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Idiopathic Pulmonary Fibrosis
Pathogenesis and Therapeutic Approaches

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Abstract

Idiopathic pulmonary fibrosis (IPF), also termed cryptogenic fibrosing alveolitis, is a clinicopathological syndrome characterised by cough, exertional dyspneoa, basilar crackles, a restrictive defect on pulmonary function tests, honeycombing on high-resolution, thin-section computed tomographic scans and the histological diagnosis of usual interstitial pneumonia on lung biopsy. The course is usually indolent but inexorable. Most patients die of progressive respiratory failure within 3–8 years of the onset of symptoms. Current therapies are of unproven benefit. Although the pathogenesis of IPF has not been elucidated, early concepts focused on lung injury leading to a cycle of chronic alveolar inflammation eventuating in fibrosis and destruction of the lung architecture. Anti-inflammatory therapies employing corticosteroids or immunosuppressive or cytotoxic agents have been disappointing. More recent hypotheses acknowledge that sequential alveolar epithelial cell injury is likely to be a key event in the pathogenesis of IPF, but the cardinal event is an aberrant host response to wound healing. In this context, abnormal epithelial-mesenchymal interactions, altered fibroblast phenotypes, exaggerated fibroblast proliferation, and excessive deposition of collagen and extracellular matrix are pivotal to the fibrotic process.

Several clinical trials are currently underway or in the planning stages, and include drugs such as interferon-γ 1b, pirfenidone, acetylcysteine, etanercept (a tumor necrosis factor-α antagonist), bosentan (an endothelin-1 receptor antagonist) and zileuton (a 5-lypoxygenase inhibitor). Future therapeutic strategies should be focused on alveolar epithelial cells aimed at enhancing re-epithelialisation and on fibroblastic/myofibroblastic foci, which play an essential role in the development of IPF. Stem cell progenitors of the alveolar epithelial cells and genetic and epigenetic therapies are attractive future approaches for this and other fibrotic lung disorders.

Idiopathic pulmonary fibrosis (IPF) is the most common of the idiopathic interstitial pneumonias (IIPs), constituting 47–71% of cases.[1-5] The terms IPF and cryptogenic fibrosing alveolitis (CFA) are synonymous.[6] Current consensus statements reserve the term IPF to refer to a specific clinical entity associated with the histopathological pattern of usual interstitial pneumonia (UIP).[6-8] Surgical lung biopsies in UIP demonstrate varying degrees of alveolar septal (interstitial) and intra-alveolar inflammation and fibrosis.[7,9] However, the pattern of the inflammatory/fibrotic process in UIP is stereotypic and distinctive, as depicted in table I. Other types of IIP include desquamative interstitial pneumonia (DIP),[10,11] respiratory bronchiolitis interstitial lung disease (ILD),[11,12] acute interstitial pneumonia,[13,14] lymphocytic interstitial pneumonia,[15] non-specific interstitial pneumonia (NSIP)[1,2,16] and cryptogenic organising pneumonia (COP).[17] These other histological patterns have a better prognosis and higher rate of response to therapy compared with UIP and are distinct entities.[6-8] A definitive diagnosis of UIP requires surgical lung biopsy,[6] but the diagnosis of UIP can be affirmed with confidence by thin-section, high-resolution computed tomography (HRCT) scans in some patients.[18-20]

1. Epidemiology and Demographics of Idiopathic Pulmonary Fibrosis (IPF)

IPF is rare, with prevalence rates of 3–20 cases per 100 000 people.[21-23] The disease is more prevalent in males,[21,23,24] in older adults[21,24] and in current or former smokers.[21-23,25] Familial IPF/UIP, which accounts for 0.5–3% of cases of IPF, is indistinguishable from nonfamilial forms, except patients with the familial variant tend to be younger.[26,27]

Table I. Histopathological features of usual interstitial pneumonia

Cardinal features	Ancillary features
Heterogeneous (patchy) involvement	Thickened, distorted alveolar walls
Predilection for peripheral (subpleural) and basilar regions	Excessive collagen and extracellular matrix
Bilateral involvement	Scattered inflammatory cells interstitium (alveolar septae) and
Fibroblastic foci (aggregates of proliferating fibroblasts and	alveolar spaces, but not a prominent feature
myofibroblasts)	Bronchiolectasis and/or bronchiectasis
Honeycomb cysts	Smooth muscle hypertrophy
	Hyperplasia and reactive metaplasia of type II pneumocytes
	Mucostasis
	Secondary pulmonary hypertensive changes
	Absence of granulomas, vasculitis, micro organisms or minerals

2. Clinical Features of IPF

Cardinal symptoms of IPF include dry cough and exertional dyspneoa, which progressively worsen over months to years.[6,28,29] On physical examination, basilar, end-inspiratory Velcro rales are present in >80% of patients with IPF/UIP; clubbing is noted in 20–50%.[6,28] Extrapulmonary involvement does not occur. Chest radiographs reveal diffuse, bilateral interstitial or reticulonodular infiltrates, with a distinct predilection for basilar and peripheral (subpleural) regions.[28] Characteristic HRCT features of UIP include: a distinct predilection for basilar and subpleural regions; patchy involvement; coarse reticular or linear opacities (intralobular and interlobular septal lines); honeycomb cysts; traction bronchiectasis or bronchiolectasis; minimal or no ground-glass opacities.[30] Severe volume loss, anatomic distortion and dilated pulmonary arteries are late findings. Zones of emphysema (typically in the upper lobes) may be present in smokers.[31,32] Pulmonary function tests in UIP reveal: reduced lung volumes (e.g. vital capacity and total lung capacity [TLC]); normal or increased expiratory flow rates; increased forced expiratory volume at 1 second (FEV_1) to forced vital capacity (FVC) ratio; reduced diffusing capacity for carbon monoxide (DL_{CO}); widened alveolar-arterial (A-a) O_2 gradient, which is accentuated by exercise; and a downward and rightward shift of the static expiratory pressure volume curve.[33] Lung volumes may be normal if emphysema coexists.[31,32]

Exertional dyspneoa progresses inexorably over months to years, with progressive fibrosis and destruction of lung parenchyma. Most patients die of respiratory insufficiency within 3–8 years from the onset of symptoms.[28,34-36] Mean survival is 2.8–3.6 years.[28,35-38] Although a subset (10–20%) of patients survive more than 10 years, there is no evidence that any form of therapy alters the natural history of the disease. Although the pathogenesis and inciting signals responsible for IPF have not been elucidated, early concepts focused on an unidentified insult which initiated a cycle of chronic alveolar inflammation leading to fibrosis, destruction and distortion of the lung architecture.[9] It was hypothesised that persistence of chronic inflammatory cells within alveolar septae and alveolar spaces (i.e. alveolitis) resulted in: damage and destruction of alveolar walls; loss of type 1 epithelial cells; proliferation of type II epithelial cells; expansion of interstitial fibroblasts and myofibroblasts; exaggerated deposition of collagen and extracellular matrix (ECM); and distortion of the alveolar architecture.[9] Therapies designed to ablate the inflammatory component (e.g. corticosteroids or immunosuppressive or cytotoxic agents) became the mainstay of therapy for IPF, but these agents have marginal or no benefit.[28,35,39] More recent hypotheses acknowledge that sequential lung injury is likely to be a key event in the pathogenesis of UIP, but an aberrant host response to wound healing results in a profibrotic environment, which propagates fibroblast proliferation and exaggerated deposition of collagen and ECM.[29,40,41] A recent hypothesis suggested that IPF may result from abnormal epithelial-mesenchymal interactions, without antecedent 'inflammation'.[40]

There may be at least two different pathogenetic mechanisms for the development of pulmonary fibrosis. First, the inflammatory pathway that mediates drug-induced ILDs, occupation/environment-associated ILD and connective tissue disease-associated ILD. In this context, an early phase of inflammatory pneumonitis is followed by a late phase of fibrosis. Secondly, the alveolar epithelial cell injury pathway appears to be critical to IPF pathogenesis. According to this paradigm, alveolar epithelial cell injury and activation is sufficient to provoke fibrotic responses.[40,42] The plausible mechanisms orchestrating the fibrotic process in IPF and potential novel therapeutic options are discussed in detail later in this manuscript (see sections 4 to 6). However, we initially review historical and conventional therapeutic approaches to this frustrating and enigmatic disease.

3. Current Treatment of IPF

Treatment of IPF is highly controversial. Traditionally, corticosteroids, immunosuppressive or cytotoxic agents have been used, but these treatment options are of unproven benefit[6,35,43] and have potentially serious toxicities.[36,44,45] Although randomised, placebo-controlled therapeutic trials have not been done, several large retrospective studies failed to document survival benefit with any of these forms of therapy. In one retrospective study, the use of corticosteroids or cyclophosphamide was associated with increased mortality.[37] A retrospective study of 487 patients with UIP found no survival benefit with any type of therapy.[35] A review of 238 patients with UIP, most of whom were treated with corticosteroids, cyclophosphamide or a combination of corticosteroids and cyclophosphamide concluded "treatment appeared to have little or no impact on survival compared to no treatment".[46] Japanese investigators confirmed the lack of benefit with corticosteroid therapy.[47] Despite the lack of proven efficacy, in several large series, 39–66% of patients with IPF were treated with corticosteroids.[35-37,48] Immunosuppressive or cytotoxic agents were used in only 2–17% of patients (primarily in unresponsive patients or experiencing adverse effects from corticosteroids).[35-37,48] Anecdotal responses have been cited with cytotoxic agents,[49-54] but the efficacy of these agents is unproven.

Recent international consensus statements concluded that existing therapies for IPF are of unproven benefit, emphasising the need to develop novel therapies.[6,43] A summary of a working conference on IPF convened by the Heart, Lung, and Blood Institute of the National Institutes of Health (Bethesda, MD, USA) in 1998 concluded: "Current therapy has minimal or no beneficial effect for patients with IPF".[55] A recent International Consensus Statement concluded "no data exist that adequately document any of the current treatment approaches improves survival or the quality of life for patients with IPF".[6]

Although the recent consensus statements acknowledge that therapy is of unproven value, they stated that a trial of therapy is reasonable for patients with clinical or physiological impairment or a deteriorating course.[6,43] In this context, both statements recommend combining an immunosuppressive agent (azathioprine) or cyclophosphamide with prednisone or prednisolone 0.5 mg/kg/day for 4 weeks, with gradual taper. When contraindications to corticosteroids exist, either azathioprine or cyclophosphamide alone should be used. These recommendations are reasonable, but have not been validated in scientific clinical trials.

3.1 Corticosteroids

Corticosteroids have been the mainstay of therapy for IPF for more than five decades.[39] Several early studies of patients with IPF/CFA cited response rates of 10–30% with corticosteroids (alone or combined with immunosuppressive agents),[51,52,56-58] but complete or sustained remission were rare. These various published series of IPF or CFA failed to classify patients according to histological entities (e.g. UIP, DIP, NSIP) and cannot be extrapolated to UIP.[28] When the diagnosis of UIP is confirmed by surgical lung biopsies, survival and response rates (to any form of therapy) are dismal (0–16%).[1,3,5,10,28,45] In two recent studies of UIP, British investigators cited favourable responses to

corticosteroids (alone or combined with immunosuppressive agents) in 1 of 14 (7%)[3] and 3 of 28 patients (11%).[1] Japanese investigators treated 30 patients with UIP with corticosteroids; no one improved.[5] A retrospective study in Japan of 234 patients with UIP cited similar mortality rates among untreated patients compared with patients treated with corticosteroids.[47]

Investigators at the Mayo Clinic (Rochester, MN, USA) retrospectively analysed efficacy of therapy among 487 patients with UIP.[35] Treatment regimens included: prednisone alone (n = 54); colchicine plus prednisone (n = 71); colchicine alone (n = 167); other treatment (n = 38); and no therapy (n = 154). By univariate analysis, the use of prednisone alone or prednisone plus colchicine was associated with a worse survival compared with no therapy (odds ratios of 1.5 and 1.4, respectively). On multivariate analysis, the following features were associated with worse survival: age, male gender, lower DL_{CO} and a history of worsening lung function.[35] When these factors were taken into account, survival among patients receiving prednisone was similar to untreated patients.

A retrospective study of 244 patients with CFA cited higher mortality rates among patients treated with either corticosteroids or cyclophosphamide.[37] The higher mortality with corticosteroids likely reflects selection bias, since patients treated with corticosteroids or cyclophosphamide may have had more advanced disease. Given the potential for debilitating adverse effects with corticosteroids,[36,45,59] we believe that high-dose corticosteroids should be discouraged in IPF. We see no role for corticosteroids in patients with a chronic course, extensive fibrosis and absence of ground glass opacities (GGO) on HRCT or patients with specific contraindications to corticosteroids. In contrast, a trial of corticosteroid therapy (combined with azathioprine or cyclophosphamide) is reasonable in patients with GGO on HRCT, a subacute or deteriorating course, young age and no contraindications to corticosteroids. In this context, a trial of prednisone (40 mg/day for 4–8 weeks, with a taper to 20mg within 3–4 months) is reasonable. The dose and duration need to be individualised, depending on the response and presence or absence of adverse effects. Therapy with corticosteroids should be continued beyond 3 months only in patients exhibiting unequivocal and objective responses to therapy.

3.2 Cyclophosphamide

Cyclophosphamide, an alkylating agent which exerts protean and complex immunomodulatory effects on immune responses,[44] has been used to treat IPF in several uncontrolled trials, with anecdotal responses.[52,60,61] However, overall experience has been disappointing.[53,54,62,63] Cyclophosphamide has generally been reserved for patients failing or experiencing adverse effects from corticosteroids or at high risk for complications from long-term corticosteroid therapy.[53,62-68] Cyclophosphamide can be administered orally (daily)[53,62,63] or as an intravenous pulse every 2–4 weeks.[67,68] Data affirming the superiority of cyclophosphamide over corticosteroids are lacking. In a retrospective study from the Mayo Clinic (Rochester, MN, USA), 30 patients were treated with cyclophosphamide, with no demonstrable benefit.[35] Similarly, investigators from the University of Colorado found no benefit with either corticosteroids or cyclophosphamide in a large cohort of patients with IPF.[46] In a retrospective study from England, 25 of 244 (10%) patients with CFA were treated with cyclophosphamide.[37] Survival was worse among patients treated with cyclophosphamide, although this probably reflects a selection bias. In a retrospective study from the University of Iowa (Iowa City, IA, USA) of 39 patients with IPF, the rate of decline of pulmonary function was faster among patients receiving cyclophosphamide compared with patients receiving corticosteroids or no therapy.[66] This does not imply that cyclophosphamide accelerated the rate of deterioration, but suggests that cyclophosphamide is ineffectual in patients with advanced disease.

Only two randomised trials evaluated cyclophosphamide for IPF.[54,64] A short-term (6 month) study at the National Institutes of Health randomised 28 patients with ‘mid-course IPF’ to prednisone alone (n = 16); prednisone plus oral cyclophosphamide

(1.5 mg/kg/day); or cyclophosphamide alone (n = 5).[64] Mean bronchoalveolar lavage (BAL) neutrophil counts declined significantly at 3 and 6 months only in the cohorts receiving cyclophosphamide, but pulmonary function tests or chest radiographs did not change in any group. British investigators randomised 43 patients with untreated IPF to oral cyclophosphamide (1–2 mg/kg/day) plus low-dose prednisolone (20mg every other day) or high-dose prednisolone alone (60 mg/day, with taper).[54] Patients failing initial therapy were crossed over to the alternative regimen. Improvement was noted in 7 of 22 (31%) patients receiving prednisolone alone and in 5 of 21 (23%) patients receiving cyclophosphamide. However, at 3-year follow up, pulmonary function tests improved above pre-treatment baseline in only one patient treated with cyclophosphamide; seven were stable, the rest worsened. Three-year mortality was higher in the prednisolone cohort (10 of 22 died) compared with the cyclophosphamide cohort (3 of 21 died), but this difference was not statistically significant. Long-term survival was poor in both groups. At 5–9 years follow up, 15 patients in each group had died. Interpretation of this study is clouded because patient groups were not well matched at study entry. Patients with more severe pulmonary dysfunction (an independent risk factor for mortality) were disproportionately enrolled in the prednisolone arm. Among 12 patients with TLC <60% predicted, nine were randomised to the prednisolone arm and only three to cyclophosphamide. All 12 failed to respond to therapy.

Several investigators cited low response rates with cyclophosphamide for corticosteroid-refractory IPF. In one study, all eight patients with corticosteroid-refractory IPF failed to respond to cyclophosphamide.[65] French investigators retrospectively analysed 17 patients with IPF treated with cyclophosphamide.[62] Six patients improved, but all six received corticosteroids concomitantly. We prospectively treated 19 patients with corticosteroid-refractory IPF with oral cyclophosphamide 2 mg/kg/day for 6 months.[63] Only one patient improved; seven remained stable and 11 deteriorated. Intravenous 'pulse' cyclophosphamide has been tried in three non-randomised studies in corticosteroid-refractory IPF, but results are unimpressive.[53,67,68] Cyclophosphamide has myriad toxicities, including myelosuppression, oncogenesis, infertility, stomatitis, bladder toxicity, pulmonary toxicity and heightened susceptibility to infections.[44] Because of its limited efficacy and considerable toxicity, we do not recommend cyclophosphamide as therapy for IPF.

3.3 Azathioprine

Azathioprine, a purine analogue which inhibits DNA synthesis and affects both cellular and humoral immunity,[44] and has been used to treat IPF for more than two decades but its efficacy is debatable.[28,49,50,60,61] Uncontrolled studies from Europe cited anecdotal responses to azathioprine combined with corticosteroids;[1,60,61] the independent effect of azathioprine was not clear. Only two prospective studies have evaluated azathioprine for IPF. In both studies, azathioprine was combined with prednisone.[49,50] In the first study, 20 patients with progressive IPF were initially treated with prednisone for 3 months; at that point azathioprine 3 mg/kg/day was added.[50] Favourable responses were achieved in 12 (60%) of patients but the independent effect of azathioprine is impossible to assess since all patients received prednisone concomitantly.[50] In a subsequent prospective, double-blind, randomised trial by these investigators, 27 patients with untreated IPF were randomised to receive azathioprine in combination with high-dose prednisone (n = 14) versus high-dose prednisone plus placebo.[49] At 1 year, FVC, DL_{CO}, A-aO_2 gradient and mortality rates were similar between the two groups (four patients died in each group). Vital capacity improved >10% above baseline in five patients receiving azathioprine plus prednisone and in two patients receiving prednisone plus placebo. DL_{CO} improved (>20% above baseline) in three patients receiving azathioprine plus prednisone and in two receiving prednisone plus placebo. Mortality after 9 years of follow up was lower in the combined therapy group (43% vs 77%), but this difference did not achieve statistical significance. Adverse effects associated with azathioprine include nausea, vomiting, diarrhoea,

leucopenia, anaemia, thrombocytopenia, elevation of hepatic enzymes and idiosyncratic reactions.[44] In contrast with cyclophosphamide, azathioprine does not induce bladder injury and is less oncogenic.[44] Although data are insufficient to judge the efficacy of azathioprine for IPF, we believe a 6-month trial of oral azathioprine 2 mg/kg/day is reasonable in patients with symptomatic or progressive disease.

3.4 Ciclosporin

Ciclosporin (cyclosporin), a fungal decapeptide which exerts potent suppressive effects on T-helper (Th) lymphocyte function and proliferation,[44] has rarely been used to treat IPF. Favourable responses have been cited, but data are limited to a few case reports[1,69,70] and retrospective series.[71-73] Ciclosporin is very expensive and causes myriad adverse effects.[44] Additional studies are required to evaluate the benefit (if any) of ciclosporin for UIP.

3.5 Mycophenolate Mofetil

Mycophenolate mofetil, a purine antagonist with potent immunosuppressive properties,[74] has not been evaluated in IPF/UIP.

3.6 Agents that Influence Collagen Synthesis or Fibrosis

3.6.1 Colchicine

Colchicine, an alkaloid derivative of the plant *Colchicum autumnale*, suppresses components of inflammatory and fibrotic responses,[75] binds microtubular proteins necessary for intracellular trafficking and cellular mitosis,[75] inhibits secretion of collagen and other important growth factors necessary for fibroblast proliferation,[76] attenuates bleomycin- and radiation-induced pulmonary fibrosis in animals,[75] inhibits the release of fibroblast growth factors by human alveolar macrophages *in vitro,*[77] and inhibited fibroblast proliferation and total collagen synthesis *in vitro* in a human lung fibroblast cell line (WI-38).[75] Data regarding the use of colchicine to treat IPF are limited to three retrospective studies from the Mayo Clinic,[35,78,79] one prospective but non-randomised study from Mexico City, Mexico,[80] and one controlled, randomised trial.[59] A retrospective study of 487 patients with UIP seen at the Mayo Clinic found no significant difference in survival between patients treated with colchicine alone (34%), colchicine plus prednisone (15%), prednisone alone (11%), other regimens (8%) or no therapy (32%).[35] By univariate analysis, the relative risk (RR) of death was similar in patients receiving colchicine alone (RR = 1.1) compared with no therapy (RR = 1.0). By univariate analysis, mortality was higher (RR = 1.7) among patients receiving colchicine plus prednisone compared with no treatment (RR = 1.0). By multivariate analysis, after adjusting for other risk factors, there was no evidence that colchicine (or any pharmacological therapy) influenced survival. In the only randomised, prospective study, 26 patients with IPF were treated with either colchicine (n = 14) or prednisone (n = 12).[59] Neither agent was beneficial. Importantly, pulmonary function tests did not improve in any patient in either group. Selman and colleagues[80] prospectively evaluated four treatment regimens in a cohort of 56 patients with IPF. Treatment regimens included: colchicine plus prednisone (n = 19); prednisone plus colchicine plus penicillamine (n = 11); penicillamine plus prednisone (n = 11); or prednisone alone (n = 15). Five-year mortality was 52% and did not differ between regimens. Although data are limited, we do not believe colchicine has a role in the treatment of UIP.

3.6.2 Penicillamine

Penicillamine (D-penicillamine) blocks collagen turnover at several points,[81] inhibits collagen biosynthesis[82] and attenuates the exaggerated collagen deposition in animal models of pulmonary fibrosis,[83] by interrupting cross-linking of collagen molecules.[84] Penicillamine has been used as a putative antifibrotic agent in progressive systemic sclerosis (scleroderma),[83,85-88] but its efficacy is controversial. Data regarding penicillamine to treat IPF are sparse. In one prospective but non-randomised study, 56 patients with biopsy-proven IPF were treated with: prednisone alone (n = 15); prednisone plus colchicine (n = 19); prednisone plus penicil-

lamine (n = 11); or prednisone plus colchicine and penicillamine (n = 11).[80] Patients were followed for up to 5 years. No benefit in survival or pulmonary function tests was noted among the four cohorts. Penicillamine is associated with myriad toxicities (e.g. loss of taste, nausea, vomiting, stomatitis, nephrotoxicity).[6] Given its adverse effect profile and the lack of data affirming its efficacy, we see no role for penicillamine as therapy for IPF.

3.7 Summary

In summary, current therapies for IPF are of unproven value. Major advances in the treatment of IPF await the development of novel therapies that prevent fibroproliferation and/or enhance alveolar re-epithelialisation.[40] In the sections that follow, we explore the putative mechanisms which elicit and orchestrate the fibrotic process operative in IPF.

4. Pathogenesis of IPF

4.1 The Alveolar Epithelial Cell Injury Pathway

Early disruption in the integrity of the alveolar epithelium with altered epithelial cell phenotypes is a distinctive feature of IPF. Alveolar epithelial cells exhibit hypertrophy/hyperplasia and ultrastructural alteration.[89] These morphological changes are accompanied by the expression of specific cytokeratins, suggesting that epithelial cells not only alter their shape, but also their state of differentiation and function.[90,91] Importantly, alveolar epithelial cells themselves express a vast armamentarium of profibrotic cytokines/growth factors. Alveolar epithelial cells in IPF are the main source of platelet-derived growth factor (PDGF), transforming growth factor-β (TGFβ)-1, tumour necrosis factor-α (TNFα), endothelin-1 (ET-1) and connective tissue growth factor (CTGF); all these mediators have been implicated in IPF pathogenesis.[92-97] Despite the prevailing concept that inflammatory cells are the principal source for these fibrogenic soluble mediators, a growing body of evidence supports the notion that injury and subsequent activation of alveolar epithelial cells play an essential role in this process.

Alveolar epithelial cells may also contribute to the formation of a procoagulant/antifibrinolytic microenvironment in the lung by synthesising tissue factor and plasminogen activator inhibitor-1.[98,99] The fibrogenic consequences of decreased alveolar plasmin and excessive fibrin deposition include lack of activation of some of the matrix metalloproteinases (MMPs) responsible for ECM degradation, impairment of epithelial cell migration and increased fibroblast proliferation.[100-102] Interestingly, a prominent fibrogenic role for epithelial cells in other human and experimental fibrotic disorders is well recognised. Idiopathic focal segmental glomerular sclerosis, a renal disease frequently associated with corticosteroid-resistant nephrotic syndrome, is characterised by a number of glomerular epithelial cell alterations that constitute the early stages in the evolution of glomerular scarring.[103,104] Inflammation is not prominent in this disorder. Inflammation is also a minor feature in experimental biliary type liver fibrosis induced by bile duct ligation.[105] In the latter case, the primary lesion promoting fibrogenesis occurs in bile duct epithelial cells. Likewise, in cystic fibrosis-associated liver disease, characterised by accumulation of myofibroblasts around bile ducts and excessive deposit of ECM, the cells triggering fibrogenesis are bile duct epithelial cells.[105,106]

4.2 Fibroblastic/Myofibroblastic Foci: A Key Feature of IPF Pathology

The presence of 'fibroblastic foci' (aggregates of proliferating fibroblasts and myofibroblasts), is a cardinal feature of UIP.[7] Fibroblastic foci appear to develop at sites of prior lung injury[107] and contain fibroblasts with an altered, 'activated' phenotype. Immunohistochemical stains have demonstrated proteoglycans,[108] integrin,[109] vinculin[7] and tenascin[110] within fibroblastic foci. These features indicate that fibrosis is actively ongoing, and not simply a sequela of old fibrosis. Fibroblastic foci are not pathognomonic, but are necessary for the diagnosis of UIP. In a recent study, profusion of fibroblastic foci was associated with progressive disease and poor clinical outcomes.[111]

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.[112] 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 is critical for restoration of normal tissue architecture.[113,114] Fibroblasts with an activated myofibroblast phenotype have been described in the fibroblastic foci that characterise UIP/IPF.[107,115] Gabbiani et al.[116] first described the transient appearance and disappearance of these so-called myofibroblasts in the granulation tissue of healing cutaneous wounds. Myofibroblasts possess ultrastructural features intermediate between fibroblasts and smooth muscle cells; they are defined by their ability to express contractile proteins.[117] This 'contractile phenotype' is functionally important for the closure of cutaneous wounds.[118] In addition, myofibroblasts represent an 'activated' fibroblast phenotype with high synthetic capacity for ECM proteins,[119,120] growth factors/cytokines,[121] growth factor receptors,[122] integrins[123] and oxidants.[124,125] 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 including the lung.[40,126]

Several studies have attempted to characterise the phenotype of fibroblasts-myofibroblasts in UIP/IPF, sometimes with conflicting results. Such differences may relate 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;[127-129] the lower rates of proliferation appear to be associated with more advanced fibrosis.[127] Moreover, fibrotic lung fibroblasts demonstrate anchorage-independent growth in soft agar, whereas normal fibroblasts do not.[130] *In vivo* apoptotic rates of fibroblasts-myofibroblasts from UIP appear to be lower than that found in the fibromyxoid connective tissue of COP;[131] paradoxically, higher apoptotic rates have been observed in *in vitro* culture of UIP/IPF fibroblasts.[128] UIP/IPF fibroblasts are highly synthetic and produce a number of ECM proteins and integrin molecules.[107,109,115,128,132] This is accompanied by reduced capacity for ECM degradation from imbalances in the production of MMPs and tissue inhibitors of metalloproteinases (TIMPs).[128,133] In particular, TIMP-2 expression by UIP/IPF fibroblasts/myofibroblasts appears to contribute to the irreversible structural remodelling of IPF.[133-136] Myofibroblasts in UIP/IPF secrete angiotensin peptides that may induce apoptosis of adjacent alveolar epithelial cells.[137-139] Other phenotypic characteristics described in UIP/IPF fibroblasts include enhanced migratory capacity,[140] increased fibroblast contractility[141] and diminished cyclo-oxygenase (COX)-2 expression/prostaglandin (PG) E_2 synthesis.[142]

There is growing recognition that fibroblasts/myofibroblasts can sustain their growth and activity in the absence of inflammatory cells.[129,143] Interestingly, fibroblasts themselves express surface receptors such as CD40 typically associated with immune cells and are capable of producing a number of chemokines and cytokines.[144-146] This suggests that autocrine mechanisms (or epithelial-derived factors) are sufficient to drive the fibrotic process, even in the absence of ongoing inflammatory stimuli.

5. Novel Approaches to Treatment of IPF

As discussed in sections 2 and 3, IPF/UIP is a progressive disorder with very poor survival rates with current therapies. Treatment based primarily on ablating inflammation has been disappointing. Recent clinical trials suggest that targeting the 'fibroproliferative' process may hold greater promise. In the following sections, we discuss recent clinical trials and potential future approaches based on advances in our understanding of the pathogenesis of IPF.

5.1 Currently in Clinical Trials

5.1.1 Interferon-γ 1b

Interferon (IFN)-γ is a cytokine with pleiotrophic antifibrotic effects, which include: inhibition of fibroblast proliferation and collagen synthesis[147-149] (mediated at least in part by blockade of TGFβ-1 signalling);[150] reduction of tissue myofibroblast numbers;[151] increased expression of MMP-1 message;[152] attenuation of fibrosis in animal models;[153] and inhibition of collagen synthesis by human fibroblasts *in vitro*.[148] Additionally, IFNγ enhances the transcription of the *c-met* proto-oncogene, the receptor for hepatocyte growth factor (HGF), a potent mitogen and motogen for epithelial cells.[154] This suggests a potential role for IFNγ in re-epithelialisation.[154] Immunohistochemical studies of lung tissue from patients with IPF show a deficiency of IFNγ.[155]

Recent studies suggest a promising role for this molecule in the treatment of IPF. An open, randomised trial from Vienna, Austria cited favourable responses to IFNγ-1b and low-dose prednisolone in a small cohort of 18 patients with IPF.[156] All patients had previously failed therapy with corticosteroids. Patients were randomised to prednisolone 7.5 mg/day alone or combined therapy with IFNγ-1b 200μg (administered subcutaneously three times weekly plus prednisolone 7.5 mg/day. At 12 months, pulmonary function tests deteriorated in all nine receiving prednisolone alone. By contrast, pulmonary function tests improved in all nine patients in the IFNγ-1b cohort. This beneficial effect was associated with a down-regulation of TGFβ-1 and CTGF gene transcription. These data are encouraging but should be viewed with caution. A retrospective review of the enrolled patients by an independent panel demonstrated that some of the cases represented non-IPF diagnoses such as NSIP. Importantly, all patients in that trial were deficient in IFNγ message measured in transbronchial biopsies.[157]

The results of two subsequent open-label, non-randomised trials using IFNγ-1b as therapy for IPF were presented at the 2001 meeting of the American College of Chest Physicians.[158,159] In one study of 17 patients with IPF treated with IFNγ-1b, symptoms and pulmonary function tests did not improve.[158] Another series analysed 33 consecutive patients treated with IFNγ-1b for progressive IPF (all had failed conventional therapy).[159] Six patients died; no patient improved in physiological parameters. Compared with the study by Ziesche and colleagues,[156] patients in both of these subsequent off-label trials[158,159] had more advanced disease. Results of a prospective, multicentre, randomised European trial were presented in September 2003 at the Annual Meeting of the European Respiratory Society.[160] In that study, 33 patients with IPF were randomised to receive either oral colchicine 1 mg/day (n = 10) or subcutaneous IFNγ-1b 200μg three times a week (n = 23). Both cohorts received prednisone for 2 months prior to enrolment and received maintenance low-dose prednisone 10 mg/day for the duration of the study. Patients in the IFNγ-1b cohort had a trend towards less dyspneoa after 6 months of treatment ($p = 0.07$), but these results are preliminary. Furthermore, given the small sample size, the significance of this finding is not clear.

Results of a large, multicentre, placebo-controlled, randomised trial evaluating IFNγ-1b as therapy for IPF were recently published.[161] In that study (GIPF-001), 330 IPF patients from 58 centres were randomised to IFNγ-1b and low-dose prednisolone, or low-dose prednisolone plus placebo. A trend towards lower mortality was noted in the IFNγ-1b cohort but results were not significant (10% compared with 17% mortality in the placebo group, $p = 0.08$). Therapy with IFNγ-1b was associated with more frequent constitutional symptoms. However, treatment adherence was similar in the two groups. More pneumonias were reported among patients in the IFNγ-1b group, but the incidence of severe or life-threatening respiratory tract infections was similar in the two groups. The authors conclude that IFNγ-1b did not affect progression-free survival, pulmonary function or the quality of life in this well defined population of IPF patients. However, owing to the size and duration of the trial, a clinically significant survival benefit could not be ruled out. IFNγ-1b is exceptionally expensive (>$US50 000

annually; 2003 values) and additional studies are required to determine the role (if any) of IFNγ-1b as therapy for IPF.

It is also possible that, under some conditions, IFNγ may contribute to fibrogenesis. Chen et al.[162] demonstrated that mice with a homozygous null mutation of the IFNγ gene developed significantly less inflammation and fibrosis than the wild type after bleomycin instillation. These findings suggest that IFNγ might play a profibrotic role under certain conditions, particularly in disorders such as sarcoidosis and other granulomatous disorders characterised by Th-1 cytokine network with enhanced production of this cytokine. Furthermore, human IFNγ gene has a variable length CA repeat in the first intron and polymorphisms of this microsatellite is associated with variations in the production of IFNγ. Interestingly, the presence of allele 2 that correlates with higher production of IFNγ, was also associated with high frequency of allograft fibrosis after lung transplantation.[163]

5.1.2 Pirfenidone

Pirfenidone attenuates pulmonary fibrosis in experimental animal models,[164,165] inhibits TGFβ-stimulated collagen synthesis,[166] reduces synthesis of collagen I and III and TNFα,[167] decreases ECM and blocks the mitogenic effect of profibrotic adult human lung fibroblasts from IPF patients.[127] In a prospective, open-label phase II trial, Raghu et al.[168] treated 54 IPF patients with pirfenidone (46 had failed conventional therapy; eight were untreated). Following institution of pirfenidone, 'conventional' therapy was discontinued in 38 of 46 (83%) patients. With pirfenidone, 1- and 2-year survival rates were 78% and 63%, respectively. After 6 months of therapy, pulmonary function tests stabilised or improved in some patients, but data are difficult to interpret as only 41 patients had repeat pulmonary function tests. Six patients (11%) died within 6 months. Chest radiographs did not improve. Adverse effects were cited in 87% of patients but were not severe. Six patients (11%) discontinued therapy due to adverse effects. These data are encouraging but further studies are required to assess efficacy.

Nagai and colleagues[169] treated 13 patients with pulmonary fibrosis (idiopathic [n = 11]; associated with scleroderma [n = 2]) with oral pirfenidone 40 mg/kg/day for 1 year. Three patients with pulmonary hypertension died of cardiac failure within 3 months and were not included. Of the remaining, ten patients were followed for 2 years; the disease progressed in eight and remained stable in two. Among the ten treated patients, six died within 2 years. Recently, Azuma et al.[170] presented preliminary results on a double-blind phase II study of pirfenidone versus placebo in Japan. In the pirfenidone cohort, functional parameters improved and there were fewer acute exacerbations of IPF in patients with moderate disease. Similarly, a recent study performed in patients with pulmonary fibrosis associated with Hermansky-Pudlak syndrome found that patients treated with pirfenidone had a slower decline in FVC, FEV_1 and DL_{CO} in comparison with the placebo group.[171] Pirfenidone is not commercially available and additional studies are required to assess its role. A double-blind, prospective, multicentre study is currently underway.

5.1.3 Acetylcysteine

Acetylcysteine, which stimulates glutathione synthesis, has been used as an antioxidant screen in IPF, but efficacy is unproven.[172,173] In a prospective open trial, 20 patients with idiopathic or collagen vascular disease-associated pulmonary fibrosis were treated with high dose oral acetylcysteine 600mg three times daily for 3 months.[174] After 3 months, levels of total glutathione and reduced glutathione were increased in BAL fluid and in epithelial lining fluid. Changes in pulmonary function tests were minimal; BAL differential counts did not change. Currently, a randomised, double-blind, placebo-controlled trial is under way in seven European countries to assess the possible role of acetylcysteine in UIP.[175] In this study, oral acetylcysteine 1800 mg/day or placebo will be added to conventional therapy with prednisone 0.5 mg/kg/day, with taper, plus azathioprine 2 mg/kg/day.

5.2 Future Therapeutic Strategies

Since no drug therapies have clearly demonstrated efficacy to alter the progressive and highly lethal nature of IPF, a number of therapeutic strategies have been advocated. Many of these strategies are designed to inhibit/antagonise cytokines or growth factors involved in fibrogenesis. Many are also under study in other tissue fibrosis. The list of these putative antifibrotic agents is constantly growing. We discuss a few of the most promising of these based on results from animal models and on their mechanism of action.

5.2.1 Targeting Alveolar Epithelial Cells

Injury to alveolar epithelial cells is an early and consistent finding in the pathology of IPF. Inability to regenerate alveolar epithelial cells can delay re-epithelialisation and promote fibrosis. Approaches to regenerate and repair the alveolar epithelium may involve administration of specific alveolar epithelial cell mitogens or progenitor cells capable of differentiating into epithelial lining cells.

Alveolar Epithelial Cell Mitogens

Two epithelial mitogens, keratinocyte growth factor (KGF) and hepatocyte growth factor (HGF), may have potential roles in the treatment of fibrotic lung disorders, including IPF. KGF is a potent growth factor for type II alveolar epithelial cells both *in vitro* and *in vivo*.[176,177] Recent data suggest that *in vivo* administration of KGF enhances the alveolar epithelial repair rate by non-mitogenic mechanisms including modification of cell adherence, spreading and migration, and through stimulation of the EGF receptor.[178] Administration of KGF before injury markedly attenuates experimentally-induced lung damage. However, KGF is not protective when it is administrated at the time of or following injury. Treatment with KGF 72 hours before hydrochloric acid instillation in rat lung reduced morphological damage, inflammation and fibrosis and improved survival; post-treatment instillation of KGF was not protective.[179] Intratracheal instillation of KGF 72 and 48 hours before radiation- or bleomycin-induced lung injury protected against fibrosis and improved survival.[180] In this study, post-injury treatment with KGF was not attempted. In a similar experiment, post-treatment with KGF did not prevent bleomycin-induced lung injury and fibrosis.[181]

In contrast with these findings, HGF, a ligand for the *c*-Met receptor tyrosine kinase, may provide protection even after the injury has been initiated. This epithelial growth and anti-apoptotic factor has been successfully used to prevent renal fibrosis and liver cirrhosis. Administration of recombinant human HGF (rhHGF) in a spontaneous mouse model of chronic renal disease during the early stages of renal insufficiency induced epithelial tubular proliferation, suppressed the expression of the profibrotic mediators, TGFβ-1 and PDGF, and reduced the profusion of myofibroblasts.[182] In this study, progression of renal fibrosis and dysfunction was attenuated. The administration of rhHGF after the development of rat liver cirrhosis induced by thioacetamide resulted in reduced collagen and TGFβ-1 synthesis, enhanced hepatocyte proliferation and decreased the degree of fibrosis.[183] Similar results have been reported in other models of chronic hepatic injury.[184]

Two exciting studies have been reported in experimental lung fibrosis as well.[185,186] First, Yaekashiwa et al.[185] induced lung fibrosis in mice by continuous infusion of bleomycin for 7 days. Some mice were treated simultaneously with rhHGF, while another group received the recombinant growth factor 7 days after the final dose of bleomycin. Both simultaneous and delayed administration of HGF significantly reduced the fibrotic response, suggesting that it may be useful to prevent or even treat lung fibrosis. In a separate study, administration of rhHGF 3 and 6 days after a single intratracheal instillation of bleomycin ameliorated the accumulation of collagen and the extent of lung fibrosis.[186] In this study, HGF also enhanced epithelial cell surface plasmin generation, expression of uPA (urokinase-type plasminogen activator) activity and cell migration *in vitro*.

Stem Cell Progenitors of the Alveolar Epithelium

Recent evidence suggests that tissue-specific stem cells can differentiate into multiple cell lin-

eages, including those other than the tissue of origin.[187,188] Murine multipotent adult progenitor cells infused intravenously into postnatal animals engraft and differentiate into cells of the haematopoietic lineage, as well as epithelial cells of the liver, lung and gut.[187] Cultured bone marrow cells injected into wild-type recipient mice after bleomycin-induced lung injury were capable of forming lung alveolar epithelium.[189] Moreover, these cells serve specifically as type I pneumocyte precursors and no donor-derived type II pneumocytes were detected at any time. Mice exposed to bleomycin were more likely to show lung engraftment than PBS-treated animals suggesting that the injury may signal the recruitment and differentiation of these bone marrow-derived progenitor cells. The requirement for an 'injury stimulus' is supported by recent findings in humans following bone marrow transplantation; in this case, marrow progenitor cells were found not to differentiate into respiratory epithelium of the healthy upper airway, although they appear capable of differentiating into other cell types.[190]

Recently, it was corroborated that mesenchymal stem cells home into lung tissue in response to bleomycin injury but, more importantly, it was demonstrated that they also reduce its fibrotic effect. Engrafted stem cells were localised to damaged areas and exhibited an epithelial-like morphology.[191]

5.2.2 Targeting Fibroblasts

Fibroblasts/myofibroblasts play an essential role in the development of IPF. They represent a key target for antifibrotic drug therapy. Potential strategies involve the inhibition of fibroblast migration/proliferation, induction of fibroblast/myofibroblast apoptosis, attenuation of ECM expression/secretion and 'deactivation' of myofibroblasts.

Inhibitors of Fibroblast Migration/Proliferation

The mechanisms implicated in the formation of subepithelial fibroblastic foci are yet uncertain. However, chemotactic and proliferative factors are likely to be involved. Selective phosphodiesterase inhibitors, such as rolipram and cilomilast, inhibit the chemotaxis of human fetal lung fibroblasts toward fibronectin.[192] Eicosanoids are lipid mediators derived from COX and lipoxygenase metabolic pathways of arachidonic acid which exhibit both antifibrotic and profibrotic effects.[193] PGE_2 has potent immunomodulatory and antifibrotic effects. PGE_2 inhibits fibroblast proliferation and collagen production, and blocks fibroblast chemotaxis.[194,195]

Interestingly, fibroblasts from IPF lungs exhibit a striking defect in their capacity to synthesise PGE_2 and fail to increase COX-2 protein expression/activity in response to a number of agonists, apparently because of diminished capacity to induce COX-2.[142] Conversely, leukotrienes (lipid mediators of inflammation derived from the 5-lipoxygenase pathway of arachidonic acid metabolism) are profibrotic. Leukotrienes promote leucocyte chemotaxis,[196] inhibit leucocyte apoptosis, generate pro-inflammatory substances such as interleukin-8[197] and TNFα,[198] and may promote fibrosis by influencing fibroblast migration, proliferation and matrix protein synthesis.[199] Patients with newly diagnosed IPF exhibit overproduction of leukotrienes in the lung.[200] Increased leukotriene B4 levels have been demonstrated in BAL fluid of patients with IPF[201] as well as asbestosis.[202] Further, leukotrienes are overproduced in bleomycin-induced pulmonary fibrosis in animal models.[199] The fibrosis is attenuated in mice rendered leukotriene deficient by knockout of the 5-lipoxygenase gene compared with wild-type control mice.[199] Further, lavage levels of the anti-inflammatory and antifibrotic molecule, PGE_2, were greater in the knockout mice. These data provide a rationale for a trial of pharmacotherapy with an inhibitor of 5-lipoxygenase metabolism. Zileuton, a direct 5-lipoxygenase inhibitor that is approved for the treatment of asthma,[203] is a candidate agent, and is currently being evaluated in a prospective, randomised trial at the University of Michigan, Ann Arbor, MI, USA.

Alternatively, anti-inflammatory molecules derived from the arachidonic acid cascade (e.g. prostacyclin [PGI_2], PGE_2), may have promise as treatment for IPF. Beneficial effects of an oral PGI_2 analogue, epoprostenol, in patients with pulmonary hypertension have been demonstrated.[204] Additionally, aerosolised epoprostenol appears to improve

pulmonary hypertension secondary to lung fibrosis without affecting gas exchange and systemic arterial pressure.[205] These agents have the potential, therefore, to exert both antifibrotic and antihypertensive effects; moreover, they are already approved for other clinical indications and have the added advantage of localised administration.

Inductors of Fibroblast/Myofibroblasts Apoptosis

At least theoretically, a logical method to reduce fibroblast/myofibroblast expansion is by induction of apoptosis, as occurs in normal wound healing.[113,206] Programmed cell death allows for the elimination of specific populations of cells without additional tissue damage. The HMG-CoA reductase inhibitors (statins) are cholesterol-lowering agents that have been in clinical use for 15 years. Interestingly, these inhibitors induce apoptosis of a number of cell types, including fibroblasts/myofibroblasts and smooth muscle cells.[207-209] Clinically achievable concentrations of lovastatin induce apoptosis in normal and fibrotic lung fibroblasts.[207] Lovastatin also induced fibroblast apoptosis *in vivo*, in a guinea pig wound chamber model.[207] Simvastatin induced regression of cardiac hypertrophy and fibrosis, improved cardiac function, and reduced extracellular signal-regulated kinase (ERK) 1/2 activity in a transgenic rabbit model of human hypertrophic cardiomyopathy.[210] However, the same drug has no effect on hepatic fibrosis induced in rats by bile duct ligation.[211]

Another putative antifibrotic effect of statins is related to their ability to inhibit the expression of CTGF by interfering with the isoprenylation of Rho proteins.[212] CTGF is rapidly induced in fibroblasts by the action of TGFβ-1, and it appears to mediate profibrotic activities of this growth factor, including ECM production/remodelling in fibrotic tissues. Statins are well tolerated apart from two uncommon but potentially serious adverse effects: elevation of liver enzymes and skeletal muscle abnormalities, which range from benign myalgias to life-threatening rhabdomyolysis.[213]

Inhibitors of Extracellular Matrix Production

Relaxin, a pregnancy-related peptide hormone, decreases the expression of interstitial collagens and fibronectin while increasing collagenase-1 (MMP-1) in human lung and dermal fibroblasts.[214,215] Relaxin alone or in combination with IFNγ reduces collagen synthesis by scleroderma-derived fibroblasts.[216] Additionally, relaxin decreases TIMP-1 and TIMP-2 secretion by activated hepatic stellate cells.[215,217] These *in vitro* antifibrogenic activities have been corroborated *in vivo* in a number of experimental models including lung fibrosis induced by bleomycin.[215,217,218] In the bleomycin model, relaxin was administered by continuous infusion 7 days after a single intravenous bleomycin injection. At steady-state serum levels of ~50 μg/L, relaxin induced a noteworthy reduction in fibrosis morphometric analysis and collagen content.[215]

Importantly, a randomised, double-blind, placebo-controlled trial has been performed in patients with systemic sclerosis.[219] Sixty-eight patients with moderate to severe diffuse sclerodermia were included. Recombinant human relaxin at 25 or 100 μg/kg or placebo was administered by continuous subcutaneous infusion over 6 months. At 4, 12 and 24 weeks the group receiving the lower dose exhibited a significant reduction in fibrotic skin scores. A smaller decrease in FVC was also noticed in this group. Surprisingly, the group receiving the highest dose did not show differences compared with placebo. Menometrorrhagia, reversible anaemia and local skin reactions were the most common drug-related adverse effects.

Deactivating Myofibroblasts

Myofibroblasts represent an 'aggressive' profibrotic phenotype that may contribute to increasing lung contractility and decreasing compliance. Phenotypic modulation of these cells offers another exciting opportunity for therapeutic intervention. IFNγ reduces the expression of α-smooth muscle actin and changes the morphology of TGFβ-1-induced myofibroblasts.[220] Two antifibrotic compounds, lufironil (HOE-077) and safironil, designed primarily as competitive inhibitors of prolyl-4-hydroxylase, prevented the activation of liver stellate cells and also accelerated their deactivation both *in vitro* and *in vivo* in a rat model of liver injury.[221]

Interestingly, this effect occurs primarily in females. The grapevine-derived polyphenol, trans-reversatrol, decreased the expression of α-smooth muscle actin and migration of fibroblasts in a monolayer wounding assay of cultured human liver myofibroblasts.[222]

5.2.3 Anticytokine Therapy

Several profibrotic growth factors/cytokines have been implicated in IPF pathogenesis and often mediate their effects through redundant pathways. It is difficult to conceive that targeting a specific profibrotic cytokine, growth factor or vasoactive peptide may be the solution for this complex disease. Some studies suggest that blocking some of these factors may have important antifibrotic effects.

5.2.4 Transforming Growth Factor-β

TGFβ is the prototypical profibrotic cytokine and has been targeted in several studies. A soluble TGFβ type II receptor construct inhibited collagen expression and fibrogenesis in a model of chronic liver injury.[223] Treatment with TGFβ-1 antiserum significantly diminished lung fibrosis provoked by repeated intranasal exposures to heat-killed bacillus Calmette-Guerin.[224] A chimeric TGFβ-1 soluble receptor that has high affinity for TGFβ-1, but lacks the ability to initiate signal transduction events, was able to significantly reduce pulmonary fibrosis when administered 2 days after bleomycin instillation in hamsters.[225] Anti-pan TGFβ antibodies administered by tail vein injection on day 1 and again on day 6 post-blood marrow transplant prevented the skin thickening as well as lung fibrosis in a murine model of sclerodermatous graft-versus-host disease.[226] Inhibition of other cytokines/growth factors may also have antifibrotic effects *in vivo*.

5.2.5 Tumour Necrosis Factor-α

TNF-α, a cytokine with pleiotropic effects on inflammatory and fibrotic processes, may stimulate fibroblast proliferation and collagen gene upregulation through a TGFβ and/or PDGF pathway.[93] However, TNFα may also suppress collagen gene expression.[227] Mice which overexpress TNFα spontaneously develop lung fibrosis accompanied by a chronic lymphocytic infiltrate.[228] Paradoxically, overexpression of TNFα was protective in a murine model of bleomycin or TGFβ-induced fibrosis.[229] TNFα gene expression rises in the murine lung after administration of bleomycin,[230] while animals missing TNFα receptors are relatively resistant to bleomycin-induced fibrosis.[230] Infusion of a 55kD human recombinant soluble TNFα receptor, rsTNFRβ, prevented the development of fibrosis in bleomycin- and silica-induced lung fibrosis in murine models.[231] In that study, the antifibrotic effect was seen even when the recombinant protein was administered 25 or more days after instillation of bleomycin or silica.

Over-expression of TNFα may play a role in the pathogenesis of IPF. Human alveolar macrophages obtained by BAL from patients with IPF or asbestosis produce increased amounts of TNFα when compared with non-diseased controls.[232] Hyperplastic type II cells of IPF patients contain significant amounts of TNFα by immunohistochemical stains.[233] Recent data suggest an association between TNFα promoter polymorphisms and an increased risk of developing IPF.[27] These insights suggest that blocking the effects of TNFα could be beneficial in IPF. Importantly, treatment with chimeric monoclonal antibodies to TNFα or soluble TNF receptor fusion protein, is beneficial in patients with rheumatoid arthritis, Crohn's disease and active ankylosing spondylitis.[234-236] An open-label pilot study evaluated the TNFα antagonist etanercept in nine patients with UIP.[237] All patients had worsening pulmonary function tests despite conventional therapy. Although data are preliminary, about half of the patients in this study showed objective functional improvement or stabilisation after an average of 2 years' follow up, suggesting that blocking TNFα may reduce or prevent further loss of lung function in IPF patients. A prospective, multicentre, double-blind, placebo-controlled trial of etanercept for the treatment of IPF is in the planning stages. Complications of therapy attributed to its immunosuppressive effects have been noted. An increased incidence of tuberculosis has been reported soon

after the initiation of infliximab, an antibody directed against TNFα.[238]

5.2.6 Protein Kinases

Blocking post-receptor signalling pathways with inhibitors of protein kinases may also be effective. Moreover, such inhibitors may also target receptor kinases. Imatinib (STI-571), a c-Abl tyrosine kinase inhibitor that also inhibits activation of the PDGF receptor, significantly reduces bone marrow fibrosis in humans.[239] Imatinib appears to be effective in the treatment of chronic myelogenous leukaemia and is US FDA-approved for this indication.

5.2.7 Endothelin-1

Endothelin-1 (ET-1) is a potent vasoconstrictor and mitogenic peptide that promotes fibroblast synthesis of collagen types I and III; it also inhibits both protein expression and activity of MMP-1.[240] Paracrine and autocrine ET-1 secretion modulates the migration and proliferation of fibroblasts.[241,242] Interestingly, transgenic mice over-expressing human pre-pro-ET-1 develop progressive diffuse lung fibrosis without any previous injury.[243] ET-1 is strongly upregulated in IPF lungs and is expressed mainly in epithelial cells.[97,244] Some studies have suggested that inhibiting ET-1 effect may have antifibrotic effects.[245,246] Use of ET-1 receptor antagonists in experimental lung fibrosis, however, give contradictory results. Bosentan, a nonselective ET(A) and ET(B) receptor antagonist, induces morphometric improvement in bleomycin-induced lung fibrosis.[247] In contrast, the same antagonist, or a specific ET(A) receptor antagonist LU-135253 failed to prevent collagen deposition and fibrosis in rat models of pulmonary fibrosis[248] and myocardial infarction,[249] respectively. Stimulated alveolar macrophages from patients with scleroderma-related lung disease secrete increased amounts of ET-1; fibroblast proliferation induced by BAL from these patients is inhibited by ET(A) receptor antagonists.[250,251] These data support a potential role of EL-1 in the development of pulmonary fibrosis. A trial of endothelin antagonists as therapy for IPF is currently underway.

5.2.8 Angiotensin II

Angiotensin II, a vasoactive peptide of the renin angiotensin system, may play an important role in fibrogenesis. Angiotensin II induces proliferation of mesenchymal cells, including human lung fibroblasts, and increases the expression of ECM proteins.[252-254] Some of these effects seem to be mediated by the autocrine/paracrine action of TGFβ-1.[254] Angiotensin peptides are involved in alveolar epithelial cell apoptosis induced by fibroblasts obtained from fibrotic lung.[139] Blockade of angiotensin II effects by ACE inhibitors and angiotensin type 1 receptor antagonists appear to be effective in a variety of experimental models of fibrosis involving the kidney, liver, heart and lung.[254-258] Moreover, treatment with an angiotensin II receptor antagonist significantly decreases plasma levels of TGFβ-1 and endothelin in transplant patients with chronic allograft nephropathy.[259]

Our results with ACE inhibitors in patients with IPF have been less successful. Nine IPF patients were treated with high doses of inhaled corticosteroids plus colchicine, and nine with inhaled corticosteroids plus captopril. No differences were found in pulmonary function tests at 1 and 2 years of follow up; mortality rates were similar.[260] Recent studies in experimental models suggest that a better approach might be to block the angiotensin II type 1 receptor rather than ACE inhibition.

5.2.9 Gene Therapy

Somatic cell gene therapy is likely to play an increasing role in the management of monogenic human disorders. This approach may be more difficult in complex diseases involving the expression/activation of several genes. IPF is likely to be polygenic with complex interactions between genetic susceptibility, environmental factors and, perhaps, the influence of aging. Nevertheless, gene therapy has been attempted with some success in several experimental fibrosis models.

HGF gene therapy has been explored in a rat model of lethal liver cirrhosis induced by dimethylnitrosamine.[261] In this study, repeated transfections of the human HGF gene into skeletal muscle induced an increase in human and rat HGF

plasma levels and tyrosine phosphorylation of the *c*-met/HGF receptor. This therapy suppressed the increases in TGFβ-1 expression and myofibroblasts, inhibited hepatocyte apoptosis and resulted in almost complete resolution of fibrosis/cirrhosis of the liver. HGF therapy appeared to be effective even when started after the fourth week of initial injury when fibrosis was already present. Similar results have been reported in experimental chronic renal fibrosis induced by unilateral ureteral obstruction in mice.[262] In this study, systemic administration of naked plasmid encoding HGF markedly ameliorated renal fibrosis.

TGFβ pathways have also been targeted by gene therapy approaches. Gene transfer of Smad 7, an antagonist of TGFβ-1 signalling, prevented fibrosis in post-obstructed rat kidney.[263] Smad 7 was introduced the next day after ureteral ligation by a recombinant adenovirus vector combined with *in vivo* electroporation. Similar attempts have been made in pulmonary fibrosis. Nakao et al.[264] examined the effect of Smad 7 introduced by a recombinant human type 5 adenovirus vector on bleomycin-induced pulmonary fibrosis in mice. In this case, Smad 7 was given at the onset of lung injury by intratracheal injection, and mice were studied at 4 weeks. Levels of exogenous Smad 7 expression persisted until day 21 and significant attenuation in lung fibrosis by histology and collagen content was noted. Liu et al.[265] tested the effect of adenoviral-mediated transfection of soluble TGFβ-III receptor on airway fibrosis in a rat model of obliterative bronchiolitis. Topical gene transfections performed on day 5 after heterotopic allogeneic tracheal transplant lead to inhibition of airway fibrosis and obliteration.

Enhanced alveolar fibrinolytic activity may improve lung repair after injury and attenuate the fibrotic response. Sisson et al.[266] transferred the *uPA* gene to the lungs of mice injured by bleomycin. The human uPA cDNA was introduced 3 weeks after bleomycin instillation and mice were analysed 1 week later. Results showed a significant attenuation of pulmonary fibrosis by histology and collagen content.

In summary, gene therapy is yet another attractive future approach for fibrotic lung disorders. The obstacles to such therapy include the efficiency of gene transfer with the need for sustained expression of the transgene, safety of the vectors utilised and localisation of delivery.

5.2.10 Epigenetic Approaches

Antisense oligonucleotides are commonly used *in vitro* to down-regulate the expression of specific genes. Such approaches may also be effective *in vivo*. Decoy oligonucleotides that bind the Sp1 transcription factor inhibited collagen 1A2 promoter activity both in cultured fibroblasts and *in vivo*, in the skin of transgenic mice, which have integrated a mouse collagen 1A2 promoter/luciferase reporter gene construct.[267] Administration of adenovirus expressing an antisense mRNA complementary to the 3′ coding sequence of TGFβ-1 reduced the expression of TGFβ-1 and the activity of TGFβ-1 responsive genes.[268] This strategy has also been tested *in vivo* with encouraging results.

Antisense oligonucleotides against heat shock protein 47, a collagen-specific molecular chaperone, attenuated experimental glomerulonephritis.[269] In this study, antisense oligodeoxynucleotides were introduced into the left renal artery of rats treated simultaneously with anti-Thy-1 antibody which induces mesangiolysis followed by acute proliferative glomerulonephritis; a significant decrease in sclerotic lesions was associated with reduction in glomeruli expressing HSP47 and type I and III collagens.

TGFβ-1 antisense oligodeoxynucleotides in a model of renal fibrosis induced by unilateral ureteral obstruction is also protective.[270] TGFβ-1 antisense was instilled through a ureteral catheter prior to ureteral ligation. Northern analysis and *in situ* hybridisation revealed a significant decrease in the expression of TGFβ-1 and type I collagen as well a decrease in the fibrotic response by histology.

Similarly, retrovirally mediated delivery of angiotensin II type 1 receptor antisense injected via the cardiac route attenuated the development of high blood pressure in a spontaneous hypertensive rat model, and consequently, ameliorated a number of pathological changes including the presence of mul-

tifocal and perivascular fibrosis.[271] Thus, antisense oligonucleotides have been shown to alter gene expression and function in *in vivo* animal models of fibrosis. The potential efficacy of such approaches in human diseases is not known.

6. Conclusion

IPF remains a therapeutic challenge. Our expanded knowledge of IPF pathogenesis and novel approaches to block fibrogenic pathways offer hope for future interventions. However, this is likely to be associated with significant problems and risks. Many drugs that have potent antifibrotic effects *in vitro* have been found to be ineffective *in vivo*. Experimental models to test the efficacy of drug targets *in vivo* have relied heavily on the bleomycin-induced lung fibrosis, a model that may not be representative of human IPF. The timing of drug administration in such models is also problematic. Drug administration before or simultaneously with initial injury does not simulate the clinical scenario in which IPF patients present most often after fibrosis is well established. Cytokines and growth factors are pleiotropic molecules that have multiple activities on diverse cell types. An anticytokine/growth factor therapy may have beneficial effects on the fibrotic process but may be harmful in other conditions or pose additional risks. This is exemplified by TGFβ-1 that has important tumour-suppressive activities in addition to its profibrotic effects. Progress in the treatment of IPF patients will require the cooperative effort of large multicentre clinical trials performed in a controlled, prospective, randomised, manner.

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