediates angiogenesis but not neutrophil recruitment [127]. Specifically, neutralization of MIP-2 activity resulted in decreased angio-genesis and attenuated pulmonary fibrosis, but had no effect on the influx of neutrophils into the lung, again suggesting that the neutrophil chemoattractant properties and angiogenic properties of the CXC chemokine family may be quite distinct.

It should be noted that the role of angiogenesis in the development of UIP/IPF remains controversial. Angio-genesis is required for initiation of wound healing and formation of granulation tissue [128]. However, as scar tissue matures, there is an ordered regression of vessels with deposition of collagen and contraction of the wound bed by myofibroblasts [128]. In support of the wound healing model of UIP/IPF, Renzoni *et al* recently observed a net vascular regression in lung biopsy specimens from patients with CFA or with fibrosing alveolitis associated with systemic sclerosis, compared with control samples [129]. These investigators demonstrated decreased vascular density as well as decreased total vascular area in specimens of CFA compared with normals. This finding is in agreement with the earlier findings of Cassan *et al*, who identified decreased mean capillary surface area in nine patients with CFA compared with normal controls [130].

Eosinophils were first postulated to be involved in the pathogenesis of IPF in 1977, when Reynolds *et al* demonstrated a significant increase in BAL fluid eosinophils from patients with pulmonary fibrosis compared with controls [103]. Further investigation revealed that a higher eosinophil count in BAL fluid predicted failure to respond to corticosteroid therapy and a greater likelihood of disease progression [131]. Elevated eosinophil counts and increased levels of an eosinophil-specific protein (eosinophil cationic protein, ECP) in BAL fluid have been shown by other investigators [132,133], but the role of eosinophils in fibrogenesis remains obscure. Wells *et al* compared BAL fluid cellularity from patients with IPF and patients with scleroderma-associated pulmonary fibrosis. These investigators observed that eosinophil

counts were higher in patients with IPF than in patients with scleroderma, suggesting that eosinophils might play a pathogenetic role in IPF and contribute to a worse prognosis [101].

Mast cells have been found in increased numbers in biopsy specimens of patients with IPF [134,135]. In addition, mast cell products, notably mast cell tryptase and histamine, have been recovered from the BAL fluid of patients with IPF [136]. Ultrastructurally, on electron microscopy, mast cells in tissue specimens of IPF show evidence of degranulation [137] and close apposition to fibroblasts [138], suggesting a potential role for mast cells in the pathogenesis of UIP. Incomplete degranulation on electron microscopy suggests a chronic 'piecemeal' degranulation process which contrasts with the total degranulation observed in anaphylaxis [139]. This 'piecemeal' degranulation may result in ongoing tissue injury that promotes fibrosis.

Therapeutic options for pulmonary fibrosis

Currently, no effective treatment for IPF exists. Therapeutic options for IPF have traditionally focused on the paradigm that chronic inflammation leads to tissue injury and fibrosis; thus, corticosteroids have been considered the mainstay of treatment for IPF. However, no prospective, randomized, placebo-controlled trials evaluating their efficacy in the treatment of IPF have been performed [25] and recent data suggest that corticosteroids may be harmful rather than beneficial in these patients [140]. Regimens containing other immunosuppressive and cytotoxic agents, such as cyclophosphamide [141], azathioprine [142], and colchicine [143], have been used but confer no benefit compared with steroids alone or, in some cases, placebo alone. A newer anti-fibrotic agent, pirfenidone, has been proposed in patients with IPF [144]; a phase III, randomized, multi-centre trial with this agent is planned. Interferon-γ has also been shown to have promising effects in the treatment of IPF, although a preliminary study showing efficacy included only 18 patients (nine who received prednisolone and nine who received prednisolone plus interferon-γ [145]. Additionally, in this pilot study, survival benefits were not assessed. Recently, a phase III, double-blinded placebo controlled multi-centre randomized trial of interferon-γ in IPF was completed, but the results of this trial have not yet been published.

Conclusion

IPF is a progressive, fibrotic process of the distal airspaces and interstitium of the lung that is characterized by the histological pattern of usual interstitial pneumonia (UIP). Therapy for this disease with glucocorticoids and other immunomodulatory agents is largely ineffective. UIP is not associated with a signifi-cant inflammatory response; rather, it appears to result from dysregulated epithelial–mesenchymal communication (Figure 3). Only recently have we begun to identify some of the key intracellular pathways and mediators that play a role in the pathogenesis of UIP [146,147]. As our understanding of the pathogenetic mechanisms in UIP/IPF increases, the development of more targeted approaches that interfere with fibrogenesis will help to combat this progressive, disabling and fatal disease.

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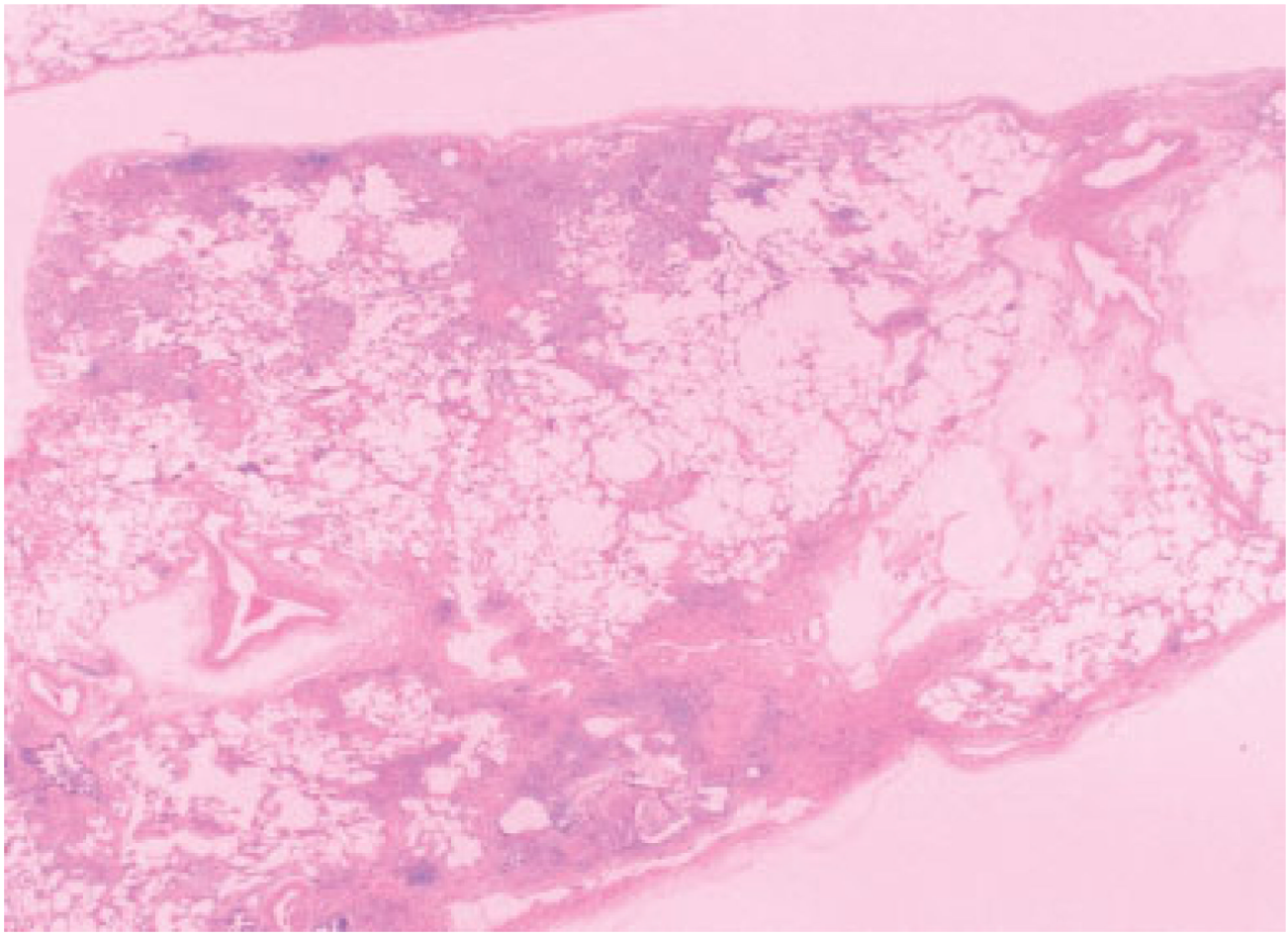

Figure 1.
Histopathology of UIP (low power). H&E-stained whole mount section of lung from a patient with IPF. Note the characteristic peripheral, subpleural location of fibrosis and honeycomb change. These changes are heterogeneous, with regions of lung parenchyma spared from fibrosis

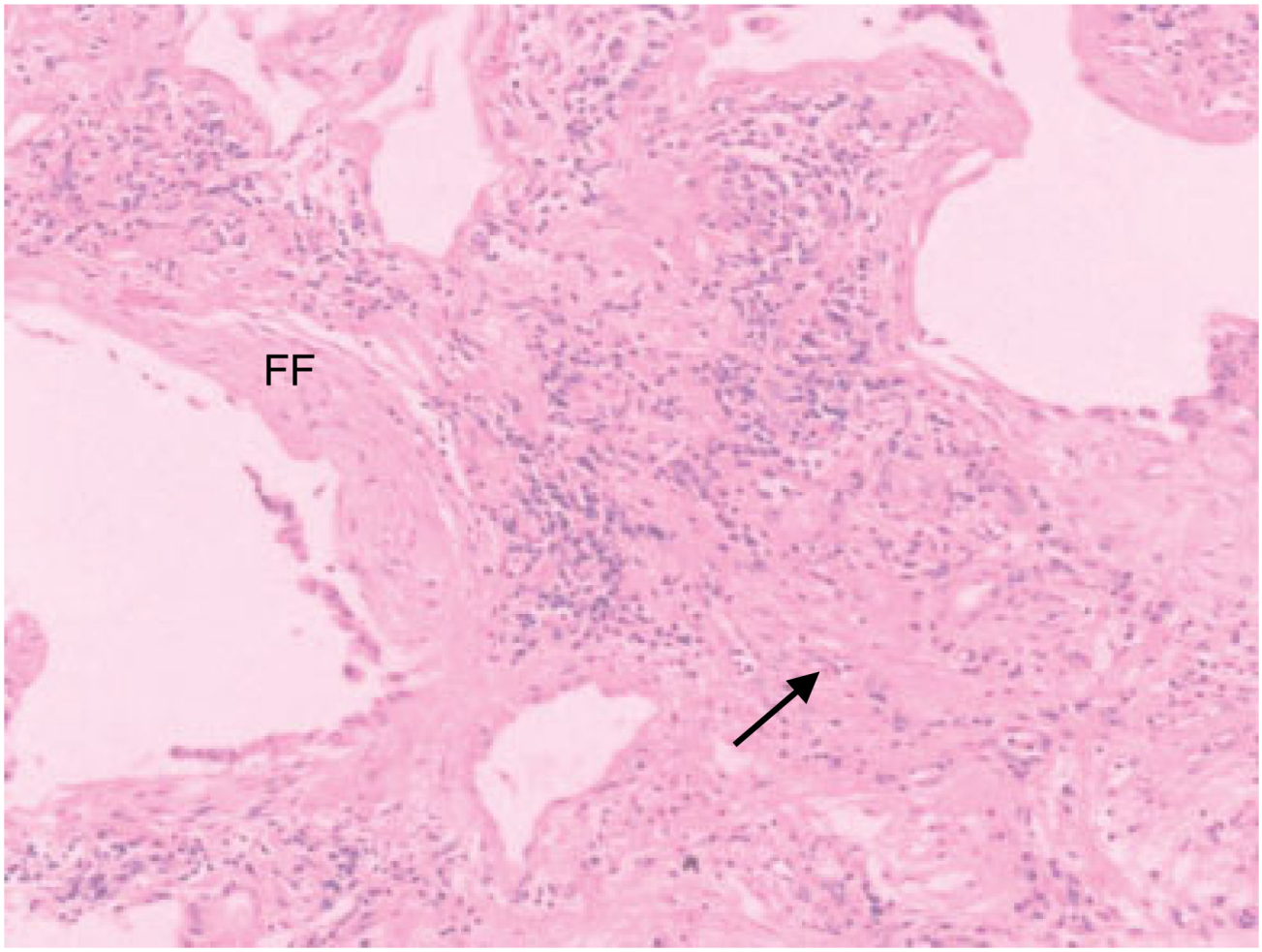

Figure 2.
Histopathology of UIP (higher power). A hallmark of UIP is the heterogeneity, represented by dense, acellular bundles of collagen with smooth muscle metaplasia (arrow) in close approximation to the fibroblastic focus (FF), a dense collection of fibroblasts and myofibroblasts. Cuboidal epithelial cells lining distorted airspaces (which may be undergoing apoptosis) are seen overlying the fibroblastic focus

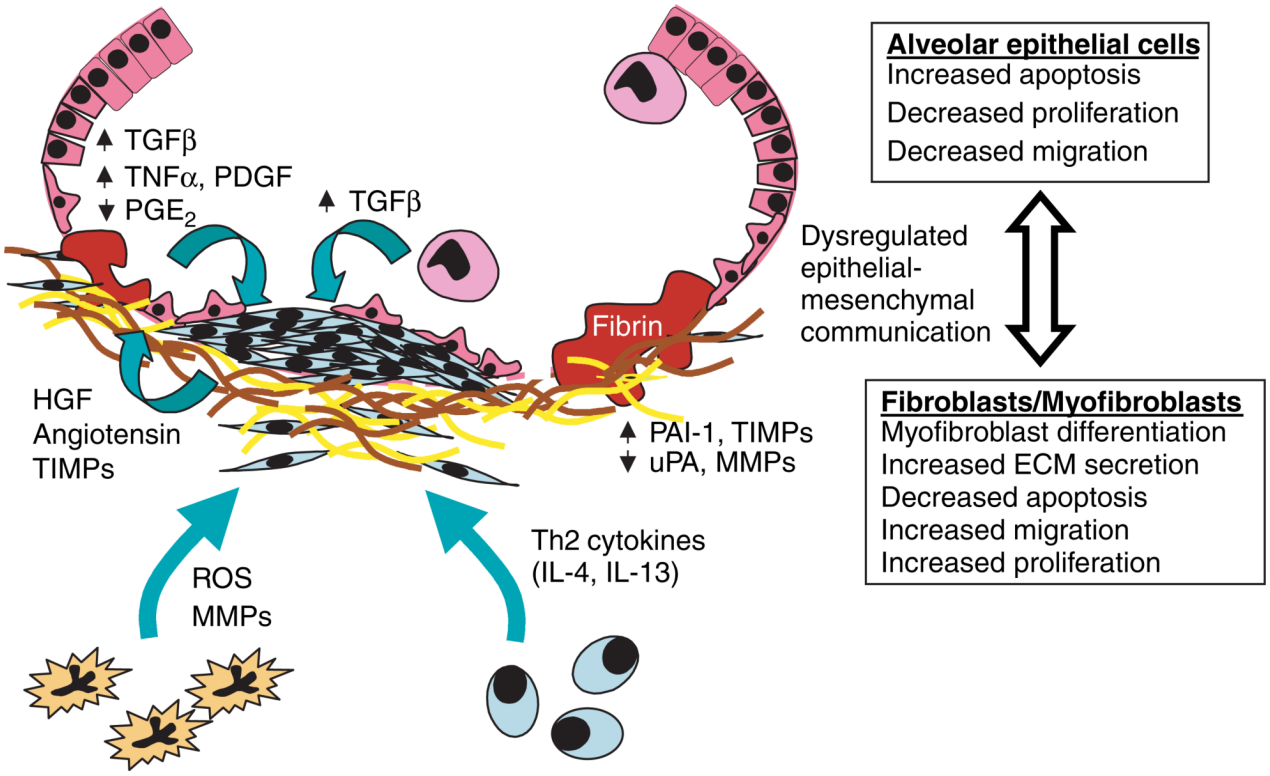

Figure 3.

Overview of some of the key pathogenetic mechanisms in UIP/IPF. Following an unidentified insult, alveolar epithelial cells become injured and delayed re-epithelialization leads to a denuded, disrupted basement membrane. A fibrin clot forms early and serves as a provisional matrix for the migration and proliferation of reparative type I alveolar epithelial cells. Angiogenic factors may be elaborated, leading to the formation of nascent vasculature early in the disease process. Neutrophils secrete pro-inflammatory mediators, reactive oxygen species (ROS) and MMPs, while recruited lymphocytes elaborate the Th2-type cytokines, IL-4 and IL-13. Fibroblasts migrate into the wound and produce extracellular matrix (ECM) proteins and mediators such as angiotensin II which may further promote alveolar epithelial cell apoptosis. Alveolar macrophages and epithelial cells secrete TGF-β1, which promotes myofibroblast differentiation, increases ECM production, and inhibits apoptosis of fibroblasts/myofibroblasts. As ECM deposition progresses, regression of blood vessels may occur. Reciprocal communication between alveolar epithelial cells and mesenchymal cells results in a ‘positive feedback loop’ that promotes ongoing fibrosis and destruction of alveolar architecture

Table 1

Original classification of interstitial pneumonia (Liebow)*

Bronchiolitis obliterans with interstitial pneumonia (BIP)
Desquamative interstitial pneumonia (DIP)
Giant cell interstitial pneumonia (GIP)
Lymphoid interstitial pneumonia (LIP)
Usual interstitial pneumonia (UIP)

*Adapted from ref 4.

Table 2

New classification of idiopathic interstitial pneumonias (ATS/ERS)*

Acute interstitial pneumonia (AIP)
Cryptogenic organizing pneumonia (COP)
Desquamative interstitial pneumonia (DIP)
Respiratory bronchiolitis–interstitial lung disease (RB-ILD)
Lymphoid interstitial pneumonia (LIP)
Non-specific interstitial pneumonia (NSIP)
Usual interstitial pneumonia (UIP)

*Adapted from ref 6.