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Idiopathic Pulmonary Fibrosis—an Epidemiological and Pathological Review

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Abstract Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease (ILD) affecting the pulmonary interstitium. Other forms of interstitial lung disease exist, and in some cases, an environmental etiology can be delineated. The diagnosis of IPF is typically established by high-resolution CT scan. IPF tends to have a worse prognosis than other forms of ILD. Familial cases of IPF also exist, suggesting a genetic predisposition; telomerase mutations have been observed to occur in familial IPF, which may also explain the increase in IPF with advancing age. Alveolar epithelial cells are believed to be the primary target of environmental agents that have been putatively associated with IPF. These agents may include toxins, viruses, or the autoantibodies found in collagen vascular diseases. The mechanism of disease is still unclear in IPF, but aberrations in fibroblast differentiation, activation, and proliferation may play a role. Epithelial–mesenchymal transition may also be an important factor in the pathogenesis, as it may lead to accumulation of fibroblasts in the lung and a disruption of normal tissue structure. Abnormalities in other components of the immune system, including T cells, B cells, and dendritic cells, as well as the development of ectopic lymphoid tissue, have also been observed to occur in IPF and may play a role in the stimulation of fibrosis that is a hallmark of the disease. It is becoming increasingly clear that the pathogenesis of IPF is indeed a complex and convoluted process that involves numerous cell types and humoral factors.

Keyword Idiopathic pulmonary fibrosis

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Introduction

A number of diseases can affect the pulmonary interstitium, and many of them lead to fibrosis. Many of these interstitial lung diseases (ILDs), also called interstitial pneumonias/pneumonitis or diffuse parenchymal lung diseases, are caused by infection, environmental or occupational toxins (e.g., silicosis), certain medications, radiation therapy of the chest, or autoimmune disease (e.g., rheumatoid arthritis, systemic sclerosis, systemic lupus erythematosus, or Sjögren's syndrome). However, a substantial portion of cases are classified as idiopathic. In 2002, an international consensus statement by the American Thoracic Society and the European Respiratory Society (ATS/ERS) classified idiopathic interstitial pneumonias into the following seven clinicopathologic entities: idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP), cryptogenic organizing pneumonia (COP), acute interstitial pneumonia (AIP), respiratory bronchiolitis-associated interstitial lung disease, desquamative interstitial pneumonia (DIP), and lymphoid interstitial pneumonia [1] (Fig. 1). In this classification system, the term "cryptogenic fibrosing alveolitis" (CFA) is synonymous with IPF, although historically CFA was used to describe a characteristic clinical presentation seen not only in IPF but also in other

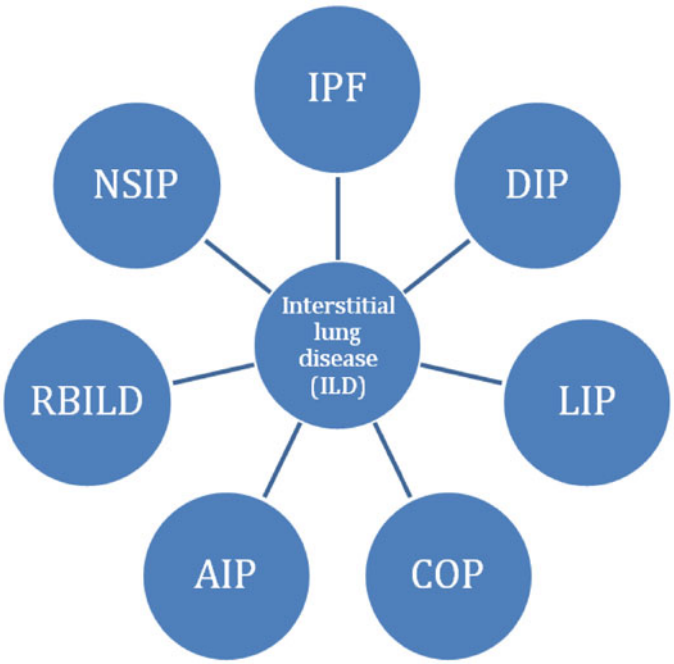

Fig. 1 ATS/ERS classification of interstitial lung disease

IPF = Idiopathic pulmonary fibrosis, NSIP = Non-specific interstitial pneumonia, COP = Cryptogenic organizing pneumonia, AIP = Acute interstitial pneumonia, DIP = Desquamative interstitial pneumonia, LIP = lymphoid interstitial pneumonia, RBILD = Respiratory bronchiolitis-associated interstitial lung disease

types of ILD and included a range of histological patterns [1, 2]. In particular, it has been estimated that NSIP made up 20–35% of patients previously diagnosed with CFA [2]. Furthermore, NSIP was considered a provisional diagnosis in the ATS/ERS publication [1], but an expert panel convened under the auspices of the ATS subsequently confirmed that it constituted a distinct clinical entity [3].

Idiopathic Pulmonary Fibrosis

Definition and Diagnostic Criteria [4]

In 2000, the ATS and ERS published a joint statement identifying the following criteria for a definite diagnosis of IPF:

1. Presence of a surgical lung biopsy showing usual interstitial pneumonia (UIP)
2. Exclusion of other known causes of ILD such as drug toxicities, environmental exposures, and collagen vascular diseases
3. Abnormal pulmonary function studies that include evidence of restriction and/or impaired gas exchange with rest or exercise or decreased diffusing capacity of the lung for CO (DL_{CO})
4. Typical abnormalities on high-resolution computed tomography (HRCT) scans as described in more detail below

Since surgical lung biopsies cannot be or are not performed in all patients, the ATS also provided a list of four major and four minor criteria (listed in Table 1) that allow the diagnosis of probable IPF if all of the major and three of the minor criteria are met.

Clinical, Radiological, and Histopathological Features [4]

Clinical symptoms of IPF including cough, dyspnea (labored breathing resulting in shortness of breath), and Velcro-type end-inspiratory crackles on chest auscultation are detected in more than 80% of patients. Clubbing is seen in 25–50% of patients. Pulmonary function tests typically show restrictive impairment (reduced vital capacity and total lung capacity by body plethysmography), although pulmonary function may be normal or near normal in the early phase. Frequently, there is a reduction in DL_{CO} corrected for hemoglobin, and gas exchange may be impaired. The lung volumes will eventually be reduced in all patients with IPF.

Almost all patients with IPF have abnormal chest radiographs at the time of presentation, typically showing peripheral reticular opacities, which are usually bilateral

Table 1 Criteria for a diagnosis of probable IPF [4]

Major criteria	Exclusion of other known causes of ILD, such as certain drug toxicities, environmental exposures, and connective tissue diseases
	Abnormal pulmonary function studies that include evidence of restriction (reduced VC often with an increased FEV1/FVC ratio) and impaired gas exchange (increased $AaPo_2$ with rest or exercise or decreased DL_{CO})
	Bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scans
	Transbronchial lung biopsy or bronchoalveolar lavage showing no features to support an alternative diagnosis
Minor criteria	Age >50 year
	Insidious onset of otherwise unexplained dyspnea on exertion
	Duration of illness ≥3 months
	Bibasilar, inspiratory crackles (dry or "Velcro" type in quality)

A definite diagnosis requires the presence of a surgical biopsy showing UIP plus the first three of the major criteria

and often asymmetric. However, a normal radiograph does not preclude the presence of IPF. Typical HRCT findings consist of patchy, predominantly peripheral, subpleural, and bibasilar reticular opacities combined with the presence of honeycombing in a basilar/subpleural distribution, the latter being virtually diagnostic of UIP and constituting a prerequisite for a HRCT diagnosis of definite UIP. Other HRCT features include traction bronchiectasis, irregular interlobular septal thickening, and minimal ground glass attenuations.

The histopathological correlate of IPF is UIP. This pattern is characterized by temporal and spatial heterogeneity, with alternating areas of normal lung, patchy fibrosis with remodeling of the lung architecture, and honeycomb change, affecting primarily the peripheral subpleural parenchyma. Interstitial inflammation generally is not prominent. The histological hallmark of UIP, particularly in IPF, is the presence of fibroblastic foci, consisting of fibroblasts and myofibroblasts covered by hyperplastic alveolar epithelium. Of note, biopsy specimens from patients with a histological pattern of UIP may show other patterns, in particular NSIP, in the same or another lobe. Since it has been shown that the presence of a UIP pattern determines the clinical course and prognosis of such patients, the default diagnosis in such cases is that of UIP.

Differential Diagnosis [1, 4]

A UIP pattern can also be seen in the context of a variety of connective tissue diseases, in particular rheumatoid arthritis, and the existence of such diseases must be ruled out. In rare cases, however, UIP/IPF is the first manifestation of a connective tissue disease, which may not manifest for several years. Of note, up to 20% of patients with IPF may exhibit circulating anti-nuclear antibodies or rheumatoid factor, usually at low titers. High titers should prompt suspicion of a connective tissue disease. Certain drug toxicities can also be associated with a UIP pattern. A UIP pattern may develop in the late stages of asbestosis, but exposure to asbestos alone does not necessarily mean that the fibrosis is due to asbestos. An appropriate exposure history and the presence of asbestos bodies upon lung biopsy may help distinguish asbestosis from IPF. Asbestosis has a more peribronchiolar distribution, honeycomb changes are uncommon except in advanced cases, fibroblastic foci are rare, and pleural plaques and/or a more diffuse visceral pleural fibrosis are common [5]. Typically, a long latency between asbestos exposure and appearance of fibrosis is consistent with IPF, not asbestosis. The fibrosing pattern of NSIP may also resemble the UIP pattern but can be distinguished by its temporal uniformity compared to the temporal heterogeneity of the lesions in UIP. In addition, the histological differential diagnosis of UIP includes all of the other idiopathic ILDs, pulmonary histiocytosis, and chronic hypersensitivity pneumonitis (HP). A full differential diagnosis for IPF is shown in Tables 2 and 3.

It has frequently been hypothesized that NPIS—at least the fibrotic form—represents an early form of IPF. In surgical biopsy specimens, UIP and NSIP patterns can be found in the same lung and even within the same lobe, but the prognosis is determined by the presence of UIP [6, 7]. The prognosis of NSIP in this and other studies is much better compared to IPF/UIP [6, 8, 9], and it has been speculated that this could represent a lead-time bias in the evolution of UIP [6]. In addition, patients with histological UIP, but an indeterminate or NSIP appearance on HRCT, survived significantly longer than patients with UIP and an HRCT scan showing typical IPF appearance (median survival 5.76 vs. 2.08 years) [8]. However, a study comparing biopsy and explant specimens from the same patients did not reveal a single instance where a NSIP pattern in the biopsy evolved into a UIP pattern at explant [7]. The authors pointed out that in order for the uniform distribution of fibrosis characteristic of the NSIP pattern to evolve into the patchy, temporally and spatially heterogeneous involvement characteristic of UIP, some areas would have to revert to normal while others progressed to irreversible fibrosis—a process that seems highly unlikely.

Another relationship between different ILDs is highlighted by the occurrence of a pattern of diffuse alveolar damage (DAD), which is the histopathological pattern

Table 2 Differential diagnosis of idiopathic pulmonary fibrosis

Other forms of interstitial lung disease
Non-specific interstitial pneumonitis
Desquamative interstitial pneumonitis
Respiratory bronchiolitis-associated interstitial lung disease
Cryptogenic organizing pneumonia
Acute interstitial pneumonitis
Lymphoid interstitial pneumonitis
Bronchiolitis obliterans organizing pneumonia
Pulmonary histiocytosis
Connective tissue diseases
Systemic lupus erythematosus
Progressive systemic sclerosis
Rheumatoid arthritis
Sarcoidosis
Hypersensitivity pneumonitis
Fungal hypersensitivity pneumonitis
Aspergillus
Organic dust
Infections
HIV infection
EBV
HCV
Parvovirus
Occupational or toxin (chemical)-related lung disease
Silicosis
Asbestosis
Cigarette smoke
Agricultural/farm products
Wood/metal dust
Sand/stone/silica
Livestock
Medication-related interstitial lung disease
Antibiotics
Bleomycin
Radiation
Cardiovascular drugs
Other conditions
Cystic fibrosis
Lymphoma
Eosinophil granuloma
Chronic aspiration
Lymphangioleiomyomatosis
Wegener's granulomatosis
Diabetes

typically seen in AIP, although it can also be caused by infections and some drugs or of organizing pneumonia (OP), the pattern of COP, or bronchiolitis obliterans with organizing pneumonia (BOOP), during acute exacerbations.

Clinical Course and Prognosis

The latency period of IPF is unknown, but several observations suggest that such a period exists. First, the onset of symptoms is generally insidious indicating that there is a very gradual increase in disease severity over time before the symptomatic stage is reached. Second, there are reports in the literature of asymptomatic cases that were discovered during routine health exams, yet had histologically proven IPF/UIP [10]. The strongest evidence for the existence of a considerable latency period comes from studies of relatives of patients affected by familial IPF [11]. An investigation of 164 subjects from 18 kindreds affected with familial IPF revealed that 22% of the asymptomatic subjects had CT abnormalities suggestive of ILD. These subjects were approximately 20 years younger on average than those with diagnosed familial IPF (46 and 67 years, respectively), but older than those without symptoms and normal HRCT findings. This suggests that it may take decades for early asymptomatic ILD to progress to symptomatic IPF. Note that subjects with preclinical familial ILD also had higher serum concentrations of MMP7, one of the most significant protein biomarkers that may distinguish IPF patients from normal controls [12]. A latency period of a decade or more is consistent with findings in other ILDs with known causes. For example, symptoms in subjects with asbestosis occur typically approximately 15 years after initial exposure [5], while chronic silicosis occurs approximately 10 years after exposure [13].

Patients with IPF generally experience a decline in lung function, which often progresses slowly, but inexorably over years, resulting in scarring, pulmonary failure, and death. However, a substantial portion of patients experience acute exacerbations, characterized by rapid deterioration in lung function and new or increased radiographic abnormalities in the absence of infection or other identifiable causes of acute lung injury. Histologically, patients with acute exacerbations show patterns of acute lung injury, such as DAD or—less frequently—OP superimposed on the UIP pattern [14]. Acute exacerbations carry an extremely poor prognosis, with mortality rates exceeding 50%. Of note, acute exacerbations do not only occur in IPF but also in NSIP, where they are also characterized by either DAD or OP [14]. A rapidly progressive decline in lung function from onset has been reported in a subset of patients with IPF. Such patients exhibit a markedly different transcriptional profile from patients with relatively stable IPF, with genes involved in integrin signaling, cell migration, connective tissue degradation, chemokine signaling, and retinoic X receptor/vitamin D receptor activation constituting the most prominent pathways in the progressive group [15, 16]. This suggests the existence of distinct subtypes of IPF.

Among the ILDs, IPF has the poorest prognosis, with median survival from the time of diagnosis generally

Table 3 Interstitial fibrosis in the lung can be caused by many different factors including

Cystic fibrosis	Paraquat	Practolol
Idiopathic pulmonary fibrosis	Bleomycin	Chloramphenicol
Mitral valve stenosis	Graft versus host disease	Caplan's disease
Lymphangiomyomatosis	Dressler's syndrome	Amyloidosis
Acute lung syndrome	Carmustine	Nitrofurantoin
Pulmonary edema	Radiotherapy	Sarcoidosis
Pneumoconiosis	Systemic sclerosis	Silicosis
Shaver's disease	Systemic lupus erythematosus	Weber–Christian disease
Tuberous sclerosis	Wegener's granulomatosis	Busulphan
Scleroderma	Penicillamine	Tropical pulmonary eosinophilia
Idiopathic pulmonary hemosiderosis	Mitomycin C	Peplomycin
Methysergide	Graphite pneumoconiosis	Bronchopulmonary dysplasia
Whipple's disease	Aluminum lung	Amiodarone
Talc pneumoconiosis	Polymyositis	Asbestosis
Rheumatoid disease	Histiocytosis X	Drugs
Sporotrichosis—pulmonary	Hexamethonium	
Interstitial lung disease	Chemical poisoning—tungsten	
Gold salts	Methotrexate	

ranging between 2.5 and 4 years [17–21]. Of note, in a cohort of IPF patients listed for lung transplantation, the age-adjusted mortality was significantly higher for blacks and Hispanics compared to Whites, and both ethnicities remained significantly associated with increased mortality risk after adjusting for a variety of demographic, clinical, and comorbidity factors [22]. The significance was lost after adjusting for predicted forced vital capacity percent, suggesting that worse lung function at the time of listing accounted for much of the heightened mortality risk. Interestingly, there are indications that UIP in association with connective tissue diseases carries a significantly better prognosis than in IPF [19, 23]. Therefore, the presence of connective tissue disease, younger age, and better lung function at baseline emerged as independent favorable prognostic factors in one of these studies [23].

Treatment of IPF is usually limited to the use of corticosteroids, which is itself associated with many side effects. Other immunosuppressants have been used to treat IPF. Oxygen may improve quality of life. Lung transplant is a therapeutic option for IPF, but the outcome is slightly worse than that performed for other causes, with a 5-year survival of only 44% in a series of 82 patients with IPF [24]. Outcome was better in the cases of double lung transplantation as compared to single lung transplantation (55% vs. 34% 5-year survival).

Epidemiology and Genetics of IPF

Demographics

IPF is the most common of the idiopathic ILDs. Its incidence increases with advancing age, being very low in people aged <35 years, increasing markedly beyond the fifth decade of life, and peaking in those aged ≥75 years. IPF is frequently found to be somewhat more common in males than in females [21, 25], although this was not observed in a population-based study from Finland [26]. The annual incidence of IPF in a recent population-based study from Olmstead County, Minnesota, for the period from 1997 to 2005 ranged between 8.8 and 17.4/10^5, depending on whether narrow or broad criteria were used for case identification [17]. A study based on large health care claims databases covering essentially the same period yielded very similar estimates, namely between 6.8 and 16.3/10^5 when extrapolated to the USA [25]. This does not differ greatly from an earlier estimate from a population-based study in Bernalillo County, New Mexico, conducted between 1988 and 1990 and showing an annual incidence of 11 and 7/10^5 in men and women, respectively [27].

Considerably lower annual incidence rates have been reported from several European countries, e.g., 2.17/10^5 in a population-based study from Denmark covering the years 2001–2005 [28], 0.93/10^5 in Greece in 2004 [29], ~3/10^5 in Spain in 2000/2001 [30], and 4.6/10^5 over the period from 1991 to 2003 in the UK [21]. Of note, whereas the UK study indicated that the incidence of IPF more than doubled during the observation period, the population-based data from the USA (Olmsted County) and Denmark show a declining incidence, particularly during the latter years of the study [17, 28].

Prevalence estimates are 17.9–63/10^5 in Olmstead County, USA [17], 14–42/10^5 extrapolated to the USA overall [25], 16–18/10^5 in Finland [26], and 3.38/10^5 in Greece [29]. Because of the different case finding methodologies, it is not possible to determine whether the marked

differences in IPF incidence and prevalence reported from various countries reflect true geographical or ethnic differences. Incidence and prevalence rates in different ethnicities living within the same geographical area have not been investigated. Interestingly, in a retrospective US cohort of >2,600 IPF patients listed for lung transplantation, Hispanic and particularly Black patients were significantly younger and more frequently female than White patients [22].

Global gene expression profiling of 20 patients with IPF/UIP and six patients with NSIP (five cellular, one fibrotic) has revealed very similar transcriptional patterns in the two diseases, with only eight genes being differentially regulated [31]. In partial contrast, a three-way comparison of transcriptional profiles from IPF/UIP, NSIP, and HP patients indicated that only two of the eight NSIP patients (apparently with fibrotic NSIP) exhibited a gene expression pattern resembling that of IPF, one NSIP sample resembled the HP pattern, while the others were distinct from both IPF and HP [32]. Of note, in this study, IPF and HP showed distinct functional profiles, with genes involved in tissue remodeling and extracellular matrix structure and turnover defining IPF, whereas genes associated with inflammation and T cell activation dominated the HP profile. It has been argued that, altogether, these findings "provide compelling evidence for the idea that a single disease entity may be responsible for causing a spectrum of histological abnormality" [33].

Familial IPF

Although most cases of IPF are sporadic, the disease can occasionally occur in familial form. The term "familial IPF" is most commonly defined as IPF affecting two or more family members. Based on this definition, familial cases were found to constitute 3.3–3.7% of all Finnish cases of IPF diagnosed according to the ATS/ERS criteria in a recent nationwide prevalence study [26]. Similarly, in the UK, it was estimated that familial cases accounted for 0.5–2.2% of all patients with CFA, although that study included an unknown proportion of patients with NSIP and DIP [34]. Although autosomal recessive or more complex modes of inheritance cannot be ruled out completely, the available data are consistent with an autosomal dominant pattern of inheritance with variable penetrance.

The clinical, radiologic, and pathologic features and survival in the familial and sporadic forms of IPF are indistinguishable [18, 35], but the age of onset tends to be earlier in familial IPF (e.g., 61.9 vs. 65.3 years in Finland [26]). The similarity between familial and sporadic IPF is further supported by the results of a recent gene expression profiling study showing that the gene expression patterns of the two groups were largely similar, although the degree of over- or underexpression was significantly greater in familial cases [31].

It has been demonstrated that different histological forms of idiopathic interstitial pneumonia can be present within the same family. In an investigation of 111 families with two or more cases with idiopathic interstitial pneumonia, 61 families were affected exclusively by IPF/UIP, while more than one type of histological pattern occurred in the remaining 50 (45%) families [35]. The most common combination was IPF and unclassified ILD, followed by IPF and NSIP, and IPF and COP. In another study, when six asymptomatic family members of patients affected by IPF underwent open lung biopsy because of clinical and radiographic findings suggestive of IPF or another ILD, they were found to have UIP in three cases and HP, NSIP, and COP in one case each [11]. These observations further suggest that there is a familial (and probably genetic) predisposition to interstitial lung disease in general, with individual susceptibilities or exposures determining which specific type of ILD develops. This supports the notion that the different histological patterns of ILD may share etiological and even pathological aspects.

Recently, familial IPF has been associated with mutations in the enzyme telomerase. Telomeres are nucleotide repeat–protein complexes at the ends of chromosomes and play a critical role in preserving chromosomal integrity. Telomeres shorten with each cell division, and once a critical length is reached, cells reach cellular senescence, i.e., loss of proliferative capacity and ultimately apoptosis. The enzyme telomerase is responsible for partially restoring telomere length after cell division. It consists of two essential components, namely telomerase reverse transcriptase (TERT) and telomerase RNA (TERC), the latter providing the template for nucleotide addition by TERT. Alterations in telomerase biology, some arising from mutations in the *TERT* or *TERC* genes, are associated with a variety of disorders, including dyskeratosis congenita, which is associated with pulmonary fibrosis in ~20% of affected individuals. Note that only ~40% of subjects affected by this disease actually carry mutations in TERT or TERC, and telomere length rather than mutations in telomerase appears to be associated with the development of dyskeratosis congenita.

A subset of patients with familial IPF (8%) show heterozygous mutations in either the *hTERT* of the *hTERC* gene [36]. Analyses of families with two or more cases of idiopathic ILD, including many with IPF, suggested even higher frequencies of such mutations (up to 24%) [37, 38]. This suggests that mutations in *hTERT* or *hTERC* confer susceptibility to ILD in general, rather than being associated with a specific histologic subtype. Of note, some of the family members of IPF patients were found to be asymptomatic despite being mutation carriers, which is consistent with the incomplete penetrance of the IPF phenotype [36]. On the other hand, they were also younger

than the subjects with a definite diagnosis of IPF, and some of them may still have been in the latency phase of disease development. Mutations in *hTERT* and *hTERC* genes were found to result in significantly shorter telomere length in peripheral blood lymphocytes and granulocytes, even though not all mutations caused decreased telomerase activity in vitro [36, 39].

Telomerase mutations were identified in only 1–3% of patients with sporadic IPF [37–39]. Nonetheless, telomere length in peripheral blood mononuclear cells was significantly shorter, and the frequency of short telomeres (below the 10th percentile of control values) was significantly higher in sporadic IPF patients compared to healthy controls [38, 39]. This indicates that short telomere length is associated with IPF even in the absence of telomerase mutations. Interestingly, a recent genome-wide association study identified a significant association between nine single nucleotide polymorphism and susceptibility to IPF in Japanese patients, including one within the TERT gene [40]. An additional case–control analysis indicated that a common variant of *TERT* was significantly associated with disease risk (odds ratio (OR) of 2.11). In addition to genetic susceptibility, aging, chronic oxidative stress, and smoking are all linked to telomere attrition, and all of these factors have been implicated in IPF. In addition, transforming growth factor-β (TGFβ), a profibrotic cytokine that is abundantly expressed in the lungs of patients with IPF, suppresses telomerase expression.

Type II alveolar epithelial cells (AECs) replenish damaged type I AECs via proliferation and differentiation and express telomerase. Therefore, defective telomerase activity in this cell type would be expected to diminish the capacity to regenerate the alveolar epithelium after repeated injury and thereby contribute to the increased rate of apoptosis of type II AECs and the defective re-epithelialization observed in IPF lungs. To date, telomerase activity and telomere length in type II AECs have not been investigated. There is, however, a report of markedly lower levels of immunoreactive telomerase in IPF compared to control type II AEC and telomerase expression correlated inversely with the rate of type II AEC apoptosis in unaffected areas of IPF lungs, whereas no such association was seen in healthy controls [41].

Environmental, Occupational, and Other Risk Factors

Even though IPF is, by definition, of unknown etiology, numerous epidemiological studies have tried to identify causative or exacerbating agents. Since alveolar epithelial cells are thought to be the main target of IPF, inhaled toxins are good candidates for being among the initiating agents. Indeed, in case–control studies, smoking is the most consistently identified risk factor for developing both the sporadic and the familial form of IPF [35]. Even asymptomatic family members with radiographic evidence of ILD more frequently had a history of smoking than family members without any signs of lung disease [11]. A recent meta-analysis of the results from five case–control studies yielded a summary estimate odds ratio of 1.58 (confidence interval (CI) 1.27–1.97) for smoking [42]. A strong risk in association with smoking was also identified in a Japanese cohort [43]. Similarly, Mexican patients with IPF were more likely to be former, though not current or ever, smokers compared to controls [44]. However, in a recent large case–control study from the UK including 920 patients with IPF and almost 3,600 controls, neither current nor former smoking was identified as a significant risk factor [45]. Even more inconsistent findings have been reported for various occupational exposures, but a meta-analysis indicated that agriculture/farming, livestock, wood and metal dust, and stone/sand/silica exposures all increased the risk of developing IPF [42].

Co-existing Conditions

In 2003, a Japanese group of researchers published the first report of an association between type II diabetes mellitus and IPF with an adjusted OR of 4.06 (CI 1.80–9.15) [43]. Since then this has been confirmed in studies from the UK [45], and type II diabetes mellitus was found to be the strongest independent predictor of IPF in Mexican patients [44].

The frequency of gastroesophageal reflux disease is significantly higher in patients with IPF compared to controls [45–48]. An analysis of data from the Patient Treatment File of the Department of Veterans Affairs showed that, among >100,000 case subjects with reflux esophagitis or esophageal strictures and an equal number of controls, pulmonary fibrosis was among the lung disorders that showed a significant association with this condition in a multivariate analysis [49]. Significant associations were also seen with chronic bronchitis, bronchial asthma, chronic obstructive pulmonary disease, bronchiectasis, pulmonary collapse, and pneumonia. Of note, only a minority of patients with IPF and gastroesophageal reflux, as determined by esophageal pH monitoring, experienced typical symptoms such as heartburn or regurgitation [46, 47]. In addition, 12 of 19 patients continued to have elevated esophageal acid exposure despite treatment with standard doses of proton pump inhibitors [47]. It has been appreciated for several decades that gastric acid can spread rapidly to the lung periphery and that aspiration of acid can cause pneumonitis and pulmonary fibrosis in animal models [50]. In addition, gastroesophageal reflux is associated with a variety of human respiratory disorders, including asthma, cystic fibrosis, and COPD. It therefore

appears plausible that chronic microaspiration could constitute, or contribute to, the repeated injury of the lung parenchyma that is thought to underlie the development of IPF.

There are also data suggesting that certain medications, in particular anti-depressants, showed significant associations with IPF, although no dose response was apparent [51, 52].

Viruses

Several viruses have been implicated in triggering, promoting, or exacerbating IPF. These include hepatitis C virus (HCV), adenovirus, human cytomegalovirus (CMV), human herpes viruses (HHV) 7 and 8, herpes simplex virus, parvovirus B19, and Epstein–Barr virus (EBV) [48, 53].

Hepatitis C Virus

The first indication for an association between HCV and IPF came from a Japanese study showing a significantly higher seroprevalence of anti-HCV antibodies in patients compared to the general population [54]. Similar findings were subsequently reported from Italian IPF patients [55], but not confirmed in a study from the UK [56]. A Japanese case–control study relying on self-reported diagnosis of HCV infection also did not find a significant association between HCV infection and the risk of developing IPF [57]. More recently, among more than 6,000 Japanese HCV-infected patients, 15 developed IPF during a mean follow-up of 8 years, whereas IPF was not diagnosed in any of more than 2,000 hepatitis B virus-infected patients followed for a mean of 6.3 years [58]. In particular, HCV patients aged ≥55 years, with ≥20 pack-years of smoking and who had liver cirrhosis were at increased risk of developing IPF. Since HCV is not known to replicate in the lung, the mechanisms by which HCV infection promotes pulmonary fibrosis are still unclear.

Parvovirus

Endothelial cells of the microvasculature are specific target of parvovirus B19. Parvovirus B19 has been implicated in the pathogenesis of certain acute and chronic vasculitic disorders such as Wegener's granulomatosus, scleroderma, Henoch–Schonlein purpura, dermatomyositis, and Kawasaki disease by virtue of its ability to generate neoantigens as a result of this affinity for endothelial cells. Recently, Magra described 12 cases of interstitial lung disease with evidence of chronic parvovirus B19 infection by serology. In those cases with IPF, there was immunofluorescence evidence of formation of anti-endothelial cell antibody. [59]

Epstein–Barr Virus

A variety of studies using nested PCR have demonstrated that lung tissue from IPF patients contains EBV DNA significantly more frequently than control lungs [60, 61]. Immunohistochemical studies confirmed the increased frequency of pulmonary EBV infection in IPF patients compared to healthy and diseased controls [62]. In addition, lytic replication of EBV, as indicated by the presence of the immunoreactive lytic cycle antigens pg340/220 and viral capsid antigen, was found to occur in a considerably higher proportion of lung specimens from IPF patients compared to controls [60]. Lung samples from 11 of 18 of the patients with IPF who were EBV DNA positive in this study contained WZhet, a rearranged form of EBV DNA that is associated with productive viral replication [63]. Peripheral blood lymphocytes from these and additional IPF patients were positive for WZhet with significantly higher frequency than the control groups, consisting of lung transplant recipients on immunosuppressive regimens or blood donors (59% compared to 0% and 4%, respectively). The absence of WZhet in transplant recipients suggests that reactivation of EBV was not attributable to immunosuppression, even though the association between WZhet positivity and immunosuppressive therapies in IPF patients came close to reaching statistical significance ($p=0.08$).

Latent membrane protein 1 (LMP1), which is expressed on the surface of EBV-infected cells in the latent and lytic phases, was detected by immunohistochemistry in nine of 29 IPF lungs, but none of the five systemic sclerosis or 14 control samples [62]. LMP1 positivity was associated with a significantly higher rate of death from respiratory failure, suggesting that EBV infection accelerates the progression of IPF. This is frequently interpreted as one of the strongest indicators of an association between viral infection and disease exacerbation. Some caution is advisable, however, since these findings were based on only ten deaths altogether, five of which were from respiratory failure, with four occurring in the nine LMP1-positive patients.

Other Viruses

Prompted by the observation that individual viruses, including the most frequently identified EBV, could be detected in lung tissue of little more than half of the IPF patients, Tang et al. [64] hypothesized that inclusion of closely related herpes virus would result in detection in all IPF patients. Indeed, using PCR for detection of a total of eight herpes viruses, they found that EBV, CMV, HHV-8, and/or HHV-7 could be detected in 97% (32 out of 33) of patients, but only 36% (nine out of 25) of controls with various other diseases. The frequency with which IPF lungs harbored two or more herpesviruses was also

significantly higher than in controls. Of note, co-infection was present in patients with sporadic IPF much more frequently than in patients with the familial form of the disease. If virus infection actually plays a role in the onset of IPF, it would seem that familial IPF has a lower threshold of antigen stimulation, possibly due to increased genetic host susceptibility.

The lungs of IPF patients were found to exhibit signs of endoplasmic reticulum stress, and the stress markers co-localized with immunoreactive herpes virus proteins in the hyperplastic alveolar endothelial cells lining areas of fibrosis in many of these patients [65]. While this suggests that viral infection plays a role in disease progression by inducing endoplasmic reticulum stress, it cannot be ruled out that this stress results from the increased protein synthesis of these type II alveolar endothelial cells. A recent genome-wide scan of Finnish multiplex families suggested that *ELMOD2* was a candidate gene for IPF susceptibility [66]. Among other tissues, the product of this gene was found to be expressed in normal lung, but was not detectable in IPF lungs. Transcription of the *ELMOD2* gene was significantly lower in IPF compared to control lungs. This gene was subsequently reported to be essential for toll like receptor 3-activated IFN-related antiviral responses [67]. Together these findings lend further support to the hypothesis that viral infections are involved in the pathogenesis of IPF and suggest that host antiviral responses play a role in determining IPF susceptibility.

Pathogenesis of IPF

According to the currently prevailing pathogenic hypothesis, UIP histology arises from repeated epithelial injury resulting in the activation of AECs. These AECs, in turn, attract and activate fibroblasts and induce their proliferation as well as their differentiation into myofibroblasts, which synthesize extracellular matrix. In the context of an injured basement membrane, re-epithelialization cannot proceed properly, resulting in the continued presence and accumulation of myofibroblasts producing excessive amounts of extracellular matrix. This makes fibroblasts the key effector cells in IPF, which is consistent with fibroblastic foci being the key histologic feature of the disease. Consequently, much of the recent research efforts in IPF have focused on understanding the sources and origins of the increased number of fibroblasts in IPF lungs and characterizing the behavior of these cells.

Fibroblasts

Lung fibroblasts are phenotypically heterogeneous [68]. The best characterized phenotype in IPF and other fibrotic diseases is the α-smooth muscle actin (α-SMA)-positive myofibroblast. This type of fibroblast constitutes an important source of type 1 procollagen, a major constituent of scar tissue. Because of their expression of α-SMA, which forms stress fibers, along with several other muscle proteins, myofibroblasts are more contractile and contribute to the increased contractility and decreased compliance of fibrotic lung tissue. Another subset of fibroblasts is characterized by the expression of TERT, the reverse transcriptase component of telomerase. A third phenotype lacks expression of Thy-1. Studies in rat lung fibroblasts indicate that Thy-1 is a negative regulator of myofibroblast differentiation in response to growth factors such as TGFβ, endothelin-1, and connective tissue growth factor (CTGF) and makes myofibroblasts more contractile as well as more resistant to apoptosis induced by collagen contraction [69]. Myofibroblasts in the fibrotic foci in lung tissue samples from IPF patients do not express Thy-1, whereas the majority of fibroblasts from normal lungs do [70]. It largely remains to be elucidated whether the different phenotypes represent distinct lineages or intermediate stages of differentiation toward the myofibroblasts.

It had long been thought that the accumulation of fibroblasts in the lung of patients with IPF and other fibrotic lung diseases arose from proliferation of resident fibroblasts. In recent years, evidence has been accumulating that tissue fibroblasts can also arise from two other sources, namely epithelial cells and bone marrow precursors.

Epithelial–Mesenchymal Transition

The ability of epithelial cells to take on mesenchymal characteristics, a process called epithelial–mesenchymal transition (EMT), is an indispensable mechanism during embryogenesis and plays an important role not only in cancer progression but also in fibrosis. It is triggered and regulated by numerous signaling pathways that exhibit extensive crosstalk and act in a cell type-specific and context-dependent manner [71]. The complex series of events taking place during EMT include loss of apical–basal polarity, dissociation of cell–cell adhesions, detachment from the basement membrane, rearrangements of the cytoskeleton, and repression of epithelial with concomitant activation of mesenchymal genes.

Primary type II alveolar endothelial cells and a type II-derived cell line have been shown to undergo EMT in response to TGFβ1, with TNFα significantly augmenting the frequency of this phenotypic transition [72]. In this and other studies, immunohistochemical analysis of a limited number of IPF lung tissue samples has provided indirect evidence for the occurrence of EMT as shown by the co-expression of mesenchymal and epithelial markers

in epithelial cells, which was not observed in control lungs [72–74]. Unfortunately, the only study that included patients with UIP or NSIP is also the only investigation not to find any evidence of EMT in either of the patient groups or in controls [75]. In a global gene expression profiling study, N-cadherin was one of the most significantly upregulated genes in patients with IPF compared to patients with HP [32]. Of note, in the process of EMT, epithelial cells lose E-cadherin expression and start expressing the mesenchymal marker N-cadherin. The detection of immunoreactive N-cadherin in flattened alveolar epithelial cells overlying fibroblastic foci provides further support for the theory that EMT participates in the accumulation of fibroblasts in IPF lungs [32]. The occurrence of EMT has also been demonstrated in experimental models of pulmonary fibrosis induced by pulmonary overexpression of active TGFβ1 or by bleomycin [74, 76].

Fibrocytes

Fibrocytes are bone marrow-derived cells that express not only the hematopoietic stem cell marker CD34, the leukocyte marker CD45, and MHC class II but also mesenchymal markers such as collagen-1. They normally represent ≤1% of circulating leukocytes. Fibrocytes, an inactive mesenchymal cell, are activated to form fibroblasts in the circulation and that fibrocytes are mediators of wound healing and tissue repair. Studies in experimental animals indicated that bone marrow-derived cells were recruited to sites of tissue injury, including bleomycin-induced lung injury, where they constituted a majority (80%) of lung fibroblasts as identified by spindle-shaped morphology [77]. In addition, they were characterized as expressing collagen-1, but not α-SMA. Instead, approximately two thirds of them were positive for TERT. Fibrocytes have been detected in IPF lungs, some of them close to fibroblastic foci but were absent from control lungs [78]. Both hyperplastic AECs and epithelial cells overlying fibroblastic foci expressed CXCL12, the ligand of CXCR4 expressed on a majority of fibrocytes. The absolute and relative counts of fibrocytes in peripheral blood of IPF patients were significantly increased compared to controls [79, 80], most strikingly in those with acute exacerbation [80]. They were also found to independently predict early mortality, although this may have been due to the high correlation between fibrocyte counts and acute exacerbations, which themselves are closely associated with increased mortality risk. Together, these data suggest that fibrocytes may constitute an important source of fibroblasts in the lung and may play an important role in the pathogenesis of IPF.

Fibroblast Behavior

Fibroblasts derived from the lungs of patients with IPF differ in numerous ways from those derived from healthy control lung specimens. The most obvious of these differences is the markedly increased presence of myofibroblasts in IPF lungs. Since comparisons between IPF and normal lung fibroblasts do not account for this difference, it is difficult to determine to what extent the differences in functional characteristics of IPF fibroblasts are attributable to their myofibroblast phenotype and to what degree they represent a pathological phenotype. The increased production of collagen-1 and other extracellular matrix molecules are typical features of myofibroblasts. But beyond this, IPF fibroblasts show hyperresponsiveness—and occasionally hyporesponsiveness—to a variety of stimuli, including TGFβ and other growth factors, cytokines, and chemokines [81–83]. In vitro studies and immunohistochemical analyses have shown that IPF fibroblasts themselves are a significant source of many of these cytokines and chemokines, which can then act in an autocrine manner. Fibroblasts derived from IPF lungs are also characterized by dysregulated expression of metalloproteinases and their inhibitors [84], which likely contributes to the deranged tissue remodeling seen in IPF. In addition, they exhibit decreased ability to upregulate COX-2 expression, resulting in reduced production of prostaglandin E2 (PGE2), an inhibitor of fibroblast migration, proliferation, collagen production, and myofibroblast differentiation. The responsiveness of IPF fibroblasts to PGE2 is also decreased [85]. Finally, cultured IPF fibroblasts are more resistant to a variety of inducers of apoptosis, including Fas [86] and plasminogen [87], and immunohistochemical studies of IPF lung tissue generally show low rates of apoptosis in fibroblasts [88, 89]. Whether cultured IPF fibroblasts show increased rates of spontaneous apoptosis remains controversial [87, 90].

In view of all these fibroblast abnormalities in patients with IPF, it is somewhat surprising that several transcriptional profiling studies failed to detect significant differences between IPF and normal fibroblasts. One of these studies compared normal lung fibroblasts to fibroblasts from lung tissues with UIP histology, unfortunately without clarifying whether or not these patients had IPF or other ILDs with a UIP pattern [91]. Even though the overall transcriptional patterns differed between UIP and control fibroblasts, the differences in the expression of individual genes were generally modest. Similarly, the transcriptional profile of lung fibroblasts from patients with IPF/UIP or systemic sclerosis/NSIP in response to stimulation with TGFβ did not differ significantly from that of normal lung fibroblasts [92].

On the other hand, significant differences between fibrotic and normal myofibroblasts were observed at the translational level (as assessed by ribosome recruitment), even though in this study the differences at the transcriptional level were again far less pronounced [93]. In particular, genes involved in cell cycle regulation were significantly more active at the translational level in control compared to IPF myofibroblasts. Another important finding was that normal myofibroblasts displayed no significant differences in gene expression and relatively few differences in ribosome recruitment on non-contractile compared to contractile matrices. In marked contrast, IPF myofibroblasts underwent extensive translational regulation depending on the matrix state. This underscores that there are pronounced phenotypic differences between normal and IPF fibroblasts. Using a systems approach to analysis of genome-wide data, it could be demonstrated that myofibroblasts derived from IPF lungs exhibited an EMT signature, lending further support to the theory that at least some of the myofibroblasts in IPF lungs derive from epithelial cells [93]. Of special note, in contrast to the other two investigations, this study specifically used fibroblasts with the α-SMA positive myofibroblast phenotype from both patients and controls, an important aspect considering that fibroblasts from IPF patients display this phenotype significantly more frequently than controls. In addition, fibroblasts were examined in contractile as well as non-contractile collagen gels. The matrix effect is a frequently neglected aspect in fibroblast studies, even though it is by now well appreciated that fibroblast behavior is greatly influenced by the composition and contractility of the matrix. The same authors had previously shown that IPF fibroblasts differed in their proliferative response to polymerized type 1 collagen, the type of matrix that surrounds them in fibroblastic foci [94]. Whereas normal fibroblast proliferated considerably less on polymerized than on monomeric collagen, this inhibitory effect was much less pronounced in IPF fibroblasts. This was shown to result from aberrant activation of the signaling pathway downstream of $\alpha 2\beta 1$ integrin ligation in the process of adhering to collagen. Of note, components of this signaling pathway have been implicated in EMT, and it could be speculated that fibroblasts arising from EMT acquire their pathological phenotype in the process of transdifferentiation.

In summary, the contributions of the individual fibroblast phenotypes to the pathology of IPF largely remain to be elucidated. Nonetheless, there are some intriguing data suggesting that the origin of the fibroblast (bone marrow, epithelial cells) may influence the phenotype, e.g., the defective proliferation regulation in fibroblasts arising from EMT or the TERT expression in fibroblasts arising from bone marrow precursors.

Epithelial Cells—Targets and Active Participants

The integrity of the alveolar epithelium is severely disrupted in IPF lungs. There is complete absence of alveolar epithelium in some areas; in others, it is primarily cuboidal rather than showing the normal arrangement of squamous type I cells interspersed with cuboidal type 2 cells. Whereas the epithelium overlying fibroblastic foci is generally referred to as alveolar, Chilosi et al. [95] identified the abnormal epithelium covering these foci as being of a bronchiolar immunophenotype. In addition, there is bronchiolization, i.e., the colonization of alveolar spaces by migrating bronchiolar cells [95]. Bronchiolar epithelium also lines honeycomb lesions. Abnormalities are also observed both in bronchiolar structures and bronchioloalveolar junctions and include bronchiolar hyperplasia, squamous metaplasia, and basal cell hyperplasia and atypia [95]. This is a major distinguishing feature between IPF and other ILDs since bronchiolar involvement was not seen in COP (BOOP), NSIP or DIP, nor in a single sample of AIP [95]. In addition, the bronchiolar cells of patients with IPF, but not any of the other ILDs, showed aberrant nuclear accumulation of γ-catenin [96], an effector molecule in the canonical Wnt signaling pathway, which has been shown to play a central role not only in early development but also in carcinogenesis and in a fibroproliferative disorders affecting a variety of organs and is increasingly implicated in IPF.

While the above highlights the extraordinary extent to which re-epithelialization is disturbed in IPF, many unresolved questions remain concerning the events that necessitate such extensive regeneration. There is still some controversy over which cell type constitutes the primary target of epithelial injury. A number of studies have shown increased rates of apoptosis of alveolar epithelial cells without characterizing the cell type [97–99]. Morphological analyses have demonstrated that type II AECs are specifically targeted and that apoptotic type II AECs can even be detected in unaffected areas of IPF lungs [41, 100]. Others, however, determined apoptosis to occur in both alveolar and bronchiolar epithelial cells, and this was associated with increased expression of pro-apoptotic molecules and downregulation of anti-apoptotic molecules in both cell types [88, 101–103]. Of note, increased apoptosis and upregulation of pro-apoptotic molecules is not specific to IPF, but is also seen in NSIP, although the degree of enhancement is significantly greater in IPF [101]. There are indications that both the Fas-mediated pathway of apoptosis and the pathway involving perforin and granzyme B may play a role in the death of bronchiolar and alveolar epithelial cells [104–106].

The question of what cell types are involved in the attempts at re-epithelialization in IPF lungs is equally

unresolved. It has been argued that the increased rate of apoptosis observed in type II AECs makes it unlikely 2 that they also constitute the hyperplastic cuboidal cells that are thought to represent rapidly proliferating cells attemp ting to regenerate injured epithelium [100]. Ultrastructural studies indicated that type II AECs participated in re-epithelialization of less severely affected areas, whereas cuboidal cells in areas of severe fibrosis were derived from bronchiolar basal cells and cuboidal cells in respiratory bronchioles [107]. Bronchiolar basal cells constitutively express ΔN-p63, a truncated isoform of p63 that antagonizes the pro-apoptotic functions of p53, which was co-expressed in a subset of basal cells detected in the bronchiolar lesions of IPF lungs [95]. It can be hypothesized that the presence of both of these molecules within the same cell may lead to conflicting signals in the regulation of apoptosis and cell cycle control and may constitute a major factor in the dysregulated parenchymal remodeling typical of IPF lungs.

Type II alveolar cells are not only one of the targets of epithelial injury but they also actively participate in shaping the profibrotic microenvironment that characterizes the lungs of patients with IPF. Numerous pathways have been shown to be involved in the fibrotic processes in IPF. It has long been appreciated that TGFβ and a variety of other growth factors, inflammatory cytokines, and chemokines along with metalloproteases and other regulators of matrix turnover play a vital role. In addition, it is increasingly appreciated that vasodilation, coagulation, oxidative stress, and vascular remodeling pathways also participate and that there is extensive crosstalk between all of these pathways. Alveolar and possibly bronchiolar epithelial cells are capable of producing many of the implicated mediators from several of these pathways.

AECs produce a variety of molecules that attract fibroblasts, stimulate their proliferation and differentiation into myofibroblasts, and induce extracellular matrix production. The most potent profibrotic mediator is TGFβ. This growth factor has many cell-type and context-dependent functions, including a vital role in downregulating inflammatory processes and inducing tissue repair and wound healing by promoting the production of extracellular matrix. But because of its ability to stimulate fibroblast chemotaxis, proliferation, myofibroblast differentiation, and synthesis of various types of collagen and other ECM proteins [82, 108], its chronic activation results in tissue fibrosis. This is most clearly illustrated in animal models where pulmonary expression of TGFβ in its active form is sufficient to induce fibrosis, which regresses once TGFβ is inactivated. TGFβ also plays an important role in tissue remodeling due to its ability to induce inhibitors of ECM protein degradation, such as tissue inhibitors of metalloproteinases [109].

Although the results of immunohistochemical analyses are somewhat conflicting, there are indications that hyperplastic AECs contribute to the enhanced production of TGFβ in the lung of patients with IPF, although alveolar macrophages represent the major source [110–112]. Alveolar and, in some cases, bronchiolar epithelial cells also produce a variety of other growth factors with profibrotic activities, including platelet-derived growth factor [113], CTGF [114], and insulin-like growth factor [115]. In addition, they can synthesize cytokines such as TNF-α or interleukin-10 [111, 116], which have profibrotic activities themselves, enhance the profibrotic effects of various growth factors, or induce the production of other profibrotic mediators.

Mediators of Fibrosis

Insulin-like growth factor binding protein (IGFBP)-5 is a fairly recently identified profibrotic mediator that not only has profibrotic effects similar to those of TGFβ but also plays a role in cellular senescence [117]. This dual role makes IGFBP of particular interest in IPF in view of the increasing incidence of IPF with advancing age and the recently identified association of the disease with telomerase, another key player in cellular senescence. Overexpression of IGFBP5 in normal fibroblasts has been shown to stimulate myofibroblast differentiation and collagen synthesis in normal lung fibroblasts [117, 118]. Expression of human IGFBP5 in mice resulted in cellular infiltration and collagen deposition particularly around the airways. This was associated with fibroblast to myofibroblast transdifferentiation [119]. In addition, type II AEC of these mice showed evidence of EMT, and the ability of IGFBP5 overexpression to induce EMT was confirmed in A549 cells in vitro. IGFBP5 and, to a lesser extent, IGFBP3 were present in greater amounts in IPF compared to healthy lungs [118]. Immunohistochemistry localized both proteins primarily to interstitial fibroblasts and alveolar as well as bronchiolar epithelial cells in lung tissue from patients with IPF, but only to cells with the appearance of type II AECs in healthy tissue.

The vasoconstrictors endothelin-1 (ET-1) and angiotensin II (ANG II) can induce in vitro fibroblast activation, proliferation, and differentiation into myofibroblasts, the cell type primarily responsible for remodeling of the extracellular matrix. Blockade of ET-1 or ANG II attenuates fibrosis in experimental animals. Expression of prepro-ET-1 mRNA was found to be increased in hyperplastic type II AECs, airway epithelium, and endothelial and inflammatory cells [120]. Bronchoalveolar lavage (BAL) fluid from patients with IPF contained significantly elevated levels of ET-1 compared to controls [121]. Pronounced upregulation of angiotensinogen transcription and protein expression was

detected in lung tissue from patients with IPF, particularly in cuboidal epithelial cells immunophenotyped as type II AEC as well as in fibroblastic foci [122]. Type II AECs also showed immunoreactivity for ANG peptide, and many of the ANG-positive cells were found to be apoptotic. ANG II bioavailability is limited by the activity of angiotensin-converting enzyme-2, and inhibition of this activity enhanced experimental fibrosis, whereas exogenous ACE-2 attenuated it [123]. In the same study, lung tissue from IPF patients was found to contain significantly lower levels of ACE-2 mRNA, immunoreactivity, and activity compared to controls.

Components of both the extrinsic and intrinsic pathways of the coagulation cascade also display potent profibrotic effects. For example, thrombin and factor Xa (FXa) stimulate fibroblast proliferation, collagen production, and differentiation into myofibroblasts in vitro [124]. These effects are primarily mediated by the protease-activated receptor (PAR)-1. Bleomycin-induced fibrosis is associated with increased expression of both thrombin and PAR-1, and inhibition of thrombin partially inhibits collagen accumulation in the lung [124]. Although increased thrombin levels have been detected in several other types of pulmonary fibrosis [124], there is as yet little information on the expression of this protease in lung tissue from patients with IPF. Bleomycin-induced fibrosis can also be attenuated by direct inhibition of FXa [125]. Transcriptional levels of factor X (FX) were fivefold higher in microdissected alveolar septae and also elevated in macrophages and myofibroblasts within fibroblastic foci in lung tissue from patients with IPF compared to controls [125]. Both bronchiolar and alveolar epithelial cell lines were shown to synthesize FX. Expression of PAR-1 in lung tissue of patients with IPF was detected in fibroblastic foci in this study, whereas others reported PAR1 immunoreactivity mainly on hyperplastic alveolar and bronchiolar epithelium [126].

PGE2 is a lipid mediator produced from arachidonic acid by cyclooxygenase (COX), an enzyme with a constitutively expressed isoform (COX-1) and an inducible isoform (COX-2). PGE2 is a potent inhibitor of fibroblast proliferation and collagen synthesis and is produced by epithelial cells, macrophages, and fibroblasts [127]. Several studies have shown that synthesis of PGE2 by fibroblasts derived from the lungs of patients with IPF is defective secondary to defective upregulation of COX-2. More recently, it was demonstrated that the level of immunoreactive COX-1 and COX-2 was significantly decreased in bronchiolar epithelial cells of patients with IPF compared to controls [128]. In addition, COX-1, but not COX-2 expression, was significantly lower in alveolar macrophages from IPF lungs compared to controls. Unfortunately, this study did not state what criteria were used for the diagnosis of IPF and it appears likely that patients with NSIP were included in the IPF group. However, similar decreases in COX-1 and COX-2 expression were seen in patients with sarcoidosis, suggesting that it is not a disease-specific feature.

In summary, both alveolar and bronchiolar epithelial cells are targets of the repeated epithelial injury that is thought to not only underlie the development of fibrosis in IPF but also actively participate in the fibrotic process by producing a variety of mediators that stimulate fibroblasts to proliferate, differentiate, and synthesize excessive amounts of extracellular matrix. As illustrated in the preceding section, AECs may contribute to the accumulation of fibroblasts in the lungs of patients with IPF by undergoing EMT, i.e., acquiring a mesenchymal phenotype.

The Role of Cellular and Humoral Components of the Immune System

Inflammation in IPF is moderate compared to other fibrotic diseases, including other idiopathic ILDs such as NSIP and DAD [129]. Nonetheless, the lungs of IPF patients display an interstitial infiltrate consisting primarily of mononuclear cells, with a recent investigation indicating that CD3 T cells and plasma cells constituted the most frequent cell types [129, 130]. In one study, CD4 T cells were found primarily in aggregates in or near lymphoid follicles, whereas CD8 T cells were diffusely scattered throughout the parenchyma [130]. This is consistent with earlier results in patients with CFA or UIP, though they were not classified according to ATS criteria [131, 132]. In another investigation, mononuclear cells were detected in small aggregates near fibroblastic foci [129]. The density of inflammatory cells was the highest in areas of dense fibrosis and decreased progressively toward the normal areas [129].

Patients with IPF were found to have increased proportions of activated peripheral and BAL CD4+ T cells, as indicated by the enhanced expression of CD38, CD154, or MHC II [11, 133], whereas only peripheral, but not BAL T cells showed evidence of increased activation in another study [134]. It has been shown that CD154 (also CD40L), via engagement of CD40 on fibroblasts, can stimulate fibroblast production of collagen and inflammatory mediators in vitro [135, 136]. Of particular note, subjects with asymptomatic ILD (some with IPF) identified during an investigation of family members of IPF patients already tended to have higher BAL lymphocyte percentages than subjects with normal HRCT scans, and this difference was significant in nonsmokers [11]. In addition, CD4 T cells, but not CD8 T cells of these subjects significantly more frequently expressed the activation markers CD38 and HLA-DR, similar to what was seen in patients with familial IPF.

BAL as well as peripheral T cells of patients with IPF also show highly biased T cell receptor (TCR) BV repertoires [133, 137]. This was also observed in patients with acute exacerbations of IPF, and some identical TCRVβ sequences were found in up to a third of the clones in sequential BAL fluid or tissue samples, suggesting the occurrence of persistent antigen-driven stimulation [138]. Such oligoclonal expansions provide evidence of a cellular immune response to one or more peptide antigen(s). Indeed, hilar lymph node CD4 T cells from IPF patients proliferated more vigorously in response to stimulation with autologous lung extracts compared to healthy controls or patients with other lung diseases [133]. In addition, peripheral CD4 T cells of some patients with IPF showed striking downregulation of CD28 expression [139]. Since suppression of this co-stimulatory molecule occurs after repeated cycles of antigen-driven proliferation, this provides further indirect evidence that IPF is associated with antigen-driven cellular immunity. Extreme downregulation of CD28 was associated with increased mortality or lung transplantation.

Plasma from 18 out of 22 (82%) of IPF patients contained IgG autoantibodies against cellular antigens, some of which were recognized by several of the plasma samples [133]. Similarly, earlier studies found that plasma from 18 out of 22 patients with CFA, but only five out of 18 samples from sarcoidosis patients, contained IgG antibodies recognizing a protein associated with alveolar epithelial cells in addition to several other lung proteins [140, 141]. The potential pathological significance of some of these antibodies is underscored by the observation that sera containing these antibodies enhanced the TGFβ and tenascin production of the type II epithelial cell line A549 and displayed cytostatic effects [142]. Others reported that all (of 18) patients with UIP, NSIP, or both were positive for anti-endothelial cell antibodies that recognized pulmonary microvascular endothelial cells and, in some cases, exhibited cytostatic or cytotoxic effects on pulmonary endothelial cells [143]. This implicates humorally mediated microvascular injury in the pathogenesis of IPF. Based on the observation in a preceding study that essentially all patients with IPF showed evidence of parvovirus B19 or CMV infection [144], the authors speculated that these infections might have resulted in the exposure of endothelial antigens. However, other investigators did not detect anti-endothelial antibodies in any of their 20 patients with UIP/IPF, whereas five out of 14 patients with UIP and four out of ten patients with NSIP associated with collagen vascular disease were positive [145]. Recently, the use of serological screening followed by recombinant expression cloning (SEREX) allowed identification of annexin 1 and Bax inhibitor 1 as autoantigens in patients with acute exacerbations of IPF [138]. Antibodies recognizing these molecules were detected in serum of 47% and 40% of IPF patients with acute exacerbations, respectively, with positivity rates in BAL fluid being similar or even slightly higher in the case of annexin 1.

Further evidence for a local humoral immune response in the lungs of patients with IPF comes from the observation of organized B cell aggregates in the majority of these patients [130, 146, 147]. Lymphoid neogenesis is frequently seen in autoimmune and other diseases characterized by chronic inflammation [148]. In such cases, the ectopic lymphoid structures are true germinal centers with proliferating B cells and naïve T cells. In contrast, the lymph node-like structures in IPF lungs were found to contain activated non-proliferating B cells, and the vast majority of T lymphocytes displayed the characteristics of recently reactivated memory T cells and also were not proliferating [147]. Immature dendritic cells (DCs) heavily infiltrated the lungs of patients with IPF, with different subsets being detected in areas of hyperplastic alveolar epithelial changes and fibrotic lesions [148]. In contrast, the lymphoid follicles contained mature DCs intercalated between T lymphocytes. These findings suggests that local maturation of DCs followed by their reactivation of memory T cells in the context of ectopic lymphoid structures is capable of sustaining chronic inflammation in IPF lungs in the absence of local lymphocyte proliferation.

While all of these findings strongly point to an autoimmune reaction in IPF patients, it remains to be elucidated whether it represents a triggering event or a secondary phenomenon. In any case, it would seem plausible that immune cells enhance fibrosis by stimulating fibroblast collagen synthesis either directly via CD154–CD40 interactions or via the release of profibrotic cytokines and other soluble mediators.

Summary

While the pathogenesis of IPF appears to be related to the proliferation, differentiation, and activation of fibroblasts, the triggering factors remain unclear. Various systems may be involved, and there is evidence that epithelial–mesenchymal transition leads to the abnormalities in cellular organization seen in IPF. The development of EMT and resulting fibrosis is under the influence of many factors, including alveolar epithelial cells, prostaglandin E2, angiotensin II, endothelin-1, and IGFBP5. An immune cellular response in IPF has been demonstrated, and this can have a stimulatory effect of fibroblast activation. Indeed, there is also evidence for a role of a humoral immune response. In fact, antibodies to several endogenous and exogenous antigens have been identified in IPF, raising the intriguing question as to whether or not IPF is an

autoimmune disease. Finally, the existence of a familial pattern in interstitial lung diseases raises the possibility of a genetic predisposition, and mutations in the telomerase gene or shorter telomere length have been observed in up to 20% of patients with familial IPF. The involvement of telomerase in preserving telomere length during cell division may similarly explain the increased incidence of IPF in patients of advancing age. In summary, the pathophysiology of IPF may involve multiple inter-related factors involved in the regulation of fibroblast behavior.

Overall, the prognosis of IPF is poor, with mean survival being only about 4 years. Pharmacologic agents used to treat IPF tend to have significant side effects themselves, and lung transplant still carries a 5-year survival rate of 44%. A better understanding of the pathophysiology may lead to other new avenues of treatment, with potentially better outcomes.

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