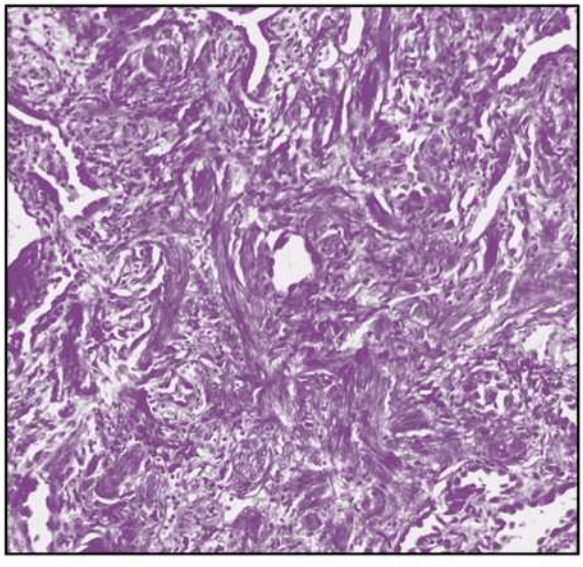
Lung Biology in Health and Disease Volume 185
Executive Editor: Claude Lenfant

Idiopathic Pulmonary Fibrosis



edited by
Joseph P. Lynch III

IDIOPATHIC PULMONARY FIBROSIS

Edited by

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MARCEL DEKKER, INC. NEW YORK · BASEL

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress.

ISBN: 0-8247-4073-4

This book is printed on acid-free paper.

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Current printing (last digit):

10 9 8 7 6 5 4 3 2 1

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INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) may very well be one of the most vexing pathologies in pulmonary medicine. It is complex, it is complicated, and the cause can be both exogenous and endogenous. This disease is frustrating for the clinician, and, most important, it is bad news for the patient.

During the last several decades, significant advances have been made in understanding the pathogenesis of many diseases of the lung and the respiratory system and in developing new treatments of them. Even though it seems that the complexity of IPF may have deterred researchers from focusing on it, considerable progress has been made in some relevant areas of research. For example, work on the inflammatory processes in the lung has been very productive and has led, in turn, to successful pursuits of better treatments of some conditions. Likewise, research on imaging now enables clinicians to diagnose disorders more precisely and earlier in the course of a disease.

The IPF puzzle is unique in the sense that we recognize many of the pieces, but not all of them, and it is very difficult to judge where the pieces fit. This volume, however, gives a very up-to-date view of the puzzle. All the pieces are described, and an image of IPF emerges quite clearly.

Dr. Joseph Lynch and the contributors to this volume provide the reader with a singular view of IPF. Not only is a vibrant state of knowledge presented, but the volume opens the door to potential new avenues of laboratory and clinical investigations.

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As Executive Editor of the series of monographs, Lung Biology in Health and Disease, I am very pleased to introduce this volume to its readership, and grateful to all the contributors for the opportunity to do so.

Claude Lenfant, M.D.
Bethesda, Maryland, U.S.A

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PREFACE

Idiopathic pulmonary fibrosis (IPF), also known as cryptogenic fibrosing alveolitis, is a devastating disease of unknown etiology, which progresses relentlessly to respiratory failure within 2 to 8 years of onset. IPF is associated with the histological pattern of usual interstitial pneumonia (UIP), and is the most common of the idiopathic interstitial pneumonias (IIPs), comprising 46–71% of IIPs. Unfortunately, despite exhaustive research efforts, the prognosis of IPF remains dismal, with 5-year mortality rates exceeding 70%. Historically, corticosteroids and immunosuppressive and cytotoxic agents have been the mainstay of therapy, but have not been proven to be effective. Within the past decade, key advances in the diagnosis of UIP include: 1) an enhanced ability to diagnose IPF/UIP by high-resolution thin-section computed tomographic (HRCT) scans and 2) the recognition that other types of IIPs—particularly nonspecific interstitial pneumonia (NSIP), respiratory bronchiolitis interstitial lung disease (RBILD), and desquamative interstitial pneumonia (DIP)—were often erroneously categorized as IPF/UIP in early publications, even though prognosis and responsiveness to therapy among these disorders are markedly different from those of UIP. More importantly, concepts of pathogenesis have evolved dramatically within the past decade. Early concepts of pathogenesis postulated that inflammatory cellular influx and release of cellular products (enzymes, oxygen radicals, cytokines, etc.), were pivotal to lung destruction and fibrosis. Unfortunately, therapies based on abrogating inflammation appear to be ineffectual. More recent studies suggest that inflammation plays at best a minor role, whereas epithelial cell injury, recruitment of fibroblasts (FBs) and myofibroblasts, and alterations in FB phenotype and activation state play cardinal roles in the pathogenesis of IPF.

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In this book, internationally recognized clinicians, scientists, and investigators discuss the latest advances and concepts in the epidemiology, diagnosis, classification, and treatment of this frustrating and enigmatic disorder. The first several chapters examine several clinically relevant aspects of IPF including epidemiology (Drs. Coultas and Hubbard), genetics of IPF and its variants (Dr. Wahidi et al.), clinical features (Dr. King), histopathology (Dr. Travis), nonspecific interstitial pneumonia (Dr. Martinez et al.), physiological aberrations (Drs. Martinez and Lynch), radiological features including HRCT (Dr. Zisman et al.), the role of other imaging and radionuclide tests (Dr. Singh et al.), and bronchoalveolar lavage (Drs. Baughman and Costabel). Additionally, Dr. Wells et al. discuss pulmonary fibrosis among connective-tissue disorders, which shares many features with IPF but differs in histopathological features and prognosis.

Following these practical and state-of-the-art articles, the next large section of the volume focuses on putative biological and immunological mechanisms that may play key roles in the pathogenesis of IPF. Chapters are devoted to several possible contributory mechanisms that may be involved in lung injury or propagation of fibrosis including: cytokine phenotypes; chemokines; polymorphonuclear leukocytes; integrins; oxidants; coagulation pathways; arachidonic acid metabolites; matrix metalloproteinases and tissue inhibitors of metalloproteinases (TIMPs); extracellular matrix components; fibroblasts and myofibroblasts, fibroblast growth factors; type II alveolar epithelial cells; surfactant apoproteins, bronchiolar epithelium; and viruses.

In the final section, Dr. Westall et al. provide an overview of *current* treatment options for IPF including pharmacological therapy and lung transplantation. Given the limited efficacy of currently available therapies for IPF, Drs. Brown and Schwarz discuss the need to identify and develop *novel* treatment options based on the evolving understanding of the pathogenesis of this disorder. Hopefully, identification of effective antifibrotic therapies will improve the outcome of what currently is a frustrating and enigmatic disease. Finally, Dr. Crystal, whose seminal insights more than 25 years ago provided a template for future studies of IPF, concludes with an elegant chapter (with Drs. Heguy and Kaplan) detailing what is known about the genetics of IPF (including genetic polymorphisms that influence susceptibility to fibrosis), animal models, assessment of gene expression, and how such information may lead to novel treatment strategies based on more specific gene targets.

This volume is comprehensive and provides invaluable insights that are relevant not only to practicing clinicians but to educators, clinical investigators, and basic scientists. We believe this volume is the most comprehensive and scholarly publication devoted to IPF/UIP, and an important addition to the field.

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1

Epidemiology of Idiopathic Pulmonary Fibrosis

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I. Introduction

A. Definition of Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is only one of a large number of diffuse parenchymal lung diseases characterized by parenchymal inflammation and fibrosis without any known cause (1). Although the term *idiopathic pulmonary fibrosis* has been in use for several decades in the United States, other terms such as *cryptogenic fibrosing alveolitis* and *idiopathic interstitial pneumonia* have been used predominately in other countries. These varying terms and evolving criteria for the diagnosis of the disease have contributed to confusion and controversy for both clinicians and investigators. In an attempt to resolve these problems, a multidisciplinary panel of experts from the American Thoracic Society and European Respiratory Society has developed consensus statements on the diagnosis and management (1) and classification of the idiopathic interstitial pneumonias, including criteria for IPF (2).

Compared to its use over the past several decades, the term IPF in this new consensus classification is defined more narrowly as "a specific form of chronic fibrosing interstitial pneumonia limited to the lung and associated with the histological appearance of usual interstitial pneumonia (UIP) on surgical (thoracoscopic or open) lung biopsy. The etiology is unknown" (1). In addition to UIP from surgical biopsy, the diagnosis of IPF requires (1) exclusion of other known causes of interstitial lung disease including drug toxicities, environmental exposures, and collagen vascular diseases, (2) characteristic abnormalities on conventional chest radiographs or high-resolution computed tomographic scans, and (3) abnormal pulmonary function studies showing restriction and/or impaired gas exchange (1). Although UIP from surgical

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Table 1 ATS/ERS Criteria for Diagnosis of IPF in Absence of Surgical Lung Biopsy[a]

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 $P(A\text{-}a)O_2$, decreased PaO_2 with rest or exercise or decreased D_{LCO}]
Bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scans
Transbronchial lung biopsy or BAL showing no features to support an alternative diagnosis
Minor Criteria
Age > 50 yr
Insidious onset of otherwise unexplained dyspnea on exertion
Duration of illness > 3 months
Bibasilar, inspiratory crackles (dry or "Velcro"-type quality)

Definition of abbreviations: BAL, bronchoalveolar lavage; D_{LCO}, diffusing capacity of the lung for CO; HRCT, high resolution computerized tomography; ILD, interstitial lung disease; $P(A\text{-}a)O_2$, alveolar-arterial pressure difference for O_2; VC, vital capacity.

[a]In the immunocompetent adult, the presence of all of the major diagnostic criteria as well as at least three of the four minor criteria increases the likelihood of a correct clinical diagnosis of IPF. *Source*: Ref. 1.

biopsy is considered the "gold" standard for diagnosing IPF, other clinical criteria may be used to establish the diagnosis with a high degree of certainty (1–3) (Table 1).

B. Epidemiology of IPF

Prior to 1990, epidemiological investigations of IPF had not been conducted. The vast majority of investigations prior to 1990 and subsequent to 1990 focused primarily on clinical characteristics, prognosis, and pathogenesis of IPF in selected patients seen at referral centers. However, during the decade of the 1990s, the scope of investigation expanded to include a number of epidemiological studies on the occurrence (4), risk factors (5–9), and prognosis of IPF (10,11). The objectives of this chapter are to review the rationale for and challenges of investigating the epidemiology of IPF, and to summarize the available epidemiological evidence on disease occurrence, risk factors, and prognosis.

Epidemiology is defined as "the study of the distribution and determinants of health-related states or events in specified populations and the application of this study to control of health problems" (12). Although the discipline of epidemiology is considered to be the "basic science" of public

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health, it also has direct relevance for clinicians (13). The term *clinical epidemiology* has been used to describe the link between the methods of clinical medicine and epidemiology. Clinical epidemiology is defined as "the science of making predictions about individual patients by counting clinical events in similar patients, using strong scientific methods for studies of groups of patients to ensure that the predictions are accurate" (13). The purpose of clinical epidemiology is "to develop and apply methods of clinical observation that will lead to valid conclusions by avoiding being misled by systematic error and chance" (13).

However, because IPF is a relatively uncommon condition and requires a surgical biopsy for definitive diagnosis, these characteristics present challenges to minimizing systematic errors (i.e., biases) and chance in the design and conduct of clinical and epidemiological investigations. As an uncommon condition it is difficult, except at large referral centers or with multicenter studies, to enroll a sufficient number of patients to have adequate statistical power to assess the role of chance. Moreover, sampling bias is a major concern, because patients seen at referral centers are not representative of all patients with IPF in the general population. In rare conditions, the case-control design is frequently used to investigate risk factors; however, an inherent limitation of this design is recall bias. This bias is a result of patients with diseases recalling past events and exposures differently than control subjects without disease. Finally, misclassification bias, incorrectly categorizing a subject's disease status or diagnosis, may result because of varying criteria used for diagnosing IPF, and particularly because the surgical lung biopsy is considered the gold standard for diagnosis, but is infrequently performed. The potential for misclassification of IPF is further complicated by the clinical and pathological overlap with diffuse parenchymal lung diseases associated with collagen vascular diseases and occupational and environmental exposures such as asbestosis and chronic hypersensitivity pneumonitis. The issue of the diagnosis of IPF and misclassification bias is considered in greater detail in the next section.

II. Diagnosis of IPF

Few investigations have been conducted to evaluate systematically any of the methods for diagnosing IPF (3,14). Although the gold standard for diagnosing IPF has been the surgical lung biopsy, until recently little has been known about the performance characteristics of this test. The standard characteristics for evaluating a diagnostic test are sensitivity, specificity, and positive/negative predictive values, which are determined using the gold standard as the reference. Therefore, as the gold standard, intraobserver and interobserver reliability offer the main test characteristics for evaluating the accuracy of the

surgical lung biopsy. However, from the patient's perspective, the ultimate test characteristic, which has not been investigated, is whether the test results lead to an improved prognosis.

The diagnosis of IPF, and particularly the pathological diagnosis, have been evolving (2). In the 2002 American Thoracic Society/European Respiratory Society (2) consensus classification of the idiopathic interstitial pneumonias, UIP is considered to be the pathological hallmark of IPF. Although this pathological term has been in use for several decades, in the past, other histological patterns such as the recently described nonspecific interstitial pneumonia were likely labeled IPF. The current classification of histological patterns is based on reports from experts in pulmonary pathology.

To determine test characteristics of the surgical lung biopsy and other diagnostic methods in IPF, Hunninghake et al. (3) conducted a prospective evaluation of 91 patients referred to eight centers for suspected IPF. All patients had a surgical lung biopsy that was interpreted independently by three pulmonary pathologists who were also blinded to the clinical information. A pathological diagnosis of IPF was made when at least two of the three pathologists agreed on the diagnosis of UIP, which was found in 54 patients (59.3%). The overall agreement among the pathologists for diagnosing IPF versus other non-IPF diagnoses was 85%. Conceivably, the level of agreement could be higher if the pathologists were provided with clinical and radiological information. However, this level of agreement may greatly overestimate the diagnostic confidence that the community physician may expect, because they may only have access to pathological interpretations performed by a single pathologist who may have little expertise in pulmonary pathology. Therefore, although the surgical lung biopsy is the gold standard for diagnosing IPF, the potential for misclassification remains.

Despite the limitations of the surgical lung biopsy, it remains the benchmark for evaluating other diagnostic methods and was used by Hunninghake et al. (3) to examine the diagnostic performance of pulmonologists with expertise in interstitial lung diseases from eight academic centers. The pulmonologists used all clinical information available to them including high resolution computed tomography and transbronchial lung biopsy. Using their clinical diagnosis, regardless of diagnostic certainty, their accuracy for diagnosing IPF was 68% with a sensitivity of 85%, specificity of 43%, and positive predictive value of 69%. When the pulmonologists were certain of the diagnosis of IPF, the probability of a correct diagnosis increased to 81%, and was only 43.5% when their clinical diagnosis was IPF, but they were not certain.

Raghu et al. (14) also examined diagnostic test characteristics for the clinical evaluation of IPF using the surgical lung biopsy as the gold standard. However, since only one pulmonary pathologist interpreted the biopsy specimens, the reliability of the surgical biopsy could not be evaluated. The

overall accuracy of the clinical diagnosis of IPF was 62%, with a sensitivity of 62% and specificity of 97%, which was substantially higher than the 43.5% specificity observed by Hunninghake et al. (3).

The diagnosis of IPF is a complex process requiring substantial expertise to minimize misclassification. Moreover, this complexity and lack of data on whether an accurate diagnosis improves health outcomes for patients have contributed to very low utilization of the lung biopsy for diagnosing IPF (4,15). Thus, the potential for diagnostic misclassification is an inherent limitation for conducting clinical and epidemiological investigations of IPF. However, standardized clinical criteria have the potential for providing a reasonably accurate method for diagnosing IPF that may be used for conducting future clinical and epidemiological investigations.

III. Descriptive Epidemiology

The occurrence of disease, including prevalence, incidence, and mortality, is the fundamental concern of descriptive epidemiology, which has relevance for clinicians, researchers, and health care planners. For clinicians, knowledge of disease occurrence and occurrence relative to other interstitial lung diseases provides a basis for estimating the probability of disease in making a clinical diagnosis (i.e., pretest probability of disease). Researchers need information on disease occurrence to design studies, and frequency of disease is necessary for planning health services. Few investigations have been conducted on the occurrence of IPF, and results from these studies on the prevalence, incidence, and mortality are reviewed in this section.

A. Prevalence

Prevalence, the proportion of patients with a disease in a defined population during a specified time period, has been determined in a limited number of investigations (4,5,16). Although measuring prevalence is relatively straightforward, it is inherently limited because of survival bias. Only patients who have less severe disease and survive are included in the calculation of prevalence, whereas patients with more severe disease and higher case fatality are not.

As part of a case-control study, Scott et al. (5) identified 46 patients with cryptogenic fibrosing alveolitis over a 2-year period in Nottingham, UK. Patients were identified through respiratory physicians or pulmonary function laboratories, and the diagnosis defined by crackles, interstitial shadowing on chest radiography, restrictive pulmonary function, and the absence of known exposure to occupational or other fibrogenic agents. The estimated prevalence of IPF in this population was 6 per 100,000. The mean age of the patients was 66.9 years, and 76% of the cases were male.

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To determine the prevalence and incidence of interstitial lung diseases, including IPF, Coultas et al. (4), in 1988, established a population-based registry of patients in Bernalillo County, New Mexico. The only criterion for a diagnosis of IPF was a physician's diagnosis, the majority of whom were pulmonologists, regardless of the diagnostic methods. In this community, the physicians used several overlapping terms, pulmonary fibrosis, IPF, and interstitial pneumonitis. However, few patients had lung biopsies to establish definitively the diagnosis (see below). Despite this limitation, these results provide the best available evidence on the prevalence and incidence of IPF in the general population.

Overall, during the first 2 years of enrollment pulmonary fibrosis, IPF, and interstitial pneumonitis were the most commonly diagnosed interstitial lung diseases (Table 2). Of 258 prevalent cases with any interstitial lung disease, one of these three terms was used in 42.2% of cases. Prevalence based on the specific term IPF was higher among males (20.2 per 100,000) compared to females (13.2 per 100,000) (Table 2). If all three diagnostic terms are combined (IPF, pulmonary fibrosis, and interstitial pneumonitis), the prevalence for males was 32.1 per 100,000 and 30.3 per 100,000 for females. The distribution of the three diagnostic terms varied between males and females (Table 2) with IPF herein used more commonly among males and IPF and pulmonary fibrosis nearly equally herein used among females. Of the three terms, interstitial pneumonitis had the lowest prevalence of use among males and females. The median age of prevalent cases was 69 years, and increased markedly with age (Fig. 1a).

Table 2 Prevalence (per 100,000) of Interstitial Lung Diseases by Sex, Bernalillo County, New Mexico, 10/1/88 through 9/30/90

Diagnosis	Male ($n = 136$)	Female ($n = 122$)
Pulmonary fibrosis		
IPF	20.2	13.2
Pulmonary fibrosis	10.1	14.3
Interstitial pneumonitis	1.8	2.8
Connective tissue diseases	7.1	11.6
Occupational and environmental	20.8	0.6
Sarcoidosis	8.3	8.8
Drug and radiation	1.2	2.2
Pulmonary hemorrhage syndromes	0.6	2.2
Other	10.7	11.6
Total	80.9	67.2

Source: Adapted from Ref. 4.

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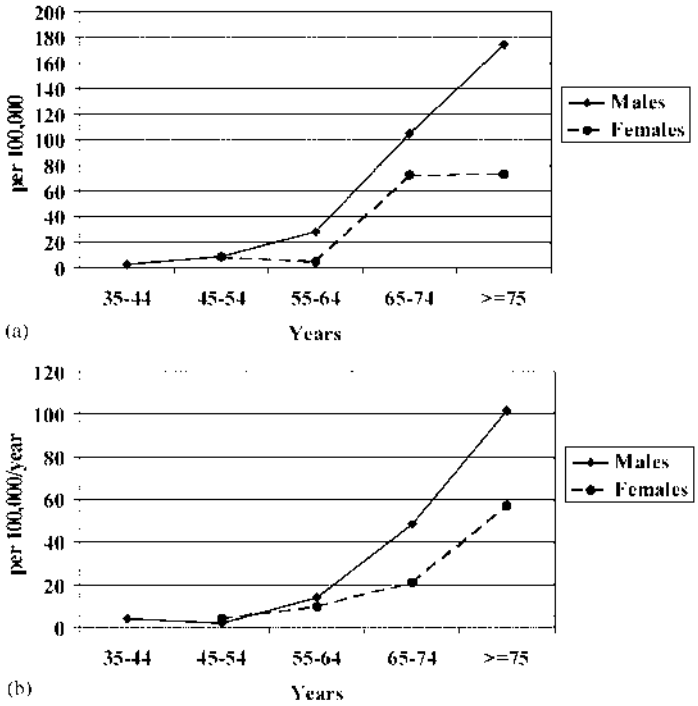

Figure 1 (a) Prevalence of IPF by sex and age, Bernalillo County, New Mexico. (b) Incidence of IPF by sex and age, Bernalillo County, New Mexico (adapted from Ref. 4).

Hansell et al. (16) used two large databases from the United Kingdom, the General Practice Research Database (GPRD) and the fourth Morbidity Survey in General Practice (MSGP4), to examine the prevalence of several respiratory diseases including fibrosing alveolitis. The GPRD and MSGP4 are estimated to include 6 and 1%, respectively, of the population of England and Wales. The diagnosis was based on the recording of a specific diagnosis by the physician. The overall prevalence estimates for fibrosing alveolitis from the two databases was similar, 15 per 100,000 patient years at risk in the GPRD and 18 per 100,000 patient years at risk in the MSGP4. Prevalence was also higher among males compared to females.

Based on the available data, the prevalence of IPF varies up to five-fold, ranging from 6 per 100,000 to as high as 32 per 100,000 if IPF-related diagnostic terms are combined. Several factors may explain these variations in prevalence estimates including differences in diagnostic approaches and labeling by physicians in different regions and countries, differences in

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diagnostic criteria used in the various investigations, and differences in exposures and susceptibility for developing IPF between populations. The effect of population differences on the occurrence of IPF was demonstrated by Roelandt et al. (17), who examined the relative occurrence of interstitial lung diseases from several regions of Belgium compared to patients from New Mexico in the United States (4). In Belgium, IPF and undefined fibrosis comprised 29% of all interstitial lung diseases compared to 42.3% in New Mexico.

B. Incidence

Scant data are available on the incidence, or rate of occurrence, of newly diagnosed cases of IPF (4). In addition to prevalence estimates, the New Mexico registry of patients with interstitial lung diseases provides information on incidence (4) (Table 3). Of 202 incident cases with any interstitial lung disease, one of the three diagnostic terms that overlaps with IPF was used in 51% of incident of cases. Incidence based on the specific term IPF was slightly higher among males (10.7 per 100,000/year) compared to females (7.4 per 100,000/year) (Table 3). If all three diagnostic terms are combined (IPF, pulmonary fibrosis, and interstitial pneumonitis), the incidence for males was 16.4 per 100,000/year and 12.9 per 100,000/year for females. The median age of incident cases was 69 years with a range of 20–95 years of age, and the incidence increased markedly with age (see Fig. 1b).

Table 3 Incidence (per 100,000/year) of Interstitial Lung Diseases by Sex, Bernalillo County, New Mexico, 10/1/88 through 9/30/90

Diagnosis	Male ($n = 106$)	Female ($n = 96$)
Pulmonary fibrosis		
IPF	10.7	7.4
Pulmonary fibrosis	3.9	4.1
Interstitial pneumonitis	1.8	1.4
Connective tissue diseases	2.1	3.0
Occupational and environmental	6.2	0.8
Sarcoidosis	0.9	3.6
Drug and radiation	1.8	1.1
Pulmonary hemorrhage syndromes	1.5	0.8
Other	2.7	3.9
Total	31.5	26.1

Source: Adapted from Ref. 4.

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C. Mortality

In contrast to estimates of prevalence and incidence of IPF, which have been determined in only a few, relatively small populations, nationwide mortality rates have been determined for a number of countries (18,19). The calculation of mortality rates for IPF has been based on vital statistic data obtained from death certificates using relevant diagnostic codes based on the International Classification of Diseases (ICD) (postinflammatory pulmonary fibrosis [ICD-9 code 515] and idiopathic pulmonary fibrosis [ICD-9 code 516.3]).

Hubbard et al. (18) examined IPF mortality rates with available data for the period 1979–1992, from seven countries including England and Wales, Scotland, Germany, Australia, New Zealand, Canada, and the United States. The mortality rates varied widely between the countries with the highest rates for IPF (516.3) in England and Wales and the lowest in Germany. Over time, the mortality rates for IPF increased in England and Wales, Scotland, Australia, and Canada but changed little in the United States, New Zealand, and Germany. The highest mortality rate for pulmonary fibrosis (515) was found in the United States, with increasing mortality over time in the United States as well as England and Wales, Scotland, Australia, and Canada. Mortality rates for pulmonary fibrosis remained unchanged over time in New Zealand and Germany. To determine whether the variation in mortality rates were influenced by changes in patterns of diagnostic coding (i.e., preferentially using one diagnostic code), also termed diagnostic transfer, they examined the ratio of mortality rates of IPF (516.3) to pulmonary fibrosis (515). Overall, small changes in the ratio were found over time suggesting that diagnostic transfer between pulmonary fibrosis and IPF could not explain the increase in mortality rates. In all countries, mortality rates for IPF and pulmonary fibrosis were higher among males compared to females.

Mannino et al. (19) examined mortality rates in the United States for pulmonary fibrosis, combining IPF (516.3) and pulmonary fibrosis (515), for the period 1979 through 1991. During this period there were over 26 million deaths, and of these deaths, 0.4% had IPF or pulmonary fibrosis being listed as the underlying cause of death, with pulmonary fibrosis being listed in 97.7%. Mortality rates were highest among men, and over time, mortality rates increased slightly among men and women. In 1979, the mortality rate for males was 48.6 per million and increased to 50.9 per million in 1991. The corresponding mortality rates for women were 21.4 per million and 27.2 per million, respectively. In addition, there was wide variation in mortality rates between regions of the country, with the highest rates being in the West and Southwest and the lowest rates in the Midwest and Northeast.

There are a number of consistent findings in these studies of mortality rates including an increase in mortality over time, geographical variation, and higher mortality among males compared to females. However, these results

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must be interpreted cautiously, since the causes for these variations in mortality rates is unknown, and may be explained by a number of factors, including true variations in occurrence and in fatality rates, increased recognition, and changes in diagnostic coding. Moreover, mortality data for interstitial lung diseases have been found to be neither sensitive nor accurate (20).

D. Synthesis

Although limited data are available on the occurrence of IPF and diagnostic misclassification is an inherent limitation for conducting population-base investigations, several consistent patterns emerge. First, the occurrence of IPF, whether measured by prevalence, incidence, or mortality, is higher among males compared to females. Second, IPF primarily occurs in the elderly, and prevalence and incidence increases markedly with advancing age (see Fig. 1). Third, the occurrence of IPF varies widely both within and between countries. These observations provide a basis for generating hypotheses on potential etiological factors in IPF and are discussed in the next section.

IV. Etiological Studies

Although the diagnosis of IPF requires "exclusion of other known causes of ILD such as certain drug toxicities, environmental exposures, and connective tissue diseases" (see Table 1), experimental evidence, clinical observations, and epidemiological investigations suggest that a number of factors may have an etiological role in IPF. The biological plausibility for a link between various exposures and IPF is based on (1) the hypothesized process for development of IPF that begins with alveolar epithelial injury followed by abnormal wound healing (21) and (2) results from animal experiments, which demonstrate that a wide variety of mineral particles are taken up by and may injure epithelial cells (22). Moreover, genetic variations that regulate the processes of epithelial particle uptake, epithelial cell injury, and wound healing may alter susceptibility to a wide variety of agents that may cause IPF. The role of genetics and IPF is considered in Chapter 2. In this section, we briefly review clinical observations, which provide further rationale for epidemiological investigations of etiological factors in IPF, and then consider the epidemiological evidence in greater detail. To date, most of the research in this area has focused on the possible role of exposure to occupational dusts and/or fumes, although other factors that have been investigated include cigarette smoking, medications, and infectious agents.

A. Occupational Exposures

There are a number of case reports linking IPF to various occupations that involve dust or fume exposure, including diamond polishing (23,24), industrial

car cleaning (25), dairy work (26), welding (27,28), gold extraction (29), and technical dental work (30). There is also a report of a patient developing IPF after exposure to a malfunctioning domestic wood burner (31). Lung mineralogical analysis of patients with IPF has revealed that these patients have an excess of silica (32) and metals, including iron and nickel (33,34), in their lungs. Taken together these case reports and mineralogical data suggest that occupational dust may be a risk factor for IPF. Further circumstantial evidence to support this hypothesis comes from the fact that the disease is most common in older men, and that in the United Kingdom, there is excess mortality from the disease in regions associated with the manufacturing industry (35).

The association between occupation and IPF has now been investigated in four separate case-control studies (Table 4), an analysis of autopsy results, and a historical cohort study. Patients in these studies were excluded if they had been exposed to known fibrogenic agents or had a connective tissue disease. The first case-control study conducted in the United Kingdom obtained lifetime occupational data via a postal questionnaire from 40 prevalent cases of IPF and 106 age-, sex-, and community-matched controls (5). There was a small association between exposure to any occupational dust and IPF (odds ratio [OR] 1.32; 95% confidence interval [CI] 0.84–2.04), but a marked increase in risk specifically with metal dust exposure (OR 10.97; 95% CI 2.30–52.4). In addition, there was a strong association with exposure to livestock (OR 10.89; 95% CI 1.24–96.0) and a moderate, but nonsignificant increase risk with wood dust exposure (OR 2.94; 95% CI 0.87–9.90).

Table 4 Summary of Results from Case-Control Studies of IPF and Occupational and Environmental Exposures

Exposure	England/Wales[a] ($n = 40/106$)	Trent region, UK[b] ($n = 218/569$)	Japan[c] ($n = 86/172$)	US[d] ($n = 248/491$)
Metal dust	10.97 (2.34–52.4)	1.68 (1.07–2.65)	1.34 (1.14–1.59)	2.0 (1.0–4.0)
Wood dust	2.94 (0.87–9.90)	1.71 (1.01–2.92)	NA	1.6 (0.8–3.3)
Livestock	10.89 (1.24–96.0)	NA	NA	2.7 (1.3–5.5)
Farming/ agricultural area	NA	NA	3.01 (1.29–7.43)	1.6 (1.0–2.5)
Textile dust	0.90 (0.24–3.44)	1.80 (1.10–2.96)	NA	1.9 (0.8–4.4)
Stone/sand	1.59 (0.52–4.79)	1.76 (1.01–3.07)	NA	3.9 (1.2–12.7)
Smoking	1.11 (0.13–1.40)	1.57 (1.01–2.43)	2.94 (1.37–6.30)	1.6 (1.1–2.4)
Wood fires	12.55 (1.04–114.0)	NA	NA	0.8 (0.4–1.6)

[a]Ref. 5.
[b]Ref. 7.
[c]Ref. 6.
[d]Ref. 9.

Iwai et al. (6) used an alternative epidemiological approach in Japan by comparing occupational data included in the autopsy records of 1311 patients with IPF to that in a systematic sample of 393,000 controls. Patients with IPF were significantly more likely to have worked as metalworkers, woodworkers, painters, and barbers/beauticians (all $p < .001$). This investigation also included a case-control study of 86 cases matched to two control groups: one of healthy community controls and one of patients with other respiratory conditions. A small increase in the risk of IPF was found in association with jobs involving working with metals (OR 1.34; 95% CI 1.14–1.59). In addition, they found a significant association with farming and living in an agricultural area (OR 3.01; 95% CI 1.29–7.43).

In a follow-up to the study by Scott et al. (5), Hubbard et al. (7) conducted a larger case-control study with 218 cases of IPF and 569 community-matched controls from the Trent region of the United Kingdom. Information on occupational dust exposure was collected twice: first using a postal questionnaire and second by telephone interview. The results confirmed a significant, independent and dose-related increase in the risk of IPF in association with both metal (OR 1.68; 95% CI 1.07–2.65) and wood dust exposure (OR 1.71; 95% CI 1.01–2.92). Significant associations were also found with exposure to textile dust and stone/sand (see Table 4).

A multicenter, case-control study was conducted in the United States by Baumgartner et al. (8,9) that included 248 cases of IPF and 491 age-, sex-, and geography-matched controls recruited using random digit dialing. As with the previous studies, metal dust was significantly associated with IPF (OR 2; 95% CI 1–4). Moreover, risk increased with duration of exposure to metal dust (< 5 years compared to ≥ 5 years), suggesting a dose-response relationship. Other significant associations (see Table 4) included exposure to stone/sand dust, livestock, and farming/agricultural areas, hairdressing (OR 4.4; 95% CI 1.2–16.3), and raising birds (OR 4.7; 95% CI 1.6–4.1).

Because recall bias is a potential limitation of case-control studies, alternative study designs are needed to strengthen confidence in these results. To examine the risk associated with metal exposure, Hubbard et al. used an occupational cohort from the pension fund archives held by a major metal engineering company (Rolls-Royce PLC) (36). This cohort contains a total of 20,526 deaths and 55 deaths from IPF. On the basis of national data, 39.5 deaths were expected resulting in a proportional mortality ratio (PMR) from IPF, which was increased among employees compared to the general population (PMR 1.39; 95% CI 1.07–1.82). In addition, lifetime occupational records were obtained for 22 cases, of whom 13 had worked with metal, and a random sample of 238 controls, of whom 125 had worked with metal. The age- and sex-adjusted OR for the impact of working with metal was 1.08 (95% CI 0.44–2.65) suggesting that in this small nested case-control study, working with metal was not a risk factor for IPF. However, among employees exposed

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to metals, there was evidence of a linear increase in the risk of IPF with duration of exposure (OR per 10 years of exposure 1.71; 95% CI 1.09–2.68), and there was no evidence of an association between duration of employment and IPF for non–metal workers.

A number of consistent associations between occupational exposures and IPF have been found in the available case-control studies (see Table 4). Significant associations were consistently found with metal dust exposure in all four studies with evidence of dose-response relationships. Significant associations have also been demonstrated in two studies with livestock exposure, farming/agricultural area, and stone/sand exposure. Although exposures to wood and textile dusts have been associated with an increased risk in at least two studies, they were not always statistically significant. Although it is difficult to exclude the possibility that these associations are not due to confounding because of concomitant exposure to known fibrogenic agents (e.g., asbestos, cobalt, thermophilic organisms), the findings offer the potential for preventing IPF by reducing or eliminating exposure to these dusts.

B. Smoking

In a number of early case series of patients with IPF (37–39) the prevalence of smoking was high, suggesting that smoking may be a risk factor for the disease. To date, smoking as a risk factor for IPF has been examined in the four case-control studies described previously (see Table 4) and in a case-control study of antidepressant exposure (40). In three of the four case-control studies of occupational and environmental exposures (6–9), smoking was significantly associated with IPF (see Table 4), but was not significantly associated in one study (5). Moreover, in a case-control study of antidepressant exposure and IPF (40), smoking was not associated with IPF. Although the studies may have a number of methodological limitations, including recall bias, selection bias from reduced response rate among smoking controls, and misclassification of smoking status (41), the available results suggest a potential etiological role for smoking in IPF. Furthermore, aside from the potential etiological role, it is also evident that smoking may be an important confounder in etiological studies of IPF, and therefore needs to be measured and adjusted for in future studies of other risk factors.

C. Medications

Interstitial lung disease is a recognized adverse effect of a number of drugs, particularly amiodarone, nitrofurantoin, and cytotoxic agents. However, interstitial lung disease has also been reported as being a rare complication of some much more commonly used drugs, including antidepressants (42–45), antibiotics (46–49), beta-blockers (50–53), anticonvulsants (54,55), and nonsteroidal anti-inflammatory drugs (NSAIDs) (56–60). These observations

suggest that some cases of IPF may be a rare complication of commonly used drugs. This hypothesis has been tested in a case-control study undertaken as an extension to the Trent occupational case-control study (61). In an attempt to minimize reverse causation, the analysis was restricted to prescriptions issued 5 years or more before the diagnosis of IPF. Among the drug groups detailed above, IPF was associated with the use of antidepressants but not beta-blockers, antibiotics, or anticonvulsants. Among antidepressants, the risk was mainly associated with the use of tricyclic and related antidepressants, and in particular with the use of imipramine and dothiepin. The biological plausibility of the hypothesis is enhanced by evidence from radiolabeling studies that about two-thirds of an orally administered dose of clomipramine is concentrated within the lung (62). Nonsteroidal anti-inflammatory drugs were also associated with an increase in the risk of IPF, but of borderline significance (61), as were a number of other drug groups, which were not considered a priori to be risk factors, including laxatives, antianginal agents, antihistamines, and barbiturates.

In order to verify the association between antidepressants and IPF, a second case control study was performed using a dataset derived from the computerized primary care records of 890 cases of IPF and 5340 controls (40). A small increased risk of disease was found in association with all antidepressants, but there were insufficient years of data to exclude bias due to reverse causation. Furthermore, the associations found in this study lacked the specificity seen in the first study. Further work is therefore required to clarify whether exposure to antidepressants is a risk factor for IPF and to identify whether other commonly used drugs may also represent potentially avoidable causes of this disease.

D. Infectious Agents

There are anecdotal reports of interstitial fibrosis as a complication of a number of infections, including *Mycoplasma pneumoniae*, *Legionella pneumophilia*, and influenza A2 virus (63–66). Several studies have also demonstrated either serological or immunocytological evidence of infection, and in some cases, replication of the Epstein-Barr virus (EBV) in the lungs of patients with IPF when compared with control subjects (67–70). However, this finding has not been consistently demonstrated in all studies (71,72), and the confounding effects of age or immunosuppressive therapy may explain some of these discrepancies (73,74). Studies of hepatitis C virus from Japan and Italy have demonstrated a higher frequency of antibodies in cases of IPF (75,76), but this observation was not confirmed in data from the United Kingdom (77). Cytomegalovirus, herpes simplex, and adenovirus have also been implicated in individual studies of patients with IPF (69,78), but have not

been independently confirmed. The role of viruses in the pathogenesis of pulmonary fibrosis is reviewed in greater detail in Chapter 23.

E. Other Factors

Other biologically plausible factors that may contribute to the development of IPF but have received limited attention are early life experiences and diet. Although early life experiences as risk factors for development of adult lung diseases such as asthma and chronic obstructive pulmonary disease have been examined, there are few data available on the impact of early life experiences (e.g., birth weight) on the lifelong risk for developing IPF. However, there are data from one study which demonstrated that shorter adult height, a surrogate marker of early environmental exposures, is associated with IPF compared to the general population (79).

The level of glutathione in bronchoalveolar lavage fluid is four times lower in patients with IPF compared to healthy subjects; suggesting that antioxidant defense mechanisms are deficient or at least under stress (80,81). This observation suggests that diets relatively deficient in antioxidants might increase the risk of developing IPF or perhaps accelerate the fibrotic process. Results from a Japanese case-control study (6) suggest that diets may have an etiological role in IPF. Consumption of fish was associated with a lower risk of IPF (OR 0.48; 95% CI 0.26–0.88). However, there were no other dietary factors significantly associated with IPF. Evidence from one ecological study also suggests that regions in the United Kingdom with higher per capita consumption of fish and fresh vegetables also have lower mortality rates from IPF (82). A multicenter trial is currently in progress to assess the efficacy of high doses of *N*-acetylcysteine, an antioxidant, in patients with IPF.

F. Synthesis

The associations observed from the available etiological studies provide support for the hypothesis that IPF may be a heterogeneous disorder caused by a number of occupational and environmental exposures. However, further evidence is needed to judge whether these associations are causal in the development of IPF. Although causation of IPF may never be directly observable, it is possible to infer causal relationships from the available scientific evidence using criteria for evaluating causality described by Hill (83). Nine criteria were originally described, but because multiple causation of disease is well established, specificity of association is not relevant. Although these criteria have been criticized, they provide a useful framework for evaluating causality.

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Of the nine criteria, five support a causal relationship between environmental exposures and IPF, including experimentation, plausibility, coherence, analogy, and consistency of association. Results from experimental studies have established the biological plausibility of environmental agents causing pulmonary fibrosis. The inhalation of environmental agents causing pulmonary fibrosis is coherent with our present knowledge of the pathogenesis of IPF based on results from experimental studies and clinical observations. Asbestos exposure, a known cause of pulmonary fibrosis, suggests by analogy that other environmental exposures may cause IPF. Consistent associations between occupational and environmental exposures and IPF have been found in epidemiological investigations from three countries.

In the context of the remaining three criteria, strength of association, biological gradient, and temporality, evaluation of the available evidence is less convincing. Overall, the strength of the associations has been low for any of the exposures that have been examined. For relative risk estimates of less than three or four systematic errors may substantially contribute to the associations. Limited evidence for metal and wood dust are available on the biological gradient, or dose-response relationship between exposure and risk of disease. Finally, the temporal relationship between exposures and IPF cannot be determined from the case-control design, and cohort studies will be needed to determine that the exposures preceded the onset of IPF.

V. Prognosis and Treatment

The ultimate goal of epidemiology is to control diseases through application of etiological information, and may include a number of approaches, including (1) elimination of exposures to prevent the occurrence of disease (2) early detection to prevent progression, and (3) treatments to lessen morbidity and lower mortality in patients with established disease. Except for pharmacological treatments, none of the other potential methods to control IPF has been examined, primarily because insufficient information has been available on etiological factors. Knowledge about prognosis and factors that influence it provides the basis for designing studies to evaluate any intervention to control IPF and for counseling patients and families.

Overall, a clinical diagnosis of IPF is associated with a poor prognosis. In early case series of patients with IPF, the median survival from the time of diagnosis was approximately 5 years (37,38), but estimates of survival from these studies were likely biased, because they were not population-based and included incident cases (i.e., newly diagnosed) and prevalent cases (i.e., previously diagnosed) (3). Prevalent cases comprise survivors, and thus will have longer survival compared to incident cases that include prevalent cases plus patients with the highest fatality rate. More recent evidence on prognosis

derived from population-based studies of incident cases have demonstrated a median life expectancy of less than 3 years (11) to 4 years (10), and compared to the general population, these patients lose on average 7 years of life (11).

In addition to survivor bias, the validity of survival results may be compromised by other biases and chance observations. For example, sampling bias, a result of systematic differences in patients seen at academic medical centers compared to community settings, is a concern. Although little overall survival difference was found in one investigation that included patients from referral centers and the general population (10), differences in short-term and long-term survival were observed between the two groups. Another limitation of many studies on prognosis in IPF is that few have had sufficient numbers of patients to assess simultaneously the influence of the multiple potential determinants of prognosis.

Prognosis of IPF and predictors of prognosis have been assessed in case series of patients seen at referral centers (37,38,84,85) and two population-based studies (10,11). The major determinants of survival that have been examined include age, gender, smoking status, pulmonary function, and histological characteristics. In addition, the influence of various treatments on prognosis has been described in case-series and tested in a limited number of small clinical trials. A summary of the evidence on determinants of prognosis, including treatments, is provided in this section. More detailed discussion of treatment options and future directions of treatment are provided in Chapters 24 and 25, respectively.

A. Age and Gender

Many of the studies of survival among patients with IPF have lacked statistical power to estimate precisely the impact of age and gender, and few have compared survival among patients with IPF to survival in the general population. For these reasons there are inconsistencies in the available data, and although some studies have demonstrated a better prognosis in younger patients (10,37,85) and among females compared to males (37,84), others have found no apparent impact of gender (10,85) or age (84).

To clarify the impact of age and sex on survival in patients with IPF, and to quantify how this survival compares to the general population, we performed a survival analysis using follow-up data for 872 cases of IPF (62% male, mean age 70 years) and 5854 general population controls (61% male, mean age 70 years) contained within the UK General Practice Research Database. Male gender and increasing age were both strong predictors of survival in both populations; however, the influence of gender, and even more so age, was less marked for patients with IPF (Table 5; unpublished data). These data suggest that although male gender and older age remain markers of poorer survival among patients with IPF, these effects are not disease specific.

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Table 5 Survival Analysis Comparing Patients with IPF to General Population Controls from UK General Practice Research Database

	IPF ($n = 872$)		Controls ($n = 5854$)	
	Hazard ratio	95% CI	Hazard ratio	95% CI
Male vs female	1.44	1.16–1.78	1.48	1.24–1.76
Age group (years)				
< 55	Reference		Reference	
55–59	1.27	0.65–2.46	3.95	1.31–11.92
60–64	1.67	0.93–3.00	5.31	1.87–15.08
65–69	2.18	1.24–3.81	10.19	3.72–27.87
70–74	3.13	1.83–5.33	13.90	5.13–37.66
75–79	3.52	2.06–6.03	21.68	8.03–58.53
80–84	4.39	2.52–7.66	30.84	11.40–83.46
85–89	7.15	3.82–13.37	40.00	14.47–110.5
< 90	8.10	3.30–19.71	72.17	23.72–219.6

B. Smoking

The question of whether smoking modifies the natural history of patients with established IPF has been examined in several studies, but with conflicting results. Among selected patients with IPF, current smokers appear to have a better survival than never smokers (84,85), and former smokers have the poorest survival. In a population-based cohort of patients with IPF, ever smokers, comprising current and former smokers, had slightly better survival compared to never smokers, but the difference was not statistically significant (10). Changes in respiratory symptoms and smoking behavior may explain these observations. For example, current smokers with IPF may present earlier than nonsmokers, because of smoking-related symptoms, and thus lead-time bias may explain the apparent improvement in survival. In contrast, quitting smoking may be a marker of more aggressive disease and poorer survival, because rapid onset of symptoms may result in quitting smoking.

C. Lung Function

In nearly all patients with IPF, the disease is progressive and accompanied by deterioration in lung function. Therefore, it is not surprising that lung function at diagnosis has been consistently found to be a predictor of survival (10,84,85), with increasing severity of forced vital capacity (FVC) impairment being associated with worsening survival. To develop a scoring system to model survival for patients with IPF, King et al. (85) examined the impact of various lung function measures together with a range of other clinical,

radiological and histological data. In the final multivariate model, the two components of lung function that remained predictive of survival were total lung capacity and Pa_{O_2} at maximal exercise (85).

D. Histological Diagnosis

Only a minority of patients with IPF undergo surgical lung biopsy (4,86). Often these patients are younger (11), and the clinical diagnosis is not certain. Therefore, prognostic results based on histological findings must be interpreted cautiously, since the process of patient selection for surgical lung biopsy may bias results on the relationship between histological findings and prognosis in patients with IPF. In early case series, a consistent finding of these studies was that patients with a histological diagnosis of desquamative interstitial pneumonitis (DIP) had a much better prognosis than those with a diagnosis of UIP (37,38).

More recently, attempts have been made to increase the specificity of a clinical diagnosis of IPF by establishing diagnostic criteria (1), and concurrently there has been a reclassification of the histological diagnoses of the idiopathic pneumonias (2). Patients with a histological diagnosis of DIP are now considered to represent a separate disease entity, and the term IPF is used specifically for patients with a histological diagnosis of UIP or those with a clinical and radiological presentation highly suggestive of this diagnosis (1). In addition, a new histological category has been established and termed nonspecific interstitial pneumonia (NSIP). Patients with NSIP share a number of clinical and radiological characteristics with UIP (87,88), but provisional evidence from several small studies has suggested that patients with NSIP may have a better prognosis (87–89). Moreover, recent evidence has been reported that among patients with a histological diagnosis of UIP, the extent of fibroblastic foci present in the open lung biopsy is an independent predictor of survival (90).

E. Associated Diseases

Connective Tissue Diseases

Although exclusion of connective tissue diseases is required for the diagnosis of IPF (see Table 1), clinical similarities has prompted investigations comparing prognosis in these patient groups, which are briefly reviewed here. The relationship between connective tissue diseases and pulmonary fibrosis are reviewed in greater detail in Chapter 9. Approximately 10–20% of patients who appear clinically to have a diagnosis of IPF have a coexisting connective tissue disease, and in most cases, this is rheumatoid arthritis (37,38,91). Early studies found no difference in survival between patients with and without a range of connective tissue diseases, but these studies had limited statistical

power (38,92). In a larger study comparing patients with systemic sclerosis and interstitial lung disease to patients with IPF (93), a large survival advantage was found among patients with systemic sclerosis even after controlling for the potential confounding effects of age, sex, and extent of lung fibrosis at diagnosis. In a population-based survival analysis of 979 patients with a clinical diagnosis of IPF, of whom 107 (11%) had a coexisting diagnosis of connective tissue disease (86 rheumatoid arthritis), connective tissue disease was associated with marginally worse survival compared to patients with no connective tissue disease (91).

Lung Cancer

Early reports suggested that patients with IPF might have an increased incidence of lung cancer (94,95). This hypothesis was first formally tested in an analysis of patients followed at the Bromptom Hospital in England, and a marked increased risk of lung cancer among patients with IPF was found (96). A more recent study based on death certificates found no association (97), but problems with death certification for patients with IPF have been reported (98). A recent analysis of 890 cases of IPF using primary care data from the United Kingdom (99) found very similar results to Turner-Warwick et al. (96), with a six- to sevenfold increase in cancer risk that was independent of smoking. Clearly, patients who have IPF will be subject to intermittent medical review and radiological investigation and, therefore, will have greater opportunity for lung cancers to be detected compared to the general population. However, the large magnitude of increase in risk for lung cancer found in the studies by Turner-Warwick et al. (96) and Hubbard et al. (99) suggests that this is a true increased risk regardless of smoking and not a result of bias.

E. Treatment and Survival

The poor prognosis of patients with IPF emphasizes the need for effective treatments; however, there is scant evidence and no proven therapies (100). Despite limited evidence, both United States (1) and British (101) guidelines recommend oral corticosteroids in conjunction with either azathioprine or cyclophosphamide. Other treatment options that have been assessed in clinical trials include colchicine and gamma-interferon.

Corticosteroids

There are no placebo-controlled trials to support the use of oral corticosteroids in the treatment of patients with IPF (102,103). The effectiveness of corticosteroids has been assessed in a number of case series (102,104) and one population-based investigation (10). Overall, in the case series, objective improvements with corticosteroids have been found in fewer than 30% of

patients. Moreover, most patients experience steroid-induced side effects (104). In a population-based cohort of incident cases of IPF, Mapel et al. (10) found that in a multivariate model controlling for age, gender, smoking, and disease severity, corticosteroid treatment was associated with higher mortality compared to patients who were not treated with corticosteroids (relative hazard 2.1; 95% CI 1.3–3.4). Based on the available evidence, recent reviews have concluded that, at present, there is insufficient evidence to support the use of oral corticosteroids for the management of patients with IPF (102,105).

Azathioprine

The evidence supporting the use of azathioprine comes from a single small randomized double-blind placebo-controlled trial in which 13 patients were allocated to receive 12 months of therapy with prednisolone alone and 14 to prednisolone plus azathioprine (106). There was little difference between treatment groups at the end of the 12-month intervention when lung function had improved slightly more in patients receiving both azathioprine and prednisolone (6.5% increase in mean percentage of predicted FVC vs. 1.7%; $P = 0.87$), and there had been four deaths in each group. However, after a period of extended follow-up to a maximum of nine years, deaths in the azathioprine plus prednisone group had increased to 6, and in the prednisone group to 10. With adjustment for age, this difference in survival had borderline statistical significance, suggesting that azathioprine may reduce longer term mortality. Adverse drug effects were common but were predominantly due to prednisolone.

Cyclophosphamide

The efficacy of cyclophosphamide was assessed in an open-label trial in which mortality at 3 years was significantly reduced in 21 patients with IPF receiving prednisolone plus cyclophosphamide (3 deaths) compared to 22 patients receiving prednisolone alone (10 deaths) (107). Clinical improvement occurred at any stage of the 3-year follow-up in seven patients receiving prednisolone only and in five patients receiving prednisolone plus cyclophosphamide. In this study, there was a higher occurrence of adverse effects, primarily hematological toxicity in the cyclophosphamide-treated group, which, in association with concerns over long-term carcinogenicity, has resulted in azathioprine emerging as the preferred second-line therapy for IPF.

Colchicine

The efficacy of colchicine has been assessed in an open-label trial in which 14 patients with IPF received colchicine and 12 received oral corticosteroids (108). The main endpoints of the trial was a composite of death, significant disease

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progression, or intolerance to treatment. Overall, survival was marginally higher among patients who received colchicine, and the incidence of side effects was significantly greater for patients who received oral corticosteroids.

Interferon Gamma-1b

One trial of interferon gamma-1b has been completed and, this included 18 nonsmoking patients with progressive IPF and who had not been treated with corticosteroids (109). Nine patients were randomized to 200 mg of interferon gamma-1b three times a week, and the remaining nine received prednisolone at a dose titrated against symptoms but which was no lower than 7.5 mg a day. An improvement in total lung capacity (TLC) was found in all patients who received interferon gamma-1b, which was in marked contrast to the patients who received oral corticosteroids alone, all of whom had a decline in TLC.

Other Treatments

Patients with IPF are often hypoxemic, particularly after exertion, and supplemental oxygen has been shown to ameliorate symptoms of breathlessness (110), and this should be considered as palliative treatment. Moreover, patients who have daytime hypoxemia are also often markedly hypoxemic at night, and nocturnal hypoxemia has been associated with symptoms of fatigue (111). Although further study of the impact of nocturnal oxygen supplementation on symptoms and long-term survival is needed, the Royal College of Physicians in the United Kingdom has recommended that patients with a resting arterial oxygen level of less than 8 kPa should be considered for long-term oxygen therapy (112).

Although lung transplantation is an option for treatment of end-stage IPF, advanced age and associated illness prevent many patients with IPF from having the procedure. Little data are available on the procedure, but it has been suggested that patients under 55 years of age may benefit from early referral to lung transplant centers (100). Results from one study suggest that if transplant is an option, patients should be reviewed by the transplant center when the diffusing capacity of the lung for carbon monoxide (DLCO) has declined to about 40% of predicted (113).

Synthesis

Although little population-based data are available on the prognosis of patients with IPF (10,11), the available evidence clearly demonstrates that the prognosis is poor. Overall, the median life expectancy is less than 3–4 years. A number of determinants of prognosis have been examined including age, gender, smoking, lung function, histological patterns, and treatments. Older

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age is a predictor of poorer survival, but is not disease specific. Gender has been inconsistently associated with prognosis. Compared to never smokers, current smokers have been found to have a better prognosis and former smokers a worse prognosis, but these results may be artifactual. Disease severity, measured with lung function, has been consistently associated with prognosis. Although histological characteristics may predict prognosis in selected patients, the limited use of surgical lung biopsy limits the generalizability and usefulness of these findings. Finally, scant evidence is available on the treatment of IPF, and there is no conclusive evidence for an effective therapy that lessens morbidity or mortality.

VI. Conclusions

Over the past decade, results from a number of population-based studies have advanced our knowledge about the epidemiology of IPF. Although the occurrence of IPF is relatively uncommon compared to obstructive lung diseases, among the elderly the condition is not rare. Moreover, gender and regional variations in prevalence and mortality suggest a role for environmental factors in the development of IPF. Supportive evidence for this hypothesis has been found in etiological studies, which have demonstrated a number of associations between occupational and environmental exposures and IPF. Once diagnosed, the prognosis of IPF is extremely poor, with a median survival of 3–4 years or less, and there are no treatments that have been substantially proven to improve morbidity or survival.

The poor prognosis and lack of effective therapies, combined with advances in our understanding of the pathogenesis of IPF, have triggered investigations to discover innovative treatments (see Chap. 25). However, results from available etiological studies suggest that further epidemiological investigations are also needed to establish whether there is a causal link between occupational and environmental exposures and IPF. Furthermore, results from studies of genetic polymorphisms and risk of IPF (see Chap. 2), which are in progress, are needed to determine susceptibility for the development of IPF. If causal links between occupational and environmental factors and genetic polymorphisms are confirmed, then prevention of IPF becomes a feasible option for controlling this devastating disease through elimination of exposures and screening.

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2

Genetics of Familial Pulmonary Fibrosis and Other Variants

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I. Introduction

Idiopathic pulmonary fibrosis (IPF) is an incurable disease with a 5-year survival of 30–50% from the time of diagnosis (1). In recent years, there has been much progress made in understanding the cellular and molecular events leading to pulmonary fibrosis (PF). However, the exact pathogenesis has yet to be determined. The initiating stimulus is unknown in the majority of patients, and only a subset of individuals (5–20%) exposed to known fibrogenic agents actually develop interstitial lung disease (ILD) and PF. Despite the expanding knowledge of the pathophysiology of these diseases, there is currently no effective treatment. Several lines of evidence suggest that inherited genetic factors play a role in the development of pulmonary fibrosis; at least in a subset of patients. The discovery of these genes would lead to attaining a greater understanding of the cellular and molecular mechanisms of this disease, counseling carriers about preventive measures and avoidance of certain exposures and occupations, identifying those with early potentially reversible interstitial lung disease, and developing novel therapeutic approaches for pulmonary fibrosis. Moreover, the role of these genes in other more common forms of interstitial lung disease, such as asbestosis, can be studied leading to similar benefits. This chapter addresses the genetic basis of pulmonary fibrosis and reviews the types of pulmonary fibrosis that have a clearly defined genetic basis.

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II. Genetic Basis of Pulmonary Fibrosis

The role of genetic factors in the development of PF is supported by the occurrence of forms of PF that appear to be inherited (familial PF and PF associated with pleiotropic genetic disorders), the apparent variation in individual susceptibility to fibrogenic dusts that are known to cause PF, and the differences in development of PF observed between inbred strains of mice following experimental exposure to fibrogenic agents.

Familial pulmonary fibrosis (FPF) was first recognized in 18-year-old twin sisters by Sandoz in 1907 (2); decades before Hamman and Rich published their cases of fulminant and fatal interstitial lung disease (3). Many case reports and series were subsequently published documenting the occurrence of PF in separately raised monozygotic twins (4,5), in closely related members of several families (6–11) in more than two generations (12,13), in several father–son pairs (13,14), and in families with disease onset in infancy (12,15).

In a more recent report, Marshall et al. described the epidemiological and clinical characteristics in 25 families with FPF within the United Kingdom (16). The investigators estimated the prevalence of FPF at 1.34 cases per 10^6 in the UK population. In addition to the familial aggregation of PF, Bitterman et al. found evidence of alveolar inflammation in bronchoalveolar lavage fluid in approximately 50% of the clinically unaffected family members of patients with FPF (7).

The description of father–son inheritance excludes sex linkage, whereas the occurrence of PF in separately raised monozygotic twins implies that the transmission of this trait is not solely due to shared environmental factors.

Assessment of the inheritance pattern in these and other reported cases in the literature suggests that FPF is inherited either as an autosomal recessive trait or as a dominant trait with reduced penetrance. This speculation can be confirmed by performing a segregation analysis that studies the transmission pattern of a trait within families and examines it in comparison to various genetic models. Segregation analysis requires systematic ascertainment of a sufficiently large sample of families of any patient with PF (lone cases and familial cluster) and would, therefore, be fairly difficult to apply to PF in view of its low prevalence.

Pulmonary fibrosis is observed in pleiotropic genetic disorders including the Hermansky-Pudlak syndrome (17), neurofibromatosis (18), tuberous sclerosis (19,20), Neimann-Pick disease (21), Gaucher's disease (22), and familial hypocalciuric hypercalcemia (23). Whereas Hermansky-Pudlak syndrome is inherited as an autosomal recessive disease and is known to be associated with a defect in 5-hydroxytryptamine (24) and lysosomal metabolism (25), familial hypocalciuric hypercalcemia is an autosomal dominant disorder that is associated with a relative deficiency in myeloperoxidase (23). Interestingly, in the Hermansky-Pudlak syndrome, there is an

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enhancement in the release of platelet-derived growth factor-β (PDGF-B) by alveolar macrophages (26,27), a growth factor that is thought to play a pathogenic role in idiopathic PF (28–31). Although these pleiotropic genetic disorders are distinct clinically and pathologically, they are all associated with PF, pointing toward a genetic basis influencing this association.

Considerable variability exists in the development of interstitial lung disease among workers reportedly exposed to similar concentrations of fibrogenic dusts. This is perhaps best documented following exposure to asbestos where similarly exposed individuals may experience very different disease outcomes. Epidemiological investigations have clearly established a causal, dose-response relationship between asbestos exposure and the development of asbestosis within several working populations (32–38). However, among workers occupationally exposed to the highest cumulative dose of asbestos, the prevalence of radiographically evident asbestosis ranges from 25 to 50%. Hence, at least one-half of those heavily exposed to asbestos appear to demonstrate ''resistance'' to its fibrogenic effect. Furthermore, individuals with similar asbestos exposures have been shown to progress radiographically at very different rates (39–41). Although youth, cigarette smoking, and more severe interstitial lung disease on initial chest radiography have been shown to be associated with progressive PF, genetic factors related to differential susceptibility for the development and progression of asbestosis have not been studied.

Inbred strains of mice clearly differ in their susceptibility to fibrogenic agents. The C57BL/6 mice have a higher propensity to develop lung fibrosis as compared to BALB/c mice (42–46) when challenged with either bleomycin or asbestos. The etiology is unknown but may be related to excessive synthesis of collagen (43), diminished levels of bleomycin hydrolase (47,48), inability to repair DNA strand breaks effectively (49), or dysregulation of proinflammatory cytokines (45,50–54). The moth-eaten mouse, which dies at 8 weeks of age with diffuse, noninfectious lung disease, provides additional evidence of genetic control over interstitial lung disease (55) that is associated with release of tumor necrosis factor-α (TNF-α) in the lung (56).

In summary, all of the aforementioned findings support a genetic basis for PF. It is logical to speculate that specific gene products modulate the inflammatory and fibrotic response to fibrogenic agents and provide the pathogenic basis for enhanced genetic risk.

III. Familial Pulmonary Fibrosis

The diagnosis of familial pulmonary fibrosis (FPF) requires that at least two members among the primary relatives in a family (biological parents, children, siblings) have clinical features of interstitial lung disease (ILD). Although some

believe that the histological diagnosis of usual interstitial pneumonitis (UIP) is essential to the diagnosis of FPF, it remains possible that FPF represents a distinct histopathological entity within the spectrum of ILD. The occurrence of FPF is well-documented but its exact incidence or prevalence is unknown. It is believed to be very rare and to affect all age groups. Bitterman and coworkers (7) performed bronchoalveolar lavage (BAL) on unaffected family members of patients with FPF. Lavage findings were consistent with inflammation in approximately one-half of family members without apparent clinical disease; giving further support for this inheritance pattern. Long-term follow-up is needed to see if these individuals eventually develop PF.

Patients with FPF usually present during the third or fourth decade of life (57), however, cases of fulminant disease have been reported during infancy (58). It is likely that these early-onset cases of ILD are distinct pathogenically from the adult-onset occurrences of FPF. The clinical symptoms, physical signs, radiographic changes, pulmonary function testing abnormalities and histopathological findings of FPF are in need of further definition. The management of patients affected by FPF is similar to that of sporadic forms of IPF (61). Lung transplantation may be an early consideration in selected patients with FPF. The course of FPF may be complicated by lung cancer including alveolar cell carcinoma, small cell carcinoma, and adenocarcinoma (59,60). It is currently not possible to predict the chances of disease expression in the presently unaffected members of the family, as a genetic or surface marker has not yet been identified. Annual screening of the primary relatives of affected family members (parents, siblings, children) with chest radiographs, pulmonary function tests, and high-resolution computed tomography (HRCT) images of the chest may detect abnormalities early enough to initiate therapeutic interventions. Despite the rarity of FPF, its overlap with IPF presents a valuable opportunity to gain insights into the etiology and pathogenesis of IPF.

IV. Search for the PF Gene

Substantial advances have been recently made in technical and strategic approaches to the molecular identification of genes and loci, and have been successfully applied to the investigation of human genetic disorders.

Some of the advances in the last decade include the improvement in processing and genotyping of DNA samples, the exponential growth of genetic maps, the continuous characterization of countless human genes, and the development of polymorphic genetic markers for linkage analysis. Two general strategies have been adopted to take advantage of these advances: candidate gene approach and positional cloning. In the candidate gene approach, specific genetic loci are identified and studied based on prior knowledge of their

biological function and possible pathogenic role in the disease under study. In positional cloning, a genome-wide search utilizing polymorphic genetic markers is performed and candidate genetic loci are identified based on cosegregation between genetic markers and a disease trait.

A. Defining Phenotypes for Human Genetic Studies of PF

Establishing a consistent phenotype (i.e., a firm diagnosis of IPF) is an essential step in conducting a valid genetic study. The American Thoracic Society (ATS) issued a consensus statement in an attempt to standardize the diagnosis and treatment of IPF (1). The definite diagnosis of IPF requires a histological appearance of usual interstitial pneumonitis (UIP) on surgical lung biopsy, as well as evidence of restriction on pulmonary function tests, compatible abnormalities on high-resolution chest computed tomography, and the exclusion of other known causes of interstitial lung diseases. The diagnosis is uncertain in the absence of a surgical lung biopsy but is highly suggested if certain major and minor criteria are fulfilled as suggested by the ATS statement. The pathological classification of IPF and the diagnostic features of each subtype have been well described in the literature (62).

In a recent study by Hunninghake et al., the probability of agreement among two of three experienced pathologist regarding the diagnosis of IPF was 85% (63). In the same study, the positive predictive value of a confident clinical diagnosis of IPF was 87% and 96% when reviewed by a core of pulmonologists and radiologists, respectively. These results were obtained in an ideal setting with experienced diagnosticians and may be falsely reassuring. Misclassified phenotypes can seriously affect the validity of a genetic study and limit the comparison or pooling of results from different genetic studies.

B. Candidate Gene Studies in PF

Based on our limited knowledge of the pathogenesis of PF, a number of candidate genes, that may predispose individual to develop this disease, can be identified. Whereas activated alveolar macrophages and epithelial cells may release polypeptide growth factors (transforming growth factor-B1 [TGF-β1], platelet-derived growth factor [PDGF-B], insulinlike growth factor-γ [IGF-γ], and fibronectin) that modulate the growth of mesenchymal cells, cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interferon-γ (IFN-γ), may serve to amplify the local inflammatory response.

TGF-β1 is a protein formed by two identical chains that has been implicated in PF through its promotion of fibroblast proliferation and collagen synthesis. TGF-β1 has been found in excessive amounts in pulmonary epithelial cells and alveolar macrophages in areas of lung regeneration and remodeling in IPF patients (64,65). Multiple studies have addressed the genetic

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variation in the TGF-β1 gene in patients with IPF. Two related studies found that homozygosity for arginine at codon 25 of the leader sequences of TGF-β1, which correlates with higher production of TGF-β1 in vitro, is more common in patients undergoing lung transplantation for fibrotic lung diseases as compared to controls and to patients undergoing lung transplantion for conditions other than lung fibrosis (66,67). In addition, the majority of lung transplantation patients who developed lung allograft fibrosis were codon 25 homozygotes (67). Using in situ hybridization and immunohistochemsitry, TGF-β1 was found in abundance in foci containing activated fibroblasts in lung biopsies obtained from IPF patients in association with a marked expression of TGF-β1 mRNA in alveolar macrophages surrounding these sites (68).

PDGF is another potent mesenchymal cell growth factor that has been shown to be released in excessive amounts by alveolar macrophages in patients with IPF (28,29). Nagaoka et al. studied the transcription rates and mRNA levels for both PDGF-A and PDGF-B genes in alveolar macrophages from normal individuals and those with IPF (30). In IPF, both PDGF-A and PDGF-B mRNA levels were markedly increased, but with preservation of the 10-fold ratio of PDGF-B over PFGF-A found in normal persons.

Fibronectin is an extracellular matrix glycoprotein that functions as an adhesive substrate and plays a role in cell proliferation, especially during tissue repair. Lung tissues from IPF patients were found to have an exaggerated expression of the fibronectin gene in both areas of early inflammation and areas of mature fibrosis rich with fibroblasts (69). Avila et al. studied polymorphisms in the fibronectin gene in patients with PF associated with systemic sclerosis (70). Utilizing restriction enzymes to assess the restriction fragment length polymorphisms, genotypes AB and CD (associated with restriction enzymes *Hae*III and *Msp*I, respectively) were found to be more frequent in systemic sclerosis patients with pulmonary fibrosis as compared with healthy control subjects (45% vs 29%; P=.0059) with an increase in relative risk of developing pulmonary fibrosis of 1.988.

IGF-1 is a growth factor that induces fibroblast proliferation and stimulates their synthesis of collagen. Bloor et al. examined the expression of four IGF-1 transcripts in BAL cells from normal subjects, IPF, and sarcoidosis patients (71). There was a differential expression of IGF-1 splice variants, where IPF patients had an increase in class 1 transcripts and failed to express class 2 IGF-1EB forms.

TNF-α and IL-1 are proinflammatory cytokines that have been implicated in the pathogenesis of IPF. Numerous studies have investigated polymorphisms in their genes. Whyte et al. conducted a case-control study on two populations: English and Italian (72). For the rarer allele of the TNF-α gene, TNF-A, the relative odds of having pulmonary fibrosis were increased in homozygous subjects by an OR of 1.85 in the English and 2.50 in the Italian

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population. Similarly, for the less common allele of the IL-1RA gene (interleukin-1 receptor agonist), IL-1*RN*, the OR was 10.95 in the English and 2.54 in the Italian population. However, in a similar study by Pantelidis et al., comparison of 74 IPF patients to 100 control subjects found no significant deviations in genotype, allele, or haplotype frequencies in four candidate genes: TNF-α, lymphotoxin-α (LT-α), TNF receptor II (TNF-RII), and IL-6 (73). Freeburn et al. hypothesized that polymorphisms in the 3′ untranslated region of the TNF-α gene would explain the previously observed TNF-α resistance to IL-10–mediated suppression in IPF (74). A single-strand confirmation polymorphism analysis was unable to detect such polymorphisms in 96 IPF patients. In a recent case-control study in a West Slavonic population by Hutyrov et al., no polymorphism in the IL-1α, IL-1β, and IL-1RA genes were found in 54 patients with IPF (75).

IFN-γ is an inflammatory cytokine that has recently drawn attention as a promising therapeutic agent for IPF. Earlier studies implicated IFN-γ in the pathogenesis of lung fibrosis, as demonstrated, in one study, by a significant increase in the frequency of allele #2 of the IFN-γ gene in a group of lung transplantation recipients who developed fibrosis after transplantation (76). However, subsequent in vitro (77–79) and in vivo (80) data demonstrated a favorable effect of INF-γ on fibrosis with inhibition of the proliferation of lung fibroblasts and downregulation of the transcription of the genes for TGF-β1.

The role of angiotensin-converting enzyme (ACE) in renal and cardiovascular diseases has been well established. Angiotensin II has been recently shown to augment the in vitro proliferation of human lung fibroblasts by means of activating the angiotensin type 1 receptors (81). The ACE gene has an insertion/deletion polymorphism on exon 16 on chromosome 17q23, resulting in three distinct genotypes: insertion/insertion (I/I), insertion/deletion (I/D), and deletion/deletion (D/D). Morrison et al. examined this polymorphism in 24 patients with usual interstitial pneumonitis (UIP) or nonspecific interstitial pneumonitis and found a higher incidence of the D allele in this population compared to controls, as well as a higher incidence of the genotype D/D (82).

The tumor suppression factor p53 protein is a transcription factor that mediates the cellular response to DNA damage by irradiation or carcinogens. The chronic DNA damage in bronchial and alveolar cells and the higher incidence of lung caner in IPF patients have led to postulations that p53 may have a pathogenic role in IPF. Hojo et al. illustrated frequent heterogeneous point mutations of the p53 gene in IPF patients (83). In the same line of investigation, Vassilakis et al. showed frequent genetic alterations in sputum samples of IPF patients (84); namely, loss of the heterozygosity and microsatellite instability which are commonly detected in malignancy of various origins, including lung carcinomas.

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Alpha$_1$-antityrpsin (α_1-AT) is a serum glycoprotein that exists in the BAL of normal humans and plays a crucial role in the inhibition of several proteolytic enzymes, particularly neutrophil elastase. The most common phenotype in the Pi loci coding for the α_1-AT gene is MM which results in normal serum levels of α_1-AT, whereas the Pi ZZ phenotype results in very low serum levels of α_1-AT. A highly significant increase in the frequency of a non-MM (MZ) phenotype was found among patients with pulmonary fibrosis with and without rheumatoid arthritis (RA) as compared to controls and RA patients with no PF (85). These findings were replicated in RA patients in a later study (86).

Investigation of the human leukocyte antigen (HLA) system, which is located on chromosome 6, has been inconclusive. IPF has been found in a higher frequency in individual with HLA B15 (87), Dw6 (88), and DR2 (89). Moreover, among patients with RA, the development of interstitial lung disease was associated with HLA B8 (88), Dw3 (88), and DQw (90). On the other hand, several studies can be cited which did not identify a relationship between specific HLA alleles and PF among patients with IPF (91,92). These inconsistencies may result from ethnic differences in the distribution of HLA alleles.

Mutation in the gene for surfactant protein C was recently reported in two family members, who developed interstitial lung disease (DIP and NSIP) in childhood (15). Recently, Lloyd and colleagues (93) reported a family with a polymorphism in the fifth exon of the SP-C gene that was associated with the development of PF. Interestingly, this family had both childhood- and adult-onset cases of PF.

In summary, candidate gene studies in human genetic diseases, including PF, tend to point toward promising genetic loci, but in general suffer from their small size and lack of consistency. Replication of such discoveries is paramount for further focused research striving to find specific genes in these intriguing genetic loci.

C. The Role of Positional Cloning in the Search of the PF Gene

The identification of a gene causing a human disease has been accomplished in the past by "functional cloning" based on the knowledge of the biochemical defect of a disease with no prior knowledge of its gene's chromosomal location. In the last two decades, it has become feasible to identify the specific genes involved in human disorders by using strategies that involve "positional cloning," which aims to locate the gene based on its map position with no functional information. Advances in molecular biology techniques and statistical genetic analysis has made positional cloning possible and led to an accelerated localization of human genetic disorders' genes such as cystic fibrosis (94) and Huntington's disease (95).

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One of the most important advances has been the availability of genetic markers: segments of DNA with known chromosomal location and no known function. They are highly polymorphic (vary in length and sequence among humans) and dispersed over chromosomes with spacing of 5–10 cM.

The first step in positional cloning involves the collection of families with two or more members affected with the trait (the disease of interest). The process of linking the disease trait to a genetic marker, and hence a chromosomal location is termed ''linkage analysis.'' DNA specimens from family members are genotyped for a series of genetic markers spanning the entire genome (''genomic screen''). The segregation of alleles of two different genes is related to the distance separating them on the chromosome. If a disease trait is segregating with a marker, it is likely that their genes reside in close physical proximity to each other. This relationship is tested using quantitative statistical tests by comparing it to known genetic models and obtaining a LOD score (logarithm of the odds of linkage). Genetic loci with a LOD score of 3 and above are considered very favorable for linkage (96). These identified linked loci provide the impetus to search that region in more detail for specific genes, utilizing the rapidly evolving knowledge of mapped human genes. These genes that map to the linked subchromosomal loci can then be prioritized on the basis of functional criteria (e.g., known oxidants, polypeptide growth factors, cytokines).

The positional candidate approach is advantageous over the candidate gene approach in PF simply because of our lack of knowledge of the pathogenesis of PF. A focused examination of genes identified based on polymorphisms in families with two or more cases of PF avoids laborious and expensive exploration of genes that may theoretically have a scientific basis but are ultimately far from the truth.

V. Known Inherited Diseases Associated with PF

Interstitial lung disease (ILD) and PF have been associated with several known and rare inherited disorders. Although a subgroup of patients affected with these inherited disorders do manifest ILD and PF, it is unclear if they have distinctive histological patterns and/or if they have UIP or nonspecific inflammatory/fibrotic features in the pulmonary parenchyma. Since the histological subgroups of idiopathic interstitial pneumonia (IIP) have been only recently defined by a consensus reached by internationally recognized experienced experts, future studies will hopefully clarify the histological patterns present in ILD patients with known inherited diseases. Regardless, when confronted with patients manifesting features of ILD, the clinician should be aware of these inherited disorders and recognize that

respiratory symptoms in these patients may be secondary to ILD associated with their inherited disorders. Futher genetic studies in these patients may offer significant insight to the genetic predisposition of PF and IIP in general.

A. Neurofibromatosis

Neurofibromatosis (NF) is an inherited neurocutaneous syndrome that is associated with PF in 7–20% of patients with this disorder (97–99). The diagnosis is made by recognizing the prominent cutaneous findings of multiple neurofibromas, axillary freckling, and café au lait spots (100,101). Lisch nodules, harmartomatous formations of the iris, are seen in 94% of patients (18,100).

It is estimated that the incidence of NF is 1 in 3000 births, and transmission is said to be autosomal dominant, although half of the cases arise by spontaneous mutation (18). There is no sex or racial predominance. A report of a mother and son with NF who both developed interstitial fibrosis strengthens a possible genetic predisposition to lung disease in this syndrome (102). The symptomatic onset of lung disease is usually between 35 and 60 years of age, although symptomatic fibrosis has developed as early as the second decade of life (103). Dyspnea is the usual presenting symptom. Radiographic findings include diffuse interstitial fibrosis and bullae. The bullae are usually apical, may appear with or without fibrosis, and are often large. A chest radiograph early in the course of the disease may show patchy airspace disease (97) and may even be normal despite lung biopsy–documented interstitial fibrosis (98). Pleural disease and mediastinal adenopathy are not known features of this disease, although neurofibromas can sometimes simulate pleural thickening or a mediastinal mass (104). Other thoracic radiographic manifestations are due to the direct effect of neurofibromas. Intrathoracic meningomyeloceles may present as a posterior mediastinal mass. ''Twisted ribbon'' rib deformities caused by the pressure of intercostal fibromas, vertebral defects, and scoliosis have been described (103–105). Pulmonary function tests show restrictive or obstructive lung defect with a reduced DLCO (97,98). The pathological features of interstitial disease in NF are nonspecific (97,98,106). Since these were described prior to the currently evolved understanding of specific histological subgroups, it is unclear if the fibrotic pattern is unclassifiable or represents one of the precisely defined histological subgroups. Like IPF, the interstitial disease in NF is sometimes complicated by scar carcinoma (102,107). The natural history of interstitial disease in NF is unclear. Progression to respiratory failure, pulmonary hypertension, and cor pulmonale may occur (108). There is no known effective treatment.

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B. Tuberous Sclerosis

Tuberous sclerosis (TS) is a very rare inherited disorder with prominent clinical manifestations of adenoma sebaceum, seizures, and mental retardation (109–112). The incidence of TS is 1 in 100,000 to 1 in 150,000 live births. Lung disease develops in only 0.1–1.0% of patients with TS. The disease is transmitted in an autosomal dominant manner with variable penetrance, although several cases may be sporadic (109,113). Three-quarters of patients die by the age of 20 years, usually due to central nervous system disease (109).

The disorder can often be recognized by its associated cutaneous findings: adenoma sebaceum is distributed symmetrically over the nose, nasolabial folds, and chin. These are seen in 80–90% of affected patients (112) and are angiofibroma by histopathological examination (100). Shagreen patches (connective tissue hamartomas), which give a leathery yellow appearance to the skin, are most often found in the lumbosacral region. They eventually develop in 50% of patients (114).

Although there is an equal gender distribution of TS, 80% of individuals that develop lung disease are women (109,110). Its striking resemblance to lymphangiomyomatosis (LAM), a disease peculiar to women of childbearing age, is intriguing. Interstitial disease arises in relatively older patients with an average age at the onset of symptoms of 34 years (110). Central nervous system disease is mild or absent in patients manifesting lung disease. Rarely, isolated lung disease may be the only manifestation of TS (110).

Exertional dyspnea is the major presenting symptom of TS. Recurrent spontaneous pneumothorax occurs in 50% affected patients, and 27% of TS patients will have hemoptysis at some time (109,110). Chylous effusions have been reported (115). The radiographic appearance is nonspecific; the diffuse interstitial infiltrates are usually uniform, but may have a basilar prominence. The presence of preserved or increased lung volumes along with interstitial infiltrates in a nonsmoking woman should raise the possibility of TS. Progression to honeycombing (110) and bullous disease have been described (111). High-resolution computed tomography (HRCT) of the chest reveals characteristic diffuse cystic changes in the pulmonary parenchyma very similar and often indistinguishable from LAM (111). Pulmonary function testing may show fixed airflow obstruction with a reduced D_{LCO} or a mixed obstructive and restrictive pattern (20,112). BAL lavage is nondiagnostic.

The pulmonary histopathology of TS is also very similar to that of LAM (110,116–118). Peribronchiolar smooth muscle proliferation that distorts and disrupts small airways, arterioles, venules, and lymphatics associated with diffuse cystic changes are the usual histological findings (110,112). Intermittent hemorrhage due to vascular involvement leads to abundant hemosiderin deposition. The striking similarities between TS and LAM have appropriately led some to consider LAM to be a forme fruste of TS (118). However, some

differences exist: There is no compelling evidence to suggest that LAM is an inherited disease. LAM can manifest as sporadic cases, be limited to the lung, and have no systemic features of TS. Chylous effusions are rare in TS but common in LAM. Finally, pulmonary involvement in TS does involve men, whereas LAM involves exclusively women (119).

The prognosis for patients with TS and lung disease is poor. The average life span after diagnosis of pulmonary involvement is 5 years, and 86% of those with pulmonary disease die of respiratory complications, with cor pulmonale and spontaneous pneumothorax being the leading causes of death (110). There is no known effective treatment at present, although treatment with progesterone acetate similar to that used in LAM may be worthwhile in some patients. Newer agents specifically aimed against smooth muscle proliferation are likely promising agents that deserve to be studied. Lung transplantation in the appropriate patient is indicated.

C. Hermansky-Pudlak Syndrome

The Hermansky-Pudlak syndrome (HPS) is characterized by the triad of oculocutaneous albinism, a bleeding diathesis secondary to platelet dysfunction, and accumulation of a ceroidlike material in the reticuloendothelial system (17,25,120–122). The disease is transmitted in an autosomal recessive manner. Heterozygotes are phenotypically normal. Over 200 cases have been reported worldwide, although most are clustered in northwest Puerto Rico and southern Holland (121,123). The pigment disorder has variable phenotypic expression depending on the patient's racial background. The pathophysiological mechanism responsible for the disease has not been definitely established, although lysosomal dysfunction is suspected to play a role (25).

Pulmonary fibrosis and a granulomatous colitis similar to Crohn's disease have been associated with HPS (25). Lung disease usually begins in the third or fourth decade of life. An insidious onset of shortness of breath with exertion and a dry cough are common presenting pulmonary symptoms. Although the most prominent symptoms are more often related to the bleeding disorder present in HPS (17,121,122), hemoptysis has not been reported. Another common physical finding on examination is a coarse nystagmus (17,123). An elevated bleeding time is the most conspicuous laboratory abnormality. The platelet defect in HPS is thought to be a storage pool deficiency. Within the platelets, there are decreased numbers of dense granules and reduced amounts of platelet adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, and calcium (124–126). Platelet numbers are normal. There is impaired secondary aggregation to ADP, collagen, and epinephrine (123). There are no known abnormalities of lymphocytic or neutrophilic function in this disorder (127). Other laboratory findings include

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weakly positive antinuclear antibodies (ANAs) in some patients, and the interesting finding of orange fluorescence of the urine under ultraviolet light due to the presence of ceroid pigment (123). Pulmonary function abnormalities include a restrictive lung defect with reduced DLCO and airflow obstruction, although the latter is seen in smokers (120,122). Radiographically, a ground-glass pattern is present early in the course of the disease (119). Evolution to a diffuse interstitial pattern and subsequent progression to honeycombing is typical. Upper lobe bullous disease and bronchiectasis have been reported (123,128). Pleural involvement and adenopathy are not manifestations of HPS.

Diffuse interstitial fibrosis and ceroid-filled alveolar macrophages are seen in lung biopsy. These macrophages stain positively with periodic acid-Schiff (PAS) (121,123) and fluoresce intensely orange-red under ultraviolet light due to engulfed ceroid material. BAL is not diagnostic, although the recovery of typical macrophages in the right clinical setting may help confirm the diagnosis (122,129). The pulmonary disease in HPS tends to be progressive and resistant to corticosteroid therapy. Although it is unclear if UIP is the histological pattern in HPS, the lack of response to corticosteroid therapy is similar to patients with IPF. The natural course of PF associated with HPS is unclear.

D. Gaucher's Disease

Gaucher's disease is the most common of the lysosomal storage diseases and is inherited as an autosomal recessive trait. The disease occurs with increased frequency in Ashkenazi Jews. Clinical manifestations of the disease are caused by the accumulation of glucocerebroside in cells of the reticuloendothelial system (22,130,131). The finding of these cells, known as Gaucher cells, is diagnostic of the condition. The primary metabolic defect is a deficiency of the enzyme glucocerebrosidase (132).

There are two forms of the disease. Infantile Gaucher's disease is characterized by prominent progressive neurological impairment and frequent pulmonary involvement. Death usually occurs by age 2 years of neurological disease. The adult form of the disease may present at any age. In contrast to the infantile form of Gaucher's disease, pulmonary involvement in the adult is rare (22). The pulmonary manifestations of this disease are caused by Gaucher cells infiltrating the interstitium, filling the alveoli, or obstructing pulmonary capillaries. Obliteration of pulmonary capillaries may result in pulmonary hypertension and cor pulmonale (126). Acute fatal bone marrow embolization of Gaucher cells to the lungs has been described (130). Pulmonary infections occur with increased frequency (133). Histopathological examination shows Gaucher cell infiltration, but inflammation and fibrosis are not usual features. The Gaucher cells stain positively with PAS and autofluoresce (22,130,131). Remarkable laboratory studies include pancytopenia caused by splenomegaly

and bone marrow involvement and elevations of liver enzymes and acid phosphatase. Gaucher cells may be found in the sputum or in bronchial washes. Chest radiographic findings are not specific. Diffuse interstitial infiltrates that occasionally have a miliary appearance have been described (22,130,131), as has mediastinal adenopathy (134). Pulmonary function tests reveal typical restrictive lung defect with a reduced DLCO (22,130,135).

The diagnosis is made in the appropriate clinical setting by the demonstration of Gaucher cells in bone marrow or other tissue samples. At present, bone marrow transplantation or glucocerebrosidase replacement therapy is possible as a treatment modality for the underlying disease (135).

E. Niemann-Pick Disease

Niemann-Pick disease is a rare lipid storage disease characterized by the accumulation of sphingomyelin in the central nervous system and the reticuloendothelial system. Inheritance is autosomal recessive (136). The incidence of lung involvement is unknown. A chest radiograph may show a reticulonodular or miliary pattern; progression to honeycombing has been described (137,138). Reticuloendothelial cells filled with sphingomyelin may fill the alveolar spaces and interstitium (139) and can be recovered with BAL (140). There is no effective treatment for this disorder.

F. Hypocalciuric Hypercalcemia

Familial hypocalciuric hypercalcemia is a very rare disorder characterized by hypocalciuric hypercalcemia, ILD/pulmonary fibrosis, and recurrent respiratory tract infections caused by granulocyte dysfunction. The disease is probably inherited in an autosomal dominant manner. Support for this comes from one family with three siblings clinically affected by ILD (141). In this relatively large family, 45% of 38 family members studied had a reduced DLCO; suggesting subclinical disease. Several asymptomatic individuals also had abnormalities of BAL fluid cell count; suggesting active alveolitis. In addition, 60% had recurrent respiratory tract infections (141). The disease usually presents in the fourth decade (141–143). Reticulonodular infiltrates that may progress to honeycombing on a chest radiograph and restrictive pulmonary physiology are typical findings. Disease progression appears to be slow with survival after diagnosis being about 10 years.

Unlike other metabolic storage diseases affecting the lung, hypercalciuric hypercalcemia is a granulomatous disorder; poorly defined granulomas, multinucleated giant cells, conchoid bodies, and foamy macrophages are seen histopathologically. Alveolar macrophages recovered at biopsy or BAL are loaded with dark cytoplasmic inclusions of an unknown nature (141). Hypercalcemia is associated with a low urinary calcium level in contrast to the high urinary calcium level seen in sarcoidosis. The granulocyte dysfunction is

characterized by a decrease in the myeloperoxidase content and in phagocytosis and killing of *Staphylococcus aureus*. Granulocyte chemotaxis is normal (141). There are too few reported cases to evaluate treatment; corticosteroids may be of potential benefit (141).

VI. Conclusions

The genetics of PF represents a very exciting area for the clinician and the investigator. Although presentations of this disease are rare and account for a subset of a low prevalent condition—interstitial lung disease—cases of FPF are recurrently observed in the practice of pulmonary medicine. Furthermore, PF is observed in several genetic disorders, and workers, as well as experimental animals, appear to respond differently to chronic inhalation of fibrogenic dusts. In aggregate, these findings strongly suggest that genetic factors predispose individuals to develop PF. Given this hypothesis, it is logical to speculate that specific gene products modulate the inflammatory/fibrotic response to fibrogenic agents and provide the pathogenic basis for enhanced genetic risk. Moreover, it is likely that the gene or genes that prove to be important in the development of FPF will also be relevant in the pathogenesis of other forms of interstitial lung disease.

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3

Clinical Features and Differential Diagnosis of Idiopathic Pulmonary Fibrosis

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I. Introduction

Idiopathic pulmonary fibrosis (IPF) (also called cryptogenic fibrosing alveolitis) is a common and well-defined diffuse parenchymal lung disease of unknown etiology (1). It has characteristic clinical, radiographic, physiological, and pathological manifestations (2). To emphasize the distinct pathophysiology and clinical course of IPF, the American Thoracic Society/European Respiratory Society has recommended reserving the term *idiopathic pulmonary fibrosis* for the clinical condition characterized by progressive dyspnea, cough, restrictive lung disease, and the histopathological pattern of usual interstitial pneumonia (UIP) (Table 1) (3).

The identification of UIP is best confirmed by the review of tissue obtained from surgical lung biopsy. The UIP pattern is characterized by heterogeneity that includes patchy chronic inflammation (''alveolitis''), progressive injury (small aggregates of proliferating myofibroblasts and fibroblasts, termed fibroblastic foci), and fibrosis (dense collagen and honeycomb change) (see Chap. 4) (4,5). It is important to separate IPF/UIP from other forms of idiopathic interstitial pneumonias, because IPF/UIP has a poor response to current therapy and a worse prognosis. In this chapter, we will review the approach to the clinical evaluation of patients with diffuse parenchymal lung disease. Only limited comments will be made about chest imaging studies, histopathological evaluation, or management, since these topics are addressed in specific chapters in this book. There have been several excellent reviews of the general approach to the diagnosis of interstitial lung disease published in the last several years (6–12).

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Table 1 Definite Diagnosis of IPF in the Presence of a Surgical Biopsy Showing UIP

Exclusion of other known causes of interstitial lung disease (such as drug toxicities, environmental exposures, and connective tissue diseases)
Abnormal pulmonary function studies that include evidence of restriction
Reduced VC often with an increased FEV_1/FVC ratio and/or
Impaired gas exchange
Increased $AaPo_2$ (alveolar-arterial pressure difference for O_2) with rest or exercise or
Decreased D_{LCO} (diffusing capacity of the lung for CO)
Abnormalities on conventional chest radiographs or high-resolution computed tomography (HRCT) scans (see text).
Patients with a history of cigarette smoking may have coexisting chronic obstructive lung disease, which will alter the manifestations of the disease as assessed by lung function and chest imaging studies.

HRCT, high-resolution computed tomography.
Source: Adapted from Ref. 3.

II. General Considerations in the Evaluation of Patients with Diffuse Parenchymal Lung Disease

The initial evaluation of a patient with diffuse parenchymal lung disease should include a complete history and physical examination followed by laboratory testing that should include routine blood tests, serological studies, chest radiography, pulmonary function testing, arterial blood gas analysis, and high-resolution computed tomography of the chest.

A. Common Symptoms

Patients with diffuse parenchymal lung diseases commonly seek medical attention because of the onset of progressive breathlessness with exertion (dyspnea) and/or a persistent and usually nonproductive cough (13,14). Often it is the identification of interstitial opacities on chest radiograph that focuses the diagnostic approach toward one of the diffuse parenchymal lung diseases versus other disorders (e.g., heart failure, asthma, or chronic obstructive pulmonary disease).

Dyspnea

A sense of shortness of breath (i.e., dyspnea) is a common complaint that patients often attribute to deconditioning, obesity, aging, or a recent upper respiratory tract illness. It is often insidious in onset in patients with diffuse parenchymal lung diseases (14). Some patients have so limited the amount of activity they perform as not to "experience" any significant dyspnea.

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Frequently, a spouse or friend brings the problem to the patient's attention. Thus, the clinician must carefully inquire as to the level of activity the patient normally participates in, the frequency of exercise or exertion of any kind (especially walking stairs, doing housework, or carrying packages), the level of exertion that causes the sensation of breathlessness, and the length of time it takes for the patient to recover (15).

Cough

A dry cough is common in IPF, and may be particularly disturbing for patients with processes that involve the airways, such as sarcoidosis, respiratory bronchiolitis, pulmonary Langerhans cell granulomatosis (LCG) (formerly called pulmonary histiocytosis X, or eosinophilic granuloma), and hypersensitivity pneumonitis. A productive cough is unusual in most diffuse parenchymal lung diseases.

Chest Pain

Chest pain is uncommon in most diffuse parenchymal lung diseases. Pleuritic chest pain may occur in diffuse parenchymal lung disease associated with some connective tissue diseases. Substernal chest pain or discomfort is common in sarcoidosis. Sudden worsening of dyspnea, especially if associated with pleural pain, may indicate a spontaneous pneumothorax. Spontaneous pneumothorax is rare in IPF, but is a characteristic finding for pulmonary LCG, tuberous sclerosis, lymphangioleiomyomatosis, and neurofibromatosis.

Hemoptysis

Gross blood or blood-streaked sputum is rarely a presenting manifestation of IPF, so its presence should suggest another diagnosis, such as diffuse alveolar hemorrhagic syndromes, lymphangioleiomyomatosis, or granulomatous vasculitides.

Wheezing

Wheezing is an uncommon manifestation of the diffuse parenchymal lung diseases, but has been described in cases of chronic eosinophilic pneumonia, Churg-Strauss syndrome, and respiratory bronchiolitis.

B. Demographic Information and Past History

Careful documentation of the past medical history is important in the initial assessment, because the cause of the illness is often recognized from the

patient's history. Key areas of focus include:

Age

Few causes of diffuse parenchymal lung disease present between the ages of 20–40 years: sarcoidosis, connective tissue disease associated with diffuse parenchymal lung disease, lymphangioleiomyomatosis, pulmonary LCG, inherited forms of diffuse parenchymal lung disease (familial IPF, Gaucher's disease, Hermansky-Pudlak syndrome). Conversely, most patients with IPF are over age 50 years (16).

Gender

Lung involvement in lymphangioleiomyomatosis, tuberous sclerosis, Hermansky-Pudlak syndrome, and the connective tissue diseases is more common in women.

Duration of Illness

The duration of illness prior to presentation and diagnosis may be helpful in narrowing the differential diagnosis of the diffuse parenchymal lung disease. *Acute* presentations (days to weeks) are unusual in IPF and should suggest acute idiopathic interstitial pneumonia, acute eosinophilic pneumonia, hypersensitivity pneumonitis, and cryptogenic organizing pneumonia. *Subacute* presentations (weeks to months) may be seen in all diffuse parenchymal lung diseases but can suggest sarcoidosis, some drug-induced diffuse parenchymal lung diseases, the alveolar hemorrhagic syndromes, cryptogenic organizing pneumonia, and connective tissue disease. In the vast majority of diffuse parenchymal lung disorders, the symptoms and signs are *chronic*; that is, months to years (IPF, sarcoidosis, pulmonary LCG).

Smoking History

Some diffuse parenchymal lung diseases occur largely among current or former smokers (pulmonary LCG, desquamative interstitial pneumonitis, IPF, and respiratory bronchiolitis) (17–22) or among never or former smokers (sarcoidosis, hypersensitivity pneumonitis) (23,24).

Prior Medication Use

All current and prior medications taken by the patients should be reviewed to exclude the possibility of drug-induced disease, including over-the-counter medications, oily nose drops, or petroleum products (25).

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Family History

The family history is occasionally helpful, since familial associations have been identified in cases of IPF, sarcoidosis, tuberous sclerosis, neurofibromatosis, Niemann-Pick disease, Gaucher's disease, and Hermansky-Pudlak syndrome (see Chap. 2) (26,27).

Occupational History and Environmental Exposures

A strict chronological listing of the patient's entire lifelong employment must be sought, including specific duties and known exposures to dusts, gases, chemicals. The degree of exposure, duration, latency of exposure, and the use of protective devices should be elicited. Review of the home and work environment, including that of the spouse and children, is invaluable (28). Family members may develop disease as a result of "passive" exposure to dusts from the hobby or occupation of another member of the family (e.g., asbestosis, berylliosis).

C. Physical Examination

The physical examination is commonly not specific. It frequently reveals tachypnea, reduced chest expansion, and bibasilar end-inspiratory dry crackles. Cyanosis is uncommon and is usually a late manifestation indicative of advanced disease. The cardiac examination is usually normal except in the mid or late stages of the disease when findings of pulmonary hypertension (i.e., augmented P_2, right-sided lift, and S_3 gallop) and cor pulmonale may become evident. Signs of pulmonary hypertension and cor pulmonale are generally secondary manifestations of advanced diffuse parenchymal lung disease, although they may be primary manifestations of a connective tissue disorder (e.g., progressive systemic sclerosis). Clubbing of the digits, that is, the distal part of the finger is enlarged compared with the proximal part, is a late manifestation suggesting advanced derangement of the lung in some patients (IPF, asbestosis) (29).

D. Laboratory Evaluation

The routine laboratory evaluation is often not helpful but should include biochemical tests to evaluate liver and renal function and hematological tests to check for evidence of anemia, polycythemia, or leukocytosis. Serological studies should be obtained if clinically indicated by features suggestive of a connective tissue disease, hypersensitivity pneumonitis, or vasculitis: sedimentation rate, antinuclear antibodies, rheumatoid factor, hypersensitivity panel, antineutrophil cytoplasmic antibodies, anti–basement membrane antibody. An elevated erythrocyte sedimentation rate and hypergammaglobulinemia are commonly observed but are nondiagnostic.

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E. Chest Imaging Studies

The diagnosis of diffuse parenchymal lung disease will often be suspected initially on the basis of an abnormal chest radiograph. A recent chest radiograph should be obtained, and it also is important to review all previous chest radiographs to assess the rate of change in disease activity. It is critical that physicians not ignore or incompletely evaluate a symptomatic patient with a normal chest radiograph or an asymptomatic patient with radiographic evidence of diffuse lung disease. Failure to evaluate completely such individuals is often the leading reason that patients with IPF present with severe, irreversible disease by the time the patient is impaired enough to seek additional medical attention. Although the chest radiograph is useful in suggesting the presence of diffuse parenchymal lung disease, the correlation between the radiographic pattern and the stage of disease (clinical or histopathological) is generally poor. High-resolution computed tomography (HRCT) offers more accuracy than conventional chest radiography in distinguishing airspace from interstitial involvement and in the earlier detection and confirmation of suspected diffuse lung disease (30) (see Chap. 7).

F. Pulmonary Function Testing

Complete lung function testing (spirometry, lung volumes, diffusing capacity) and resting room air arterial blood gases should be obtained. Most of the diffuse parenchymal lung disorders have a restrictive defect with reduced total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV). Flow rates are decreased (FEV_1 and FVC), but this is related to the decreased lung volumes. The FEV_1/FVC ratio is usually normal or increased. Reductions in lung volumes increase as lung stiffness worsens with disease progression. Smoking history must be considered when interpreting the functional studies. A reduction in the diffusing capacity of the lung for carbon monoxide (D_{LCO}) is a very common but nonspecific finding. The resting arterial blood gas may be normal or reveal hypoxemia (secondary to a mismatching of ventilation to perfusion) and respiratory alkalosis. Carbon dioxide retention is rare, and is usually a manifestation of far-advanced end-stage disease. Importantly, because resting hypoxemia is not always evident and because severe exercise-induced hypoxemia may go undetected, it is important to perform exercise testing with serial measurement of arterial blood gases. In fact, increasing evidence suggests that serial assessments of resting and exercise gas exchange are the best methods to identify disease activity and responsiveness to treatment, especially in IPF (31). Oximetry testing is not a reliable method to identify exercise-induced hypoxemia in patients with diffuse parenchymal lung disease (see Chap. 6).

G. Bronchoalveolar Lavage

In selected cases, bronchoalveolar lavage (BAL) cellular analysis studies may be useful to narrow the differential diagnostic possibilities between various types of diffuse parenchymal lung diseases. However, the utility of BAL in the clinical assessment and management of IPF patients remains to be established (32–35) (see Chap. 9).

III. Idiopathic Pulmonary Fibrosis

A. Clinical Features

IPF usually presents in patients between 50 and 70 years of age. In fact, it is quite uncommon for a patient to present before the age of 40 years. The typical patient presents with the insidious onset of breathlessness with exertion and a nonproductive cough. Constitutional symptoms are uncommon. However, weight loss, fever, fatigue, myalgias, or arthralgia is occasionally present. Most patients have these symptoms for months to years prior to definitive evaluation, usually appropriately 12–18 months (31).

Patients with IPF are tachypneic, with rapid shallow breaths, probably because of the increased work of breathing (36,37). Most patients have bibasilar late inspiratory fine crackles (Velcro rales) on chest examination. Clubbing of the fingers is seen in 40–75% of patients and is a late finding in the disease course. Similarly, cyanosis is a late manifestation indicative of advanced disease. Cardiac examination is usually normal except in the middle or late stages of the disease when findings of pulmonary hypertension (i.e., augmented P_2, right-sided lift, and S_3 gallop) and cor pulmonale may become evident. Spontaneous pneumothorax rarely occurs.

B. Blood and Serological Studies

An elevated erythrocyte sedimentation rate, hypergammaglobulinemia, low titer-positive antinuclear antibodies (21% of patients with IPF), rheumatoid factor, circulating immune complexes, and cryoimmunoglobulins have been identified in these patients (38).

C. Chest Imaging Studies

The most common radiographic abnormalities in IPF are a reticular (i.e., netlike appearance) of linear or curvilinear densities. These usually appear as subpleural opacities with a predilection for the lower lung zones. Multiple cystic or honeycombed areas (i.e., coarse reticular pattern with translucencies measuring 0.5–1.0 cm in diameter) are also common radiographic findings, and the extent of involvement tends to correlate with advanced disease and poor prognosis. Chest radiographic evidence of reduced lung volumes is usually

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present unless there is associated obstructive airway disease. Pleural involvement is uncommon in IPF; its presence should suggest another diagnosis, such as collagen vascular disease (especially rheumatoid arthritis or systemic lupus erythematosus), mitral valve disease, congestive heart failure, asbestosis, infection, drug-induced lung disease, or lymphangitic carcinomatosis (see Chap. 7).

High-resolution CT (HRCT) is useful in the differentiation of IPF from other interstitial lung diseases, the determination of the extent and severity of disease activity, and, most importantly, the detection of disease, especially in patients with normal or minimal change on plain chest radiographs (39,40–42). HRCT findings in IPF include a patchy, peripheral, and especially subpleural distribution of the reticular opacities. There are almost always areas of honeycomb changes (cystic spaces 2–4 mm in diameter) (43–45). Atypical findings that should suggest another diagnosis include extensive ground-glass opacities, nodules, upper lobe or mid zone predominance of findings, and significant hilar or mediastinal lymphadenopathy.

Studies evaluating the ability of HRCT scanning to diagnose accurately IPF have found that HRCT significantly increases the level of diagnostic confidence compared with the chest radiograph. However, HRCT has not replaced lung biopsy in the diagnosis and assessment of most interstitial lung diseases (46,47). Connective tissue diseases (particularly scleroderma and rheumatoid arthritis) (48,49) and asbestosis (50–53) may cause a similar HRCT appearance except for the presence of parenchymal bands of fibrosis and pleural plaques in patients with asbestosis. Patients with subacute or chronic hypersensitivity pneumonitis can have a similar reticular opacity or honeycombing, but often lack the bibasilar predominance seen in IPF (54). The accuracy of a confident diagnosis of IPF made on HRCT by a trained observer appears to be approximately 90% (47,54–57). Less experienced observers are substantially less accurate than experienced observers (55).

D. Pulmonary Function Tests

The lung volumes (TLC, FRC, and RV) are reduced. The lung volumes may be normal in the early stages of the disease or in patients with superimposed chronic obstructive pulmonary disease. Lung volumes are higher in smokers with IPF compared with those who have never smoked (58). In general, as the disease progresses, lung compliance decreases and lung volumes fall. Expiratory flow rates, FEV_1 and FVC, may be decreased because of the reduction in lung volume, but the FEV_1/FVC ratio is maintained. Because of the increased static elastic recoil found in these patients, at any given lung volume, flow rates are often increased.

The (D_{LCO}) is reduced, which may actually precede the loss of lung volume. The decrease in the D_{LCO} results from both a contraction of the pulmonary capillary volume and the presence of ventilation-perfusion

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abnormalities. The resting arterial blood gases are usually abnormal and reveal hypoxemia and respiratory alkalosis. Exercise testing is more sensitive than assessments at rest in the detection of abnormalities in oxygen transfer and is a better parameter than other lung function tests for following the clinical course of the disease (31,59,60). Pulmonary hypertension rarely occurs at rest but is common during exercise even in the early stages of IPF (61,62). Many patients with IPF, especially those with low daytime arterial S_{O_2} or a history of snoring during sleep, develop sleep disturbances that are characterized by reduced rapid eye movement (REM) sleep, lighter and more fragmented sleep, and hypoxemia during REM sleep.

E. Role of Lung Biopsy in Diagnosis of IPF

The history, physical and laboratory evaluation, although of critical importance in the evaluation of interstitial lung disease (ILD), when viewed in isolation, has little diagnostic accuracy for IPF (63). Lung biopsy need not be performed in all patients with suspected diffuse interstitial lung disease. However, following the initial evaluation, it is important to confirm the diagnosis and establish the stage of disease (64–66). This is often only possible following careful examination of adequate lung tissue (46,47).

Two recent studies have addressed the accuracy of the clinical diagnosis of IPF (46,47). In both studies, all patients underwent surgical lung biopsies and the clinicians had access to the clinical information but were blinded to the results of the lung biopsy. When compared to the histopathological diagnosis, Raghu and coworkers (46) showed that the clinical diagnosis of IPF had a sensitivity of 62% and a specificity of 97%. Hunninghake and colleagues (47) performed a similar study and showed that when compared to histopathology, the *confident* clinical diagnosis of IPF had a sensitivity of 48% and a specificity of 89%. These studies demonstrate that, when additional clinical information is consistent with the radiographic diagnosis, many cases of IPF can arguably be diagnosed without histopathological confirmation (66).

The recent American Thoracic Society consensus statement described major and minor criteria for the clinical diagnosis of IPF (Table 2) (3). The presence of all four major criteria and at least three of four minor criteria increases the likelihood of a correct clinical diagnosis. When a confident clinical diagnosis of IPF is made by experienced pulmonologists and radiologists, the accuracy is high (specificity of 89–97%). Unfortunately, a *confident* clinical diagnosis can be made in only a minority of the patients. Importantly, the authors of the consensus statement note that in the absence of a surgical lung biopsy, the diagnosis of IPF remains uncertain (66).

Fiberoptic bronchoscopy with transbronchial lung biopsy is often the initial procedure of choice (3,12,34). Several reports have evaluated the diagnostic yield of transbronchial lung biopsy in diffuse parenchymal lung

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Table 2 Criteria Supporting the Clinical Diagnosis of Idiopathic Pulmonary Fibrosis

Major Criteria (must have all four)
1. Exclusion of other known causes of ILD (such as certain drug toxicities, environmental exposures, and connective tissue diseases)
2. Abnormal pulmonary function studies that include evidence of restriction (reduced VC often with an increased FEV_1/FVC ratio) and impaired gas exchange (increased $AaPo_2$ with rest or exercise or decreased DLCO)
3. Bibasilar reticular abnormalities with minimal ground-glass opacities on HRCT scans
4. Transbronchial biopsy or bronchoalveolar lavage (BAL) showing no features to support an alternative diagnosis

Minor Criteria (must have at least three of four)
1. Age over 50 years
2. Insidious onset of otherwise unexplained dyspnea on exertion
3. Duration of illness > 3 months
4. Bibasilar, inspiratory crackles (dry or Velcro-type in quality)

Source: Adapted from Ref. 3.

diseases (67–69). The major limitations of transbronchial lung biopsy are the small size of the biopsy obtained and the lack of histological preservation, so that the key features required allowing a histological diagnosis are often not present. Because of these factors, transbronchial lung biopsy specimens have a poor diagnostic accuracy for IPF. As with BAL, transbronchial lung biopsy can aid in the diagnosis of alternative causes of ILD, especially when sarcoidosis, lymphangitic carcinomatosis, eosinophilic pneumonia, Goodpasture's syndrome, or infection is suspected.

If a specific diagnosis is not made by transbronchial biopsy, then an open or more commonly a thoracoscopic lung biopsy is indicated. Surgical lung biopsy is important to the diagnosis and management of the approximately 50–75% of patients with idiopathic interstitial pneumonia who do not have the classic clinical, radiological, and physiological features of UIP (70). Open or thoracoscopic lung biopsy is the most definitive method to diagnose and stage the disease so that appropriate prognostic and therapeutic decisions can be made (3,12) (see Chap. 4). The thoracic surgeon should generally make the choice between video-assisted thoracic surgery (VATS) and open lung biopsy based on individual patient characteristics. There are three important situations in which thoracoscopic lung biopsy is contraindicated: inability to tolerate single-lung ventilation (e.g., severe hypoxemia, high airway pressures), coagulopathy, and pleural adhesions or scarring from previous thoracic surgery or pleurodesis (66).

Ultimately, it remains up to the individual clinician and patient to decide with what degree of diagnostic specificity they are comfortable. Given the importance of accurate histopathological diagnosis and the weight of the

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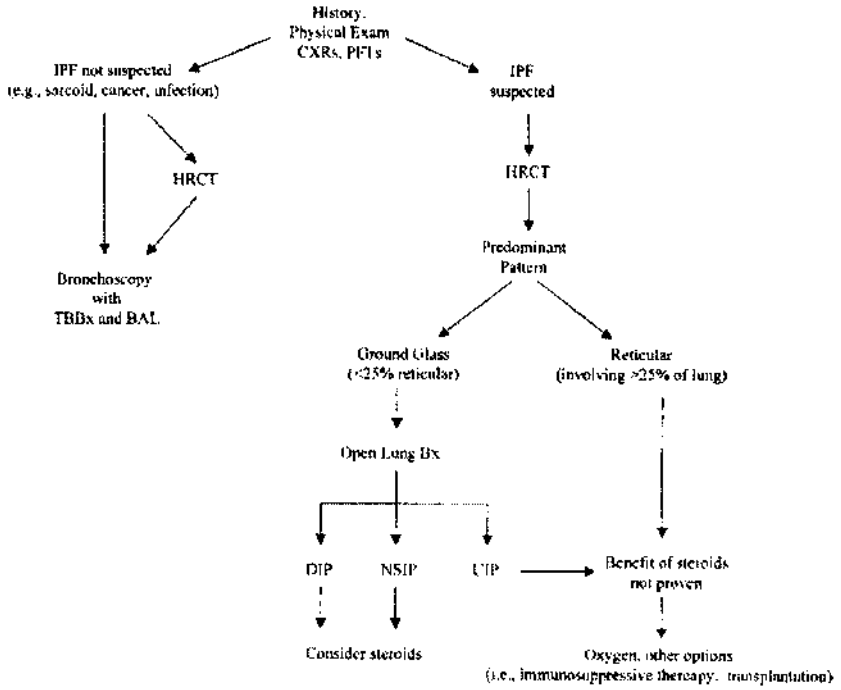

Figure 1 A simplified algorithm for the diagnosis and management of IPF. Histological confirmation of the specific pattern of lung injury should be pursued in all patients with progressive disease who show an atypical pattern or predominantly ground-glass appearance by HRCT. Importantly, immunocompetent patients with a classic HRCT appearance for IPF/UIP or with findings suggestive of end-stage disease (see text) are less likely to benefit from an surgical lung biopsy, since this may not provide any additional prognostic information. CXR, chest radiograph; PFT, pulmonary function testing; Bx, biopsy; TBBx, transbronchial biopsy; open lung biopsy, thoracotomy or video-assisted thoracoscopy. (From Ref. 6.)

evidence showing that surgical lung biopsy is a well-tolerated, relatively low-risk procedure in nonacute settings, it is our opinion that surgical lung biopsy should be pursued if the diagnosis of IPF remains in doubt after careful clinical and radiographic evaluation (66). A simplified algorithm for the diagnosis and management of IPF is shown in Figure 1 (6).

IV. Differential Diagnosis of IPF

A. Nonspecific Interstitial Pneumonia

Nonspecific interstitial pneumonia (NSIP) derived as a category of idiopathic interstitial pneumonia until recently has been "...defined more in terms of

what it is not rather than what it is'' (71,72). To date, there are no clearly recognized and distinctive clinical descriptions of patients presenting with NSIP. However, the term *nonspecific interstitial pneumonia* has evolved from its original use, which was intended to indicate a histological pattern with a variety of etiologies (e.g., hypersensitivity pneumonitis, drug reaction, associated with connective tissue diseases, and survivors of acute respiratory distress syndrome) (72) to now being used to identify a specific form of idiopathic interstitial pneumonia (5,73–78). It is characterized by varying degrees of inflammation and fibrosis, with some forms being primarily inflammatory (cellular NSIP) and others primarily fibrotic (fibrotic NSIP).

The presentation of NSIP is quite similar to other forms of idiopathic pneumonias, especially UIP. Most patients are middle-aged adults with a subacute of chronic onset of symptoms of prior to diagnosis (72,73,79,80). There is a female predominance. Dyspnea and cough are the most common presenting complaints. Fever and systemic flulike symptoms may occur and should suggest connective tissue disease or hypersensitivity pneumonitis (72,80,81).

Pulmonary function testing shows a restrictive pattern similar to that seen in IPF. The chest radiograph usually shows bilateral reticular or hazy opacities (72,73,79,80). HRCT scanning reveals a predominance of ground-glass abnormality, most commonly bilateral and subpleural in distribution, and is associated with lower lobe volume loss (79,82,83). Patchy areas of airspace consolidation and reticular abnormalities may be present, but honeycombing is unusual (82). Importantly, there is a continuum of findings in NSIP, with some cases being radiographically indistinguishable from IPF (83).

Unlike patients with IPF (UIP), the majority of patients with nonspecific or nonclassifiable interstitial pneumonia have a good prognosis with most showing improvement after treatment with corticosteroids (5,73–78). The prognosis appears to depend on the extent and severity of the fibrosis at presentation (5,73,75–78).

B. Respiratory Bronchiolitis–Associated Interstitial Lung Disease and Desquamative Interstitial Pneumonia

Respiratory bronchiolitis (RB) is a common inflammatory lesion of the respiratory bronchioles that occurs almost exclusively in current or former cigarette smokers (22,84). Respiratory bronchiolitis–associated interstitial lung disease (RB-ILD) and ''desquamative'' interstitial pneumonia (DIP) are distinct clinical syndromes found in current heavy cigarette smokers (20,21, 85–88). Previously, many investigators considered desquamative interstitial pneumonitis (DIP) or RB-ILD to represent an early stage of IPF; however, it is now widely viewed as being a distinct clinical and pathological entity (12). RB-ILD and DIP are considered together, because we believe they represent different stages of the same process. Also, the term *RB-ILD* is more

anatomically accurate and it conveys important pathogenetic implications compared to the older term *DIP* (5). Because of the marked differences in clinical course and prognosis, it must be separated from other idiopathic interstitial pneumonias.

RB-ILD and DIP afflicts primarily cigarette smokers in their fourth or fifth decade of life. There is a male preponderance. Patients with the disease commonly present with cough and dyspnea. Coarse, bibasilar end-inspiratory crepitations are frequent findings on physical examination. Finger clubbing, a relatively common finding in IPF, is very rare in RB-ILD and more common in DIP (5).

Pulmonary function testing may be normal but usually shows mild to moderate restriction, normal or slightly reduced diffusing capacity, and mild hypoxemia. A mixed obstructive-restrictive pattern is common, while an isolated increase in RV may be seen.

The chest radiograph shows less severe changes compared to IPF and may be normal in up to 20% of cases. A pattern of fine granular opacities suggesting an acinar filling process may be the early finding. Air bronchograms can be found when this process surrounds the airways. Most often the chest radiograph shows diffuse, fine reticular interstitial opacities usually with normal-appearing lung volumes. Bronchial wall thickening, prominence of peribronchovascular interstitium, small regular and irregular opacities, and small peripheral ring shadows are distinctive features of respiratory bronchiolitis (89). As the disease progresses, linear densities and reticulation occur. The honeycombed pattern is rare.

The computed tomographic (CT) features of RB-ILD are quite different from IPF is that they show diffuse bronchial wall thickening, centrilobular nodules, ground-glass opacity, upper lung predominant emphysema, and lower lung predominant air trapping (90,91). The ground-glass opacities may be diffuse or patchy, often with a peripheral predominance in the lung (92). Similar ground-glass opacification, without basal or peripheral predominance, may be found in patients with hypersensitivity pneumonitis (54,93). Even on HRCT, the honeycombed pattern is rare (90,91,94,95).

Smoking plays a major role in the pathogenesis of RB-ILD, and smoking cessation has been associated with resolution of the symptoms and improvement in the radiographic and physiological abnormalities. Since there has not been a longitudinal study of a large group of subjects, the clinical course and prognosis of RB-ILD is unknown. Corticosteroids may be required in refractory or recurrent cases. Most studies suggest a favorable response to corticosteroids; with documented improvement in lung function and chest radiographs (86,88). The mortality rate is poorly defined but appears to be markedly less than for UIP, with an average survival of 12 years in one series (96). Other studies have shown similar results, but this needs additional study of a larger number of subjects (88,94,95,97).

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C. Acute Interstitial Pneumonia (Hamman-Rich Syndrome)

Acute interstitial pneumonia (AIP) is a rare fulminant form of lung injury that presents acutely (days to weeks from onset of symptoms), usually in a previously healthy individual (98). AIP likely represents the subset of cases of idiopathic adult respiratory distress syndrome (ARDS) and is what Hamman and Rich described and termed ''acute diffuse interstitial fibrosis'' (99).

Most patients are over the age of 40 years (mean age 50 years; range 7–83 years). There is no sexual predilection. A prodromal illness, lasting usually 7–14 days before presentation, is common. The clinical signs and symptoms include fever, cough, and shortness of breath. Routine laboratory studies are nonspecific and generally not helpful.

Radiographs typically show bilateral patchy densities that progressed to a diffuse alveolar filling pattern in nearly all cases (100,101). CT scans show bilateral, patchy, symmetrical areas of ground-glass attenuation. Bilateral areas of airspace consolidation may also be present. A predominantly subpleural distribution may be seen. Mild honeycombing, usually involving $< 10\%$ of the lung, may be seen on CT examination. These radiographic findings are similar to those seen in ARDS.

Most patients have moderate to severe hypoxemia and develop respiratory failure. Mechanical ventilation is often required. A surgical lung biopsy is required to confirm the diagnosis of diffuse alveolar damage (DAD) (101,102).

The mortality from AIP is high ($> 60\%$), with the majority of patients dying within 3 months of presentation. However, those who recover usually do not have recurrence of the disease and most have substantial or complete recovery of lung function. It is not clear that corticosteroid therapy is effective in AIP. The main treatment is supportive care. A recent study showed that survivors of AIP could experience recurrences and chronic, progressive interstitial lung disease (101). No clinical or pathological feature predicted the longitudinal course in survivors or mortality in these patients (101).

D. Cryptogenic Organizing Pneumonia

Cryptogenic organizing pneumonitis (COP), or idiopathic bronchiolitis obliterans with organizing pneumonia (BOOP), is a specific clinicopathological syndrome characterized by a ''pneumonia-like'' illness, with excessive proliferation of granulation tissue within small airways and alveolar ducts associated with chronic inflammation in the surrounding alveoli (87,103–112).

The incidence of COP is similar in men and women and can be seen at any age (113). Patients with COP are frequently very specific about the duration of their illness—usually less than 2 months. The onset is often dramatic with the development of a flulike illness characterized by cough, fever, malaise, fatigue, and weight loss. Inspiratory crackles are frequently

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present on chest examination. Finger clubbing is rare in patients with COP. Routine laboratory studies are nonspecific (104,114,115). Leukocytosis without an increase in eosinophils is seen in approximately half the patients. The initial erythrocyte sedimentation rate (ESR) is frequently elevated in patients with COP.

Pulmonary function is usually impaired, with a restrictive defect being most common, although obstructive defect (FEV_1/FVC ratio less than 70%) is found in almost one-fifth of subjects with COP, mostly in current or former smokers. Gas exchange abnormalities are extremely common with resting and exercise arterial hypoxemia. The DLCO is reduced (< 80% of predicted) in three-fourth of the patients.

The radiographic manifestations are distinctive with bilateral, diffuse alveolar opacities in the presence of normal lung volume (107,108,116). A peripheral distribution of the opacities, very similar to that thought to be virtually pathognomic for chronic eosinophilic pneumonia, is commonly seen in COP (104,108,109). Irregular linear or nodular interstitial opacities are rarely present as the only radiographic manifestation. Honeycombing is rare and is seen only as a late manifestation in the few patients with progressive disease. Frequently, the CT findings are far more extensive then are expected by review of the plain chest radiograph.

Corticosteroid therapy results in complete clinical, radiological, and physiological recovery in two-thirds of patients. One-third demonstrates recurrent or persistent disease. In general, clinical improvement is rapid; that is, within several days or a few weeks. Occasionally, recovery is quite dramatic. Relapses can occur when the corticosteroids are withdrawn, usually within 1–3 months. Most patients will improve when retreated with corticosteroids. Spontaneous improvement may occur over 3–6 months in some patients (114,116). Few of the patients with COP die as a result of this illness, but those prone to develop a rapidly progressive fatal form of BOOP have a clinical course similar to the Hamman-Rich syndrome (117). In such patients, the diagnosis is usually delayed or missed.

E. Lymphocytic Interstitial Pneumonia

Lymphocytic interstitial pneumonia (LIP) is an uncommon pathological process characterized by the presence of widespread, monotonous sheets of lymphocytic infiltration in the interstitium of the lung (118,119). In addition to being differentiated from IPF, it must be distinguished from lymphocytic infiltrations associated with pseudolymphoma, primary lymphomas, lymphomatoid granulomatosis, benign lymphocytic angiitis and granulomatosis (120), plasma cell interstitial pneumonia (121), and angioimmunoblastic lymphadenopathy (122). LIP is commonly found in association with other

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immunological disorders, especially hypo- or hypergammaglobulinemic conditions and Sjögren's syndrome.

LIP occurs more commonly in women (123,124), usually in the fourth to sixth decade of life (123–126). It is also seen in children, particularly those with hypogammaglobulinemia or the acquired immunodeficiency syndrome (AIDS) (127–130). The clinical manifestations of LIP are dominated by those related to the underlying disease. Progressive dyspnea and cough are the most common presenting symptoms, although weight loss, pleuritic pain, arthralgias, and fever also occur. Bibasilar rales on chest examination, cyanosis, and finger clubbing are common physical findings.

The chest radiogram is nonspecific with reticular opacities being the most frequent abnormality. A mixed alveolar-interstitial pattern appears as the disease progresses as a result of the coalescence of the opacities. Cysts, honeycombing, and pulmonary hypertension are also late manifestations (131). Pleural effusions are infrequent and suggest a complicating lymphoma. A restrictive defect is commonly seen often associated with a reduction in the carbon monoxide diffusing capacity and arterial hypoxemia. A striking T-cell lymphocytosis is seen on BAL.

The clinical course of idiopathic LIP is unknown. In cases where LIP is associated with another disease, the underlying disease largely determines the outcome. Marked improvement or complete resolution followed corticosteroid therapy in many case reports (123,124,126,132–136). Progressive pulmonary fibrosis, cor pulmonale, and death can occur despite therapy (119,123,124). Infection is a common complication in these patients, especially in those with associated dysproteinemia. Progression to pulmonary and/or systemic lymphoma occurs (137).

F. Connective Tissue Diseases

Clinical findings suggestive of a connective tissue disease (musculoskeletal pain, weakness, fatigue, fever, joint pains or swelling, photosensitivity, Raynaud's phenomenon, pleuritis, dry eyes, dry mouth) should be carefully elicited in patients who present with diffuse parenchymal lung disease and pulmonary symptoms. The connective tissue diseases may be difficult to rule out, since the pulmonary manifestations occasionally precede the more typical systemic manifestations by months or years (particularly in rheumatoid arthritis, systemic lupus erythematosus, and polymyositis-dermatomyositis). The frequency, clinical presentation, prognosis, and response to therapy vary depending on underlying connective tissue disease (scleroderma, rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis-polymyositis, Sjögren's syndrome or mixed connective tissue disease) and the histological pattern of injury (e.g., usual interstitial pneumonia, desquamative interstitial pneumonia, organizing pneumonia, diffuse alveolar damage) (138). A review

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of the specific connective tissue diseases is beyond the scope of this chapter (138–140).

G. Hypersensitivity Pneumonitis

Hypersensitivity pneumonitis is associated largely with repeated inhalation of finely dispersed organic dusts. This produces diffuse patchy interstitial and/or alveolar opacities in the lung following the formation of antigen-antibody complexes (Arthus reaction). Farmer's lung (exposure to moldy hay containing fungal spores) is the prototype. In urban areas, bird fancier's lung (due to exposure to bird proteins) and air conditioner or humidifier lung disease (due to fungal overgrowth and aerosolization) are more common (14,141).

Acute Hypersensitivity Pneumonitis

Heavy exposure to an organic antigen can result in an acute reaction (4–6 h later) characterized by the abrupt onset of fever, chills, malaise, nausea, cough, chest tightness, and dyspnea without wheezing. These symptoms subside over hours or days. Diffuse fine crackles are heard throughout the chest, mild hypoxemia, and a restrictive ventilatory defect may accompany symptomatic episodes. A fleeting, micronodular, interstitial pattern in the lower and mid lower zone may be identified on chest radiograph. HRCT is more sensitive than chest radiography in detecting abnormalities and assessing the type and extent of abnormalities in HP. Ground-glass opacity, predominating in the lower lobes, was the most frequent feature on HRCT scans. Other common features include centrilobular nodules, airspace consolidation, mosaic perfusion (inspiratory scans), and air trapping (on expiratory scans). Emphysema and mediastinal lymphadenopathy are commonly found (142). Removal from exposure usually results in complete resolution. Pathologically, this stage is characterized by noncaseating interstitial granulomatous pneumonitis. The clinical presentation usually allows easy recognition.

Chronic Hypersensitivity Pneumonitis

An insidious or chronic form results if repeated acute episodes or continued antigen exposure occurs and can be difficult to distinguish from IPF (54). Disabling and frequently irreversible respiratory findings may occur. Mixed obstructive and restrictive physiology is often present on pulmonary function testing, as well as reduced diffusing capacity and hypoxemia. The chest radiograph shows progressive reticular abnormalities with nodular densities and loss of lung volume, particularly of the upper lobes. HRCT is similar to that described above for acute hypersensitivity pneumonitis, but in many cases, it is difficult to distinguish from those findings typically seen in IPF.

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Parenchymal micronodules or ground-glass densities are less common, and there is often progressive loss of lung volume, particularly in upper lobes. Emphysema (without a history of smoking or other occupational exposure) and honeycomb changes are not uncommon (143). Diagnosis of the chronic form of hypersensitivity pneumonitis usually requires lung biopsy. A lavage lymphocytosis (usually $> 40\%$) may be very helpful in suggesting the diagnosis. Early diagnosis is critical, since irreversible or progressive disease can occur if diagnosed late in the clinical course (144). It often requires intensive detective work to uncover the source of the antigen (145). Serum precipitins to a general panel of possible antigens are often not helpful.

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4

Pathology of Usual Interstitial Pneumonia

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I. Introduction

Usual interstitial pneumonia (UIP) is a distinctive pathological pattern that is seen in the lungs of patients with diffuse parenchymal lung disease (DPLD) (9,19,43,44). It is the most common and most important of the group of DPLDs regarded as idiopathic interstitial pneumonias (IIPs) (Table 1) (43,44). The lung injury in UIP is often distributed along the subpleural and paraseptal regions and is characterized by patchy, temporally heterogeneous fibrosis with scattered fibroblastic foci at the edges of dense fibrotic scars that cause remodeling of the lung architecture and microscopic honeycombing (Table 1) (9,19,43,44). The UIP pattern can be seen in patients with collagen vascular disease, chronic hypersensitivity pneumonitis, pneumoconiosis (particularly asbestosis), drug toxicity, and with no apparent cause. In the latter setting, the clinical-radiological-pathological terms idiopathic pulmonary fibrosis (IPF) or cryptogenic fibrosing alveolitis (CFA) are appropriate (23,44).

The concept of UIP has evolved considerably over the past three decades since Liebow originally described it in the late 1960s (29). Liebow thought that diffuse alveolar damage (DAD) was an early pattern of UIP; however, this was before the concept of DAD as a form of acute lung injury was recognized (29). He described UIP as a "highly variegated lesion with evidence of hyaline membrane formation and varying degrees of exudation" (29). Liebow's observation of "variegation" is preserved in the current pathological definition of the UIP pattern, but the presence of hyaline membranes either indicates either the process of DAD alone or accelerated decline of UIP or IPF (43,44).

The histological criteria for UIP have been narrowed over the past decade (43,44). Several significant developments have contributed considerably

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Table 1 ATS/ERS Histological and Clinical Classification of IIPs

Histological patterns	Clinical diagnoses
Usual interstitial pneumonia	Idiopathic pulmonary fibrosis Cryptogenic fibrosing alveolitis
Nonspecific interstitial pneumonia	Nonspecific interstitial pneumonia (Provisional)[a]
Organizing pneumonia	Cryptogenic organizing pneumonia[b]
Diffuse alveolar damage	Acute interstitial pneumonia
Respiratory bronchiolitis	Respiratory bronchiolitis interstitial lung disease
Desquamative interstitial pneumonia	Desquamative interstitial pneumonia
Lymphocytic interstitial pneumonia	Lymphocytic interstitial pneumonia
UNCLASSIFIABLE INTERSTITIAL PNEUMONIA	

[a]This represents a heterogeneous group with poorly characterized clinical and radiological features that needs further study.
[b]This is the preferred term, but it is synonymous with idiopathic bronchiolitis obliterans organizing pneumonia.
Source: Ref. 44.

to this evolution, including the description of the entity of nonspecific interstitial pneumonia (NSIP) and the advent of high-resolution computerized tomography (HRCT). Katzenstein and Fiorelli described NSIP in 1994 as a spectrum of non-UIP inflammatory and fibrosing form of DPLD (20). The connective tissue was described as being temporally uniform in contrast to the temporal heterogeneity of the UIP pattern (20). HRCT allowed for better recognition of the gross distribution of the pathology of UIP. Now it is known that approximately half of patients with IPF have very distinctive HRCT findings that are virtually pathognomonic for this disease (17,40). It has also shown the importance of the peripheral bibasilar reticular abnormalities with minimal ground glass opacities seen by HRCT that are also reflected in the gross and histological pathology of UIP (43).

The term UIP has been used in two major ways: as a pathological pattern and as a clinicopathological syndrome (44,45). Unfortunately, both the terms UIP and IPF have been used indiscriminately resulting in considerable confusion about how separation from other IIPs. In a recent American Thoracic Society (ATS) statement, King et al. clarified that the term IPF should be restricted to patients with the HRCT and/or lung biopsy pattern of UIP (23). A subsequent ATS statement put the entity of UIP/IPF in the context of other IIPs (44). In the setting where other known causes of DPLDs, such as drug toxicity, environmental exposures, and collagen vascular diseases, have been excluded; the clinicopathological term idiopathic pulmonary fibrosis (IPF) or cryptogenic fibrosing alveolitis (CFA) is appropriate. Confusion

occurs when it is not clearly stated whether one is using the term *UIP* to describe a clinicopathological entity or a histological pattern. Pathologists can work around this problem by using the term *UIP pattern* when referring only to lung histology.

If a patient has DPLD with a UIP pattern in the setting of underlying disease (e.g., rheumatoid arthritis), then the clinicopathological diagnosis of IPF is inappropriate and the term *UIP* should be used with mention of the specific associated condition (e.g., *UIP with associated rheumatoid arthritis*).

Since the clinical features and epidemiology of UIP/IPF are addressed elsewhere in this book, this chapter will focus on pathological aspects of this disorder.

II. Pathological Aspects of UIP

A. Gross Features

The lungs are severely fibrotic and the lung size is small in UIP. The fibrosis typically is distributed in the subpleural and paraseptal regions and the lower lobes are most severely affected (Fig. 1). The pleural surface of the lungs has a cobble-stoned appearance due to the retraction of scars along the interlobular septa. The fibrosis is patchy with areas of relatively normal lung adjacent to fibrotic lung. The fibrotic lung is firm, rubbery, white connective tissue frequently associated with honeycomb cystic changes.

B. Histological Features

Histologically, the UIP pattern consists of patchy interstitial fibrosis with fibrotic lung alternating with areas of normal lung (Fig. 2). These features are best appreciated at low-power magnification (Table 2) (9,19,45). The fibrosis is often in a subpleural and/or paraseptal distribution. The fibrous connective tissue is temporally heterogeneous with dense scarring in addition to fibroblastic foci scattered at the edges of the dense scars (Figs. 2–4). The dense fibrosis causes remodeling of the lung architecture resulting in collapse of the alveolar walls and formation of cystic spaces or honeycombing (Fig. 5). The fibroblastic foci consist of loose fibrosis that contains myofibroblasts within a myxoid stroma. These fibroblastic foci are highlighted by the Movat stain (Fig. 6). Smooth muscle proliferation is often present in areas of dense fibrotic scarring, and this has been called muscular cirrhosis of the lung (10).

A variety of secondary changes can be seen in UIP, including vascular intimal fibrosis and medial thickening (Fig. 7) (45). Hyperplastic cuboidal epithelial cells or a bronchiolar type of epithelium are frequently seen along fibrotic alveolar septa. However, the epithelial cells overlying the fibroblastic

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Figure 1 UIP pattern. There is a predominantly lower lobe, peripheral, subpleural patchy process with fibrotic areas alternating with relatively normal lung. Fibrotic areas are characterized by tan, firm scars causing remodeling of the lung architecture and honeycomb cystic changes.

foci are usually flat or low cuboidal. The epithelium lining cystic honeycomb spaces are typically bronchiolar or cuboidal epithelium. Interstitial inflammation is usually mild to moderate and consists of lymphocytes and a few plasma cells. Lymphoid aggregates may be present. Interstitial mast cells, eosinophils, and neutrophils are inconspicuous or absent. Alveolar macrophages are often present, particularly if the patient is a cigarette smoker. Isolated giant cells or granulomas may be seen, but when present, they raise the differential diagnosis of hypersensitivity pneumonitis or collagen vascular disease. In rare cases, prominent noncaseating granulomas may be present in a lung biopsy showing a UIP pattern, but whether this is true UIP, fibrosing sarcoid, or a collagen vascular disease needs to determined based on careful clinical, radiological, and pathological correlation. The cysts in areas of

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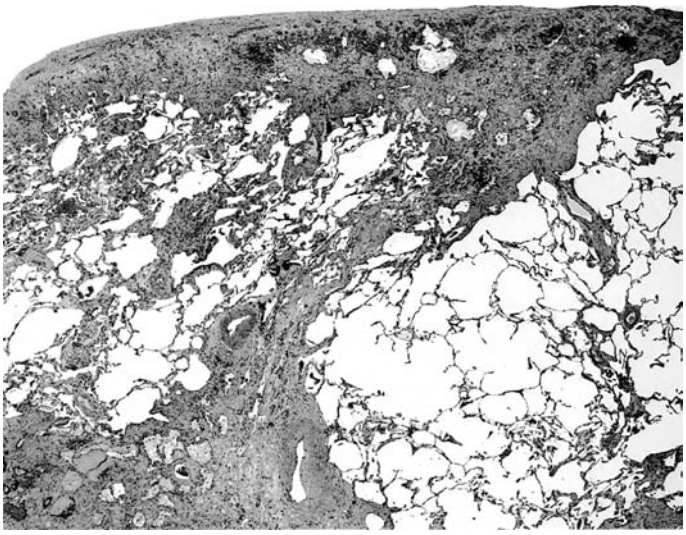

Figure 2 UIP pattern. Patchy fibrosis with remodeling of the lung architecture shows a striking subpleural distribution.

microscopic honeycombing may be empty or they may contain mucus and inflammatory cells, particularly macrophages or neutrophils.

If only severe honeycombing is present in a lung biopsy, this may reflect a sampling problem if the surgeon took the biopsy from an area of very fibrotic lung. Some areas of relatively normal lung must be present. In order to exclude the presence of active lesions of other interstitial disorders and to make a definitive pathological diagnosis of a UIP pattern. This is important, since end-stage fibrosis can be seen as the advanced phase of other interstitial disorders.

Lung Carcinoma

Lung carcinomas can develop in 13–31% of patients with IPF (28,38). All histological types of lung carcinoma can occur. In some cases, prominent reactive epithelial proliferations may be difficult to distinguish from well-differentiated adenocarcinomas (Fig. 8). Criteria that favor adenocarcinoma include exuberant micropapillary proliferation of glandular epithelium, relatively sparse inflammation, prominent mucinous epithelium, invasive growth, and cytological atypia.

Table 2 UIP Pattern: Histological Features

Major features
Patchy lung involvement
Frequent subpleural, paraseptal,and/or peribronchiolar distribution
Dense fibrosis causing remodeling of lung architecture with frequent honeycomb fibrosis
Fibroblastic foci scattered at the edges of the dense scars
Interstitial inflammation, mild/moderate
Minor features
Focal alveolar macrophage accumulation
Lymphoid aggregates
Smooth muscle proliferation
Vascular medial and intimal thickening
Bronchiolar metaplasia
Pleuritis, mild/moderate
Pleural fibrosis, mild/moderate
Type II pneumocyte hyperplasia
Subpleural fatty metaplasia
Squamous metaplasia
Dilated pleural or septal lymphatics
Alveolar neutrophils
Cholesterol clefts
Rare interstitial or alveolar eosinophils
Metaplastic bone or calcification
Hyaline cytoplasmic inclusions in pneumocytes
Focal alveolar fibrin
Subpleural blebs
Pertinent negative findings
Lack of active lesions of other interstitial diseases (i.e., sarcoidosis or Langerhans histiocytosis)
Lack of marked interstitial chronic inflammation
Granulomas: inconspicuous or absent
Lack of substantial inorganic dust deposits; i.e., asbestos bodies (except for carbon black pigment)

Accelerated Decline of IPF

A small percentage of IPF patients develop an accelerated decline of their disease, and no specific cause such as an opportunistic infection can be found (2,24,25,48). This has sometimes been called "acute exacerbation of IPF." In some cases patients, there is a well-documented previous diagnosis of UIP based on classic HRCT findings and/or a previous lung biopsy. In other cases, there is a clinical history consistent with a preexisting UIP pattern, and the biopsy is first performed during the acute exacerbation. In other cases, there is

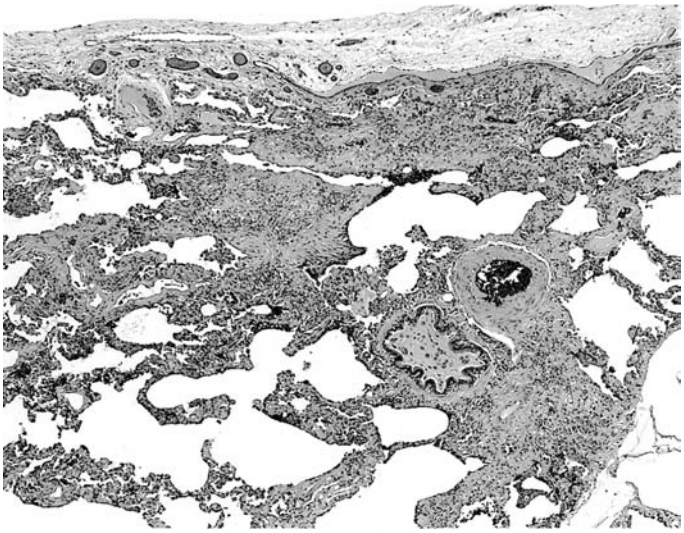

Figure 3 UIP pattern. Patchy, subpleural fibrosis consists of dense collagenous scarring with remodeling of the lung architecture and small cystic changes. Interstitial chronic inflammation is mild with a few lymphoid aggregates. Proliferation of bronchiolar epithelium is present on the surface of some of the scarred areas. Areas of ''normal'' lung are present that lack active lesions of other interstitial lung disorders.

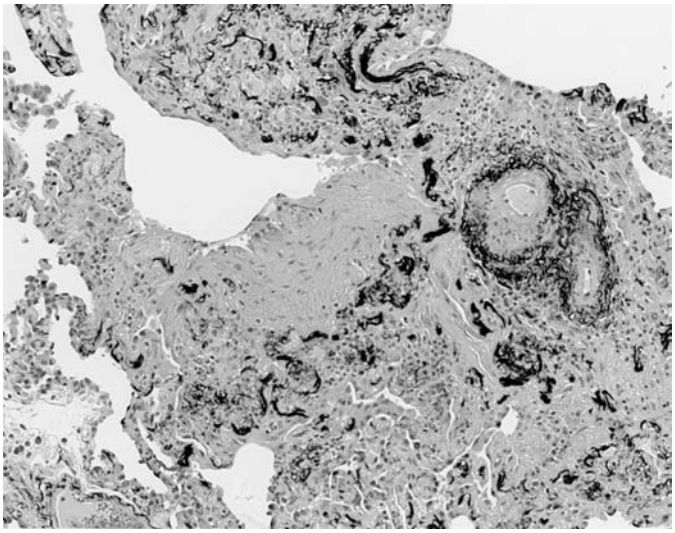

Figure 4 UIP pattern. The dense collagenous scar is juxtaposed with a fibroblastic focus of loose organizing connective tissue.

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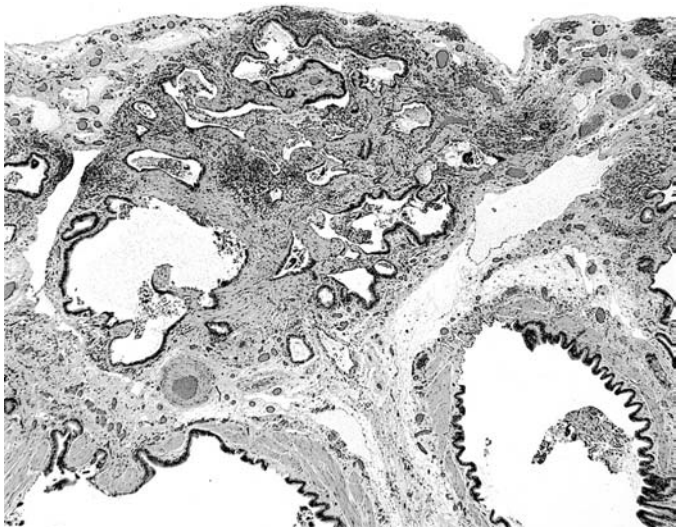

Figure 5 UIP pattern. Microscopic honeycombing consists of dense fibrosis with complete loss of lung architecture that is replaced by cysts of varying size that are lined by a metaplastic cuboidal to bronchiolar type of epithelium.

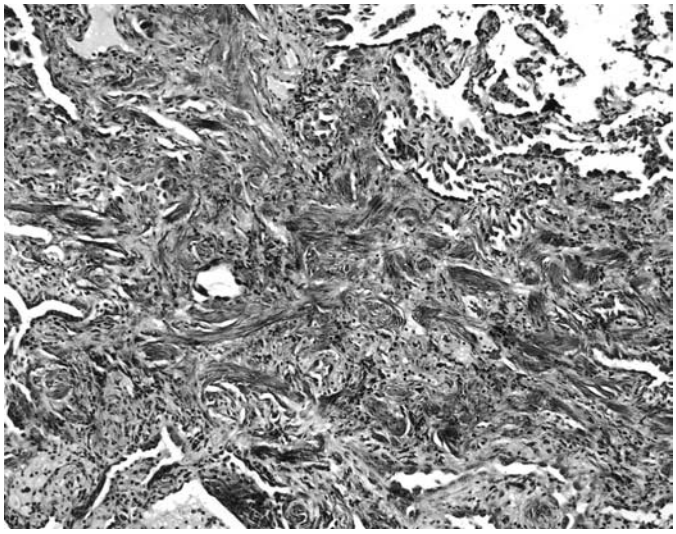

Figure 6 UIP pattern. (a) The dark blue staining with the Masson trichrome stain highlights the abundant interstitial dense collagen. The metaplastic smooth muscle stains red. (b) The Movat stain highlights the fibroblastic foci in contrast to the dense fibrosis.

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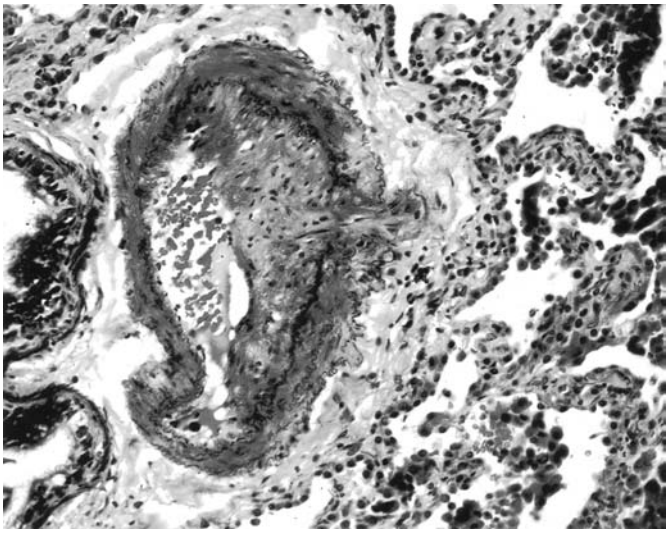

Figure 7 UIP pattern. Marked medial thickening and intimal proliferation reflect hypertensive vascular changes.

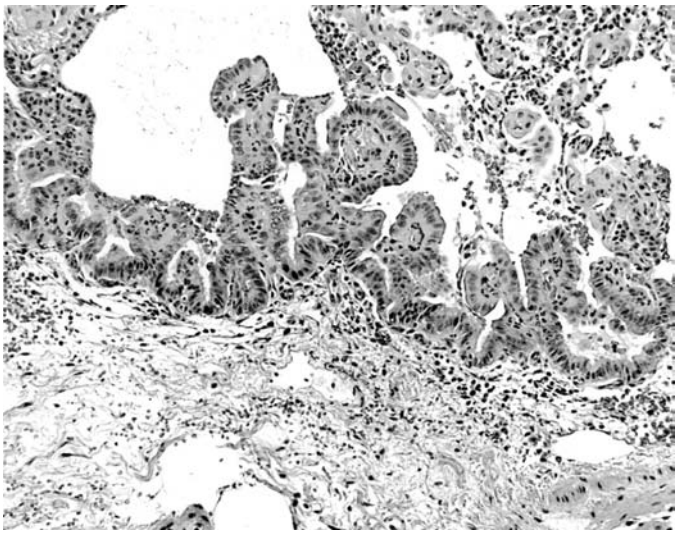

Figure 8 UIP pattern with adenocarcinoma. In addition to a UIP pattern, this case showed a very exuberant atypical epithelial proliferation. The glandular proliferation shows micropapillary growth within the lumens of the airspaces and prominently mucinous epithelial cells with foci of invasion that indicates adenocarcinoma rather than a reactive process.

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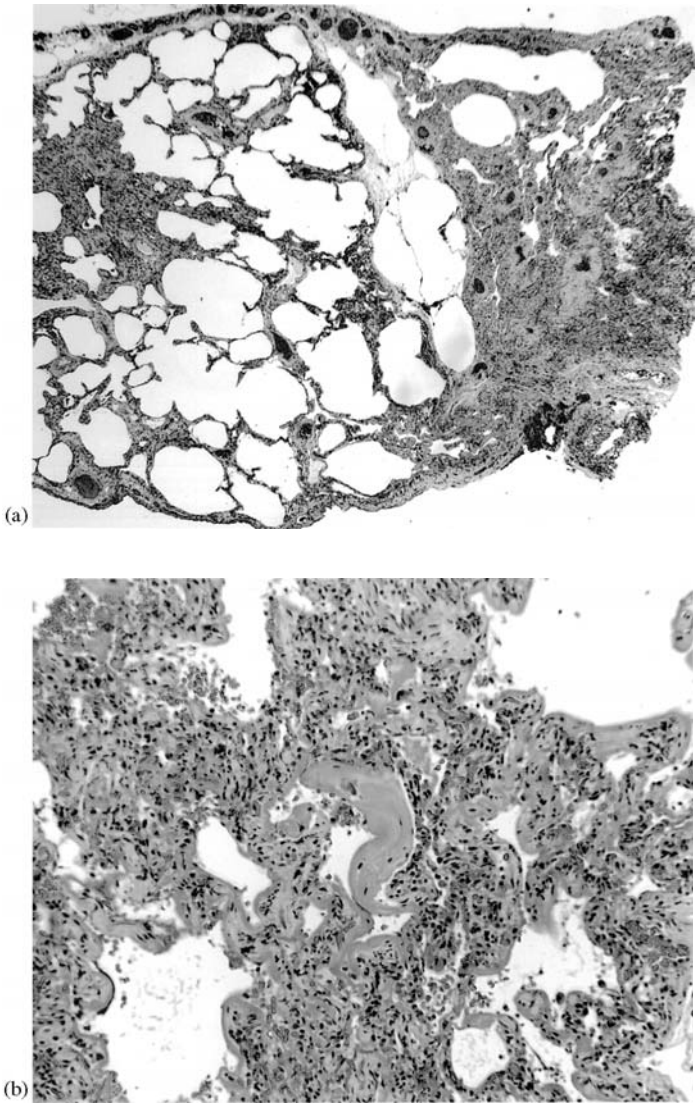

Figure 9 Usual interstitial pneumonia pattern, accelerated decline. (a) An underlying UIP pattern is present (left), with a superimposed acute lung injury pattern (right), which consists of diffuse alveolar damage with focal hyaline membranes (b).

no prior history of lung disease, and one can only speculate about the possibility of preexisting UIP/IPF. Usually, lung biopsies show two major patterns: a chronic fibrosing process with a UIP pattern and a superimposed acute lesion. Several types of acute lesions have been described, including

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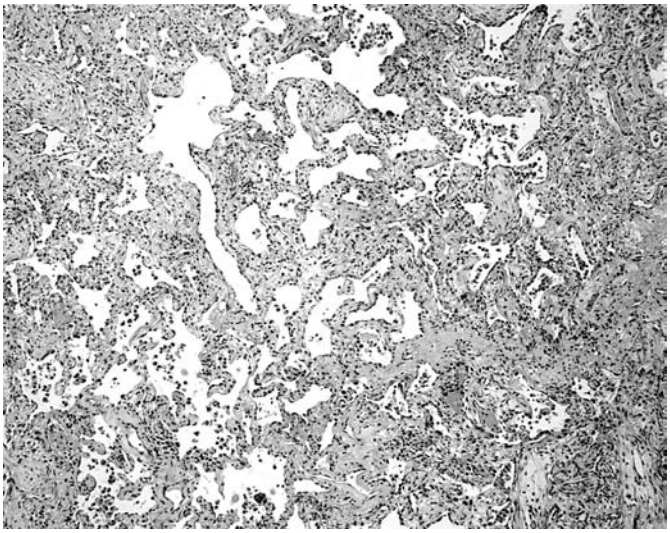

Figure 10 Discordant UIP with area showing fibrosing NIP. This specimen shows a fibrosing NSIP pattern with alveolar septal fibrosis causing uniform fibrosis without fibroblastic foci. Other areas from this patient's lung showed classic UIP.

organizing pneumonia, DAD (Fig. 9), and hemorrhage with capillaritis (2,24,48). It is often very difficult to sort out the chronic versus acute histological lesions, making it difficult to establish a definitive pathological, diagnosis without good clinical correlation.

Histological Heterogeneity in Different Lobes of Lung in IPF Patients

It has recently been recognized that there is histological heterogeneity in UIP with areas of the lung that may show an NSIP pattern (Fig. 10). Flaherty et al. reported that in 26% of patients where UIP was found in at least one lobe, an NSIP pattern was found in a separate lobe (12). They proposed that patients with a UIP pattern in all lobes should be categorized as concordant UIP and those with a pattern of UIP in at least one lobe but an NSIP pattern in another lobe should be categorized as discordant UIP (12). This finding emphasizes the importance of taking specimens from more than one lobe of lung at the time of surgical lung biopsy. It also explains why, in some cases, it is so difficult to separate UIP from NSIP.

D. Correlations Between Histology and Prognostic or Clinical Parameters

It has been very difficult to identify histological predictors of prognosis in patients with UIP/IPF (42,46,47). Unfortunately, most of the prior studies

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have not been based on the current concept of IPF, recognizing the importance of separating the UIP pattern from other DPLDs, especially NSIP. Therefore, the suggestion that patients with a cellular pattern on lung biopsy have a better prognosis than those patients with a fibrotic pattern may reflect differences in survival between cases with a UIP pattern versus a DIP or NSIP pattern. What is needed are studies that examine for histological predictors of survival based on series of patients with careful histopathological classification of their lung biopsy findings according to current concepts.

The following correlations have been made between histological findings on lung biopsy and various clinical parameters. A correlation between clubbing of the fingers and prominent smooth muscle proliferation in the fibrotic portions of lung biopsies from patients with IPF and a UIP pattern of fibrosis ($P < .01$) was shown by Kanematsu T et al. (18). Cherniack et al. demonstrated that the diffusing capacity of the lung to carbon monoxide (DLCO) correlated with "desquamation" ($P = .003$) and total pathology scores ($P = .0004$), whereas total lung capacity ($P = .04$) and forced vital capacity ($P = .0001$) correlated with a cellularity factor score (8). Watters et al. found that a total pathology score correlated with a composite clinical-radiographic-physiological score ($P < .001$) (46). Gallium-67 citrate scanning was demonstrated by Line et al. to correlate with the degree of interstitial cellularity ($P < .05$) and the degree of alveolar cellularity ($P = .005$) (30). Honeycombing ($P = .049$) or dense interstitial inflammation ($P = .045$) involving 60% or greater of the biopsy specimen; and intrapleural fat ($P = .01$) was found by Travis et al. to correlate with a poor prognosis in patients with idiopathic UIP (45). In this study, multivariate analysis using the Cox method showed that the presence of subpleural fat ($P = .0143$) was independent of the other significant histological factors (45). In addition, Fulmer et al. demonstrated a high correlation between exercise-induced changes in arterial oxygen pressure per liter of oxygen consumed and the degree of fibrosis ($P = .001$) as well as the degree of cellularity ($P = .009$) (13).

In the study by Flaherty et al. on the histological variability among lung biopsies from multiple lobes in IPF patients, survival was better in NSIP compared to both concordant and discordant UIP groups ($P < .001$) (12). Discordant UIP patients experienced better survival than concordant UIP earlier in follow-up ($P = .09$), although the survival curves converged near the end of the follow-up period. These findings support the conclusion that patients with a histological pattern of UIP in any lobe should be classified as UIP (12).

Pathogenesis

The etiology of IPF/UIP is unknown, but a variety of viral, genetic, and immunological factors have been considered. The importance of genetic factors

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is suggested by cases of familial IPF (31), reports of HLA associations (14,32), and the presence of pulmonary fibrosis in inherited syndromes such as neurofibromatosis (33) and the Hermansky-Pudlak syndrome (41). Viral infection may play a role as suggested by presentation in a number of patients with a flulike illness (22), in addition to reports of viral agents is suggested by reports of associations with hepatitis C (35), Epstein-Barr virus (11), and adenovirus (16). The frequency of collagen vascular disease in patients with UIP is strong evidence that immunological factors are important in the pathogenesis. In addition, circulating autoantibodies such as antinuclear antibodies (7–25%), rheumatoid factor (14%), and cryoimmunoglobulins (41%) can often be found in patients with IPF (22).

One theory is that after an initial injury, immune complexes activate alveolar macrophages to produce a variety of factors, including chemotactic factors that attract neutrophils. Immune complexes have been demonstrated in bronchoalveolar lavage and the alveolar epithelial cells as well as the luminal side of the capillary wall (22). These events initiate an ongoing fibrosing process in UIP that is controlled by a variety of growth factors (3), cytokines (1), and metalloproteinases and tissue inhibitors of metalloproteinase (15) that have very complex interactions (34).

The fibroblastic foci in IPF/UIP are thought to represent the leading edge of ongoing microscopic lung injury that are the sites of destruction of the lung architecture, collapse of alveolar walls, and deposition of dense collagen (4–6,15,21,26,27,37). Thus, these fibroblastic foci are thought to account for the progressive destruction of the lungs that occurs in UIP. These fibroblastic foci not only reflect the temporal heterogeneity of UIP but they also represent sites of progressive ongoing injury in contrast to DAD, NSIP, COP, and DIP which are thought to follow lung injury at a single point in time resulting in connective tissue of a uniform age (21).

III. Differential Diagnosis

Both histological and etiological, considerations are important in the differential diagnosis for the UIP pattern (Tables 3 and 4). Therefore, clinical issues as well as histological criteria must be addressed. The clinical history should be examined for drug intake, inhaled antigen exposure, and occupational exposures to minerals such as beryllium, cobalt, asbestos, silicosis, and aluminum. In addition, clinical and serological manifestations of collagen vascular diseases should be pursued. In many cases, when a biopsy does not show definitive histological findings, correlation with a HRCT an provide invaluable information, especially if it shows a classic UIP pattern (44).

Table 3 Clinical, Radiographic, and Histological Differential Diagnosis of Idiopathic UIP, Idiopathic BOOP, and AIP

Feature	IPF/UIP	COP/OP	AIP/DAD
Clinical presentation			
Age, mean (range)	64 yrs	56 yrs	50 yrs
Duration of symptoms	1–3 yrs	1–6 months	1–4 weeks
Mechanical Ventilation	Rare	Rare	Yes
HRCT			
Main findings	Irregular lines, honeycombing	Consolidation	Consolidation
Predominant distribution	Middle and lower lung zones; subpleural in ~90% of cases	Patchy, peribronchial or subpleural in ~60% of cases	Middle and lower lung zones Dependent lung regions
Histology			
Distribution	Patchy, subpleural, paraseptal	Patchy	Diffuse
Hyaline membranes	No	No	Yes
Temporal appearance	Heterogeneous	Uniform	Uniform
Fibroblastic foci	Characteristic, at edge of scars	Absent	Absent
Fibroblastic proliferation	Focal	Patchy, airspace	Diffuse
Interstitial	Minimal	No	Yes
Airspace	Focal	Yes	Sometimes
Type II cell proliferation	Characteristic	Focal	Extensive,diffuse
Dense fibrosis	No	Absent	Only in late fibrotic phase
Thrombi		No	Yes
Clinical outcome	20–50% 5-yr survival	90% 5-yr survival	10–50% recovery
Response to steroids	Poor	Excellent	Poor

IPF, idiopathic pulmonary fibrosis; UIP, usual interstitial pneumonia pattern; COP, cryptogenic organizing pneumonia/organizing pneumonia pattern; AIP, acute interstitial pneumonia; DAD, diffuse alveolar damage.
Source: Refs. 19 and 36.

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Table 4 Separation of NIP Pattern from UIP Pattern

Features	NSIP, fibrosing pattern	UIP
Clinical		
Age (range)	49 yrs (11–78)	51 yrs (27–70)
Sex(M:F)	28:27	72:37
Duration of symptoms, average (range)	8 mos (1 week–5 yrs)	2.5 yrs
Radiology		
Main findings	Ground-glass attenuation	Irregular lines, honeycombing
Predominant distribution	Diffuse or patchy	Middle and lower lung zones, subpleural in ~90% of cases
Pathology		
Temporal appearance	Uniform	Heterogeneous
Interstitial inflammation	Mild to marked	Mild
Dense interstitial fibrosis	Variable, diffuse or patchy	Patchy
Organizing pneumonia	Not uncommon	Uncommon
Fibroblast foci	Absent or inconspicuous	Characteristic feature
Honeycomb fibrosis	Rare, inconspicuous	Frequent
Alveolar macrophages	Occasional, patchy	Occasional, focal
Prognosis		
5-yr survival	90%	20–45%
10-yr survival	35%	10–15%

Source: Refs. 7, 20, 36, and 45.

Since the UIP pattern has such ominous clinical implications, it is important to determine whether a UIP or a non-UIP pattern is present in a lung biopsy. When the classic UIP pattern is present, it is usually readily recognized. However, there are cases where the issue of separating the UIP pattern from other interstitial pneumonias, especially the fibrosing NSIP pattern, is challenging. Because of this difficulty, there are varying levels of certainty in a pathologist's assessment as to whether a UIP pattern is present or not. It can be useful to communicate this level of certainty to clinicians in a comment in the pathology report: (1) definite UIP pattern, (2) probable UIP pattern, (3) possible UIP pattern, (4) definitely not a UIP pattern. When the pathological diagnosis is difficult, careful clinical and radiological correlation is advised. In a small percentage of cases, a pathologist may only be able to say fibrosing interstitial pneumonia, not further classified and either favor UIP or NSIP. Things that can make it difficult to interpret a biopsy include a small sample a specimen atelectasis, and mixed patterns of lung injury.

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The fibrosing pattern of NSIP is the most difficult to separate from the UIP pattern. The UIP pattern frequently shows a subpleural, paraseptal, or peribronchiolar distribution that is seen less often in the fibrosing NSIP pattern. However, the key distinguishing feature is the fibrosing NSIP pattern lacks the temporal heterogeneity with prominent fibroblast foci and dense fibrosis characteristic of the UIP pattern. Fibroblastic foci may be focal or inconspicuous in cases with the NSIP, fibrosing pattern (19–21,39,45). However, when present, care should be taken to make the distinction from the UIP pattern. It is likely that cases of idiopathic NSIP, fibrosing pattern were included in previous studies of idiopathic UIP, since only recently have the criteria for the UIP pattern been narrowed to a more precise definition.

The separation between the UIP and fibrosing NSIP patterns is primarily based on histological examination, but it also requires careful correlation with clinical and radiographic data, especially HRCT scans. Some lung biopsies interpreted as showing a fibrosing NSIP pattern may represent cases of poorly biopsied UIP. When the quality of the lung biopsy is poor, it should be stated in the pathology report that the distinction between a fibrosing NSIP and UIP cannot be made with certainty.

Biopsies that show a fibrosing NSIP pattern in patients with clinical and radiographic features suggestive of idiopathic UIP (IPF) probably should be diagnosed as idiopathic UIP (IPF). This may reflect the problem of histological heterogeneity or discordant UIP in IPF where biopsies from at least one lobe of the lung show a UIP pattern and biopsies from other lobes show a NSIP pattern (12). In some cases, precise classification may be difficult at the time of pattern lung biopsy and ultimately may be established only after the clinical outcome is clear.

The separation of the UIP and DIP patterns is usually straightforward, but some UIP cases have prominent alveolar macrophages and some DIP cases may show some focal scarring or honeycomb changes. A marked interstitial inflammatory infiltrate or the presence of granulomatous inflammation should raise the consideration of hypersensitivity pneumonitis, pneumoconiosis, drug-induced pneumonitis, infection, or collagen vascular disease. Dust deposits should be searched for; in particular asbestos bodies. If there is a suspicion for asbestos exposure, iron stains should be performed unless asbestos bodies are seen on H&E–stained sections. Polarization microscopy may also help to identify birefringent particles that could suggest significant dust deposits. However, a few biregringent particles are not uncommonly found in lung biopsies from patients with UIP. The presence of infectious agents should be excluded, which may require special stains for organisms, particularly *Pneumocystis carinii*.

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Patchy scars with a stellate configuration and situated on bronchioles should raise the differential diagnosis of a fibrotic phase of Langerhans' cell histiocytosis. The combination of airspace organization and granulomas with peribronchiolar inflammation should suggest hypersensitivity pneumonitis. Prominent giant cell formation in alveolar macrophages should raise concern for giant cell interstitial pneumonia or hard metal pneumoconiosis. Occasionally honeycomb changes and fibrosis associated with bronchiectasis can be confused with a UIP pattern.

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5

Nonspecific Interstitial Pneumonia

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I. Introduction

Nonspecific interstitial pneumonia (NSIP) represents a subset of idiopathic interstitial pneumonias (IIPs) which has only recently been categorized (1). Since the initial description of NSIP in 1994 (1), several investigators have confirmed that NSIP has an improved prognosis and responsiveness to therapy compared to usual interstitial pneumonia (UIP) (2–9). In addition, virtually all series of idiopathic pulmonary fibrosis (IPF) or cryptogenic fibrosing alveolitis (CFA) included a mix of histological entities, including NSIP (2,10). Classification schema utilizing pathological criteria were developed more than 25 years ago (11) and continue to be refined (12). The first pathological schema, developed by the eminent pathologist Liebow, recognized five distinct histological categories among IIPs including UIP, bronchiolitis obliterans with interstitial pneumonia (BIP), desquamative insterstitial pneumonia (DIP), giant cell interstitial pneumonia (GIP), and lymphocytic interstitial pneumonia (LIP) (11). Subsequent investigators affirmed that GIP was the pathological hallmark of hard metal pneumoconiosis (13) and should not be included as an IIP. Further, BIP, now considered to be synonymous with bronchiolitis obliterans organizing pneumonia (BOOP) (14) or cryptogenic organizing pneumonia (COP) (15,16), is an intraluminal disease rather than an interstitial disorder and has been dropped from the IIP classification schema (2). LIP, a

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lymphoproliferative disorder often associated with immunodeficiencies (particularly human immunodeficiency virus [HIV] infections) (5,17,18), was dropped from the IIP classification schema of Katzenstein and Myers (2) but was retained in the most recent consensus statement of IIPs endorsed by the American Thoracic Society (ATS) and European Respiratory Society (ERS) (12). Acute interstitial pneumonia (AIP), a rapidly progressive form of IIP which exhibits histopathological features of diffuse alveolar damage (DAD), is indistinguishable from the acute respiratory distress syndrome (ARDS) (19–22). Although a subset of patients with IPF/UIP develop an accelerated course, often as a terminal event, with features of DAD on lung biopsy or necropsy (23,24), clinical and radiographic features of AIP differ sharply from other IIPs (2). Another variant of IIP, termed respiratory bronchiolitis interstitial lung disease (RB-ILD), was described in the 1980s (25,26), principally in smokers, characterized by pigmented alveolar macrophages within respiratory bronchioles, and with histological features which overlap with desquamative interstitial pneumonia (DIP) (27,28). In 1998, Katzenstein and Myers published a classic article recognizing four distinct forms of IIPs (i.e., UIP, NSIP, DIP/RB-ILD, and AIP) (2). In that article, they emphasized "... the term 'idiopathic pulmonary fibrosis' should be reserved for cases of UIP, the most common idiopathic interstitial pneumonia" (2). In 2000, an international consensus statement produced by a collaborative effort from the ATS, ERS, and the American College of Chest Physicians (ACCP) stated that "... usual interstitial pneumonia (UIP) is the histopathological pattern that identified patients with IPF" and that other histopathological patterns should be considered distinct from IPF (29). More recently, a joint consensus statement from the ATS and ERS classified IIP into seven clinical-radiological-pathological entities: IPF, NSIP, COP, AIP, RB-ILD, DIP, and LIP (12). These experts reaffirmed that UIP is the histological pattern observed in IPF. They also recognized that the histological pattern NSIP may be observed in response to occupational exposures or in the context of collagen vascular disorders (CVDs) as well as a form of IIP. They stated "... after considerable debate, ... use of the term NSIP was acceptable as a provisional measure until there is further clarity on the nature of the corresponding clinical condition" (12). They also pointed out that information was lacking in several important aspects of NSIP, including incidence or prevalence, whether clinical features can distinguish NSIP from other IIPs, whether further subclassification of NSIP is warranted or possible, and what relationship exists (if any) between NSIP and UIP.

Since NSIP was only recently described, prospective studies assessing clinical course, treatment responsiveness, and prognosis are lacking. Any relationship of NSIP to UIP remains conjectural. Anecdotal evidence suggests that at least a subset of patients with NSIP may progress to UIP. However, complete and lasting remissions have been achieved with therapy in NSIP (30),

whereas responses to therapy with UIP are infrequent and incomplete (10,31). Important clinical questions remain unanswered. Do all (or most) cases of NSIP progress to UIP in the absence of therapy? Does therapy with corticosteroids or immunosuppressive agents alter the natural history of NSIP? Is NSIP an early phase of UIP or can it evolve in other directions? It is possible that NSIP may represent a pathological response to diverse insults, and prognosis between variants may differ. Further, as will be discussed, histological variants of NSIP (i.e., cellular and fibrotic) differ in prognosis and responsiveness to therapy. Yet the distinction between NSIP-fibrotic and UIP can be difficult (8). Given the absence of prospective studies and limited long-term follow-up, definitive answers to these various questions are lacking. However, in this chapter, we attempt to outline the salient features of NSIP (i.e., demographic, physiological, and radiographic features; prognosis and responsiveness to therapy) and provide a framework for future study of this entity.

II. Historical Description of NSIP

Nonspecific interstitial pneumonitis/fibrosis (NSIP) was first described in 1994 by Katzenstein and Fiorelli (1) to refer to cases of IIP that did not fit the histopathological criteria for the other categories of IIP. In NSIP, an interstitial inflammatory cell infiltrate (with or without fibrosis) may be present, but features of other IIPs are lacking. In contrast to UIP, NSIP displays temporal homogeneity (1,2). The lesions in NSIP are of similar age (i.e., temporally uniform), whereas in UIP, both recent and old lesions are present concomitantly (1–4). The temporal and geographical heterogeneity of UIP can be appreciated at low-power magnification; in UIP, areas of dense scarring, honeycomb cysts, apparently normal lung, and ''fibroblastic foci'' (aggregates of proliferating myofibroblasts and fibroblasts) are observed concomitantly (2,3). Although other IIPs (e.g., DIP/RB-ILD, AIP) exhibit temporal homogeneity, these entities differ from NSIP in other important histological aspects (see Chap. 4). Other features distinguish NSIP from UIP (2,3). For example, fibroblastic foci are a prominent feature of UIP but are rarely prominent in NSIP (2). In addition, honeycomb change (HC), a prominent feature of UIP, is absent or mild in NSIP (2–5,7). In contrast to UIP, the alveolar architecture is preserved in NSIP (2). The salient histopathological features of NSIP will be discussed in greater detail later in this chapter. From a clinical perspective, the designation NSIP is important, because prognosis and responsiveness to therapy are improved compared to UIP (discussed in detail later). Further, histological variants of NSIP (i.e., cellular NSIP and fibrotic NSIP) exhibit distinct differences in prognosis (4,5). The natural history of NSIP has not been elucidated. It is possible that cellular NSIP evolves to fibrotic NSIP and ultimately progresses to UIP, but this hypothesis has not yet been validated.

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In the sentinel description, Katzenstein and Fiorelli emphasized that the term *NSIP* referred to a clinical syndrome resembling IPF in immunocompetent patients (1). They suggested that NSIP "should not be considered a specific disease, however, because it may have varying etiologies including connective tissue diseases, organic dust or other exposures, and prior acute lung injury; less often it may reflect a nonrepresentative biopsy of another process" (1). Histological features of NSIP indistinguishable from idiopathic NSIP may be observed in collagen vascular diseases (32–34) or as a response to diverse lung injuries or toxins (2). Early descriptions of interstitial lung disease (ILD) associated with rheumatoid arthritis (33) and dermatomyositis (DM) or polymyositis (PM) (34,35) observed histological features consistent with cellular interstitial pneumonia or NSIP. In a recent study, surgical lung biopsies were obtained from patients with DM or PM with ILD; NSIP was observed in 18 of 22 patients (82%) (36). The term NSIP is confusing, because historically, it referred to a nonspecific histological lesion in immunocompromised hosts, including HIV-infected patients or bone marrow transplant recipients (17,37–40). The terms unclassified pneumonia (41) or cellular interstitial pneumonia (2,33,34) were considered to be synonymous with NSIP (42). In a classic review, Katzenstein and Myers emphasized that the term *NSIP* should not be used as a wastebasket term to describe "nondiagnostic" biopsies from transbronchial or surgical lung biopsies (3). Controversy remains as to whether NSIP comprises a distinct clinical entity given the overlap with patients suffering interstitial lung disease and systemic inflammatory disease (1,32,36,43–45), hypersensitivity reactions (1,46), drug reactions (1,47,48), occupational exposures (1,49,50), and infection, including HIV (17).

III. Clinical Series of NSIP

Undoubtedly, previous series of IPF or CFA (some of which lacked surgical biopsy confirmation) included a subset of patients with NSIP (31, 51–53). Following the initial description of idiopathic NSIP (1), several groups reexamined surgical lung biopsies from patients previously diagnosed as having IPF or CFA (3–9,32). In these various studies, NSIP was identified in 12–36% of cases. The published series are described in Table 1.

The initial description of NSIP analyzed 101 open lung biopsies diagnosed as chronic interstitial pneumonia, not otherwise specified, or nonspecific interstitial pneumonia derived from the consultation files of Katzenstein from 1981 to 1991 (1). Following an initial review of available slides from 95 patients, 31 cases were excluded for the following reasons: alternative diagnoses, that is, UIP ($n = 13$), BOOP ($n = 7$), DIP ($n = 1$); underlying malignancy and chemotherapy ($n = 4$); pulmonary-renal syndrome ($n = 1$); acquired immunodeficiency syndrome (AIDS) ($n = 1$); infants < 3 years

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of age (n = 3). The remaining 64 cases were grouped into three groups: (1) predominantly cellular interstitial pneumonia (n = 31); (2) a mixture of inflammation and fibrosis (n = 24); (3) predominant fibrosis (n = 9).

In 1994, Izumi and colleagues organized the Kyoto conference, whereby a panel of nine pathologists evaluated surgical lung biopsies gleaned from 18 medical centers in Japan to identify cases of NSIP. Forty-five cases of NSIP were detected (idiopathic 13; associated with collagen vascular disease 14) (4). The incidence of NSIP was not determined, as the denominator of the total cases of IIP evaluated at these institutions was not provided. At a second international conference in Kyoto in 1995, the 31 patients with idiopathic NSIP were analyzed in detail, and salient clinical features and prognosis were described (4).

In 1998, French investigators identified 12 cases of NSIP upon rereview of pathological slides of surgical lung biopsies performed for evaluation of IIPs at one hospital in Lyon, France between 1979 and 1996 (32). Six cases of NSIP were idiopathic; three were associated with CVD; two were consistent with occupational exposure or hypersensitivity pneumonia; and one with recurrent ARDS (32). Again, the ''denominator'' was not provided, so the incidence of the disease could not be established.

In 1998, investigators at the Mayo Clinic reviewed 102 open lung biopsies from patients previously diagnosed as having IPF from 1976 to 1985 (3). Patients with CVD or environmental exposures were excluded. Fourteen cases were reclassified as NSIP; 63 had UIP; 8, DIP; 2, RB-ILD; 2, AIP (3). In a recent report, these investigators detected 18 patients with NSIP gleaned from a cohort of 22 surgical lung biopsies obtained between 1990 and 1998 from patients with ILD complicating PM or DM (36).

Investigators at the Royal Brompton Hospital in England identified 15 patients with idiopathic NSIP in a retrospective review of 113 open or thoracoscopic lung biopsies performed from 1990 to 1995 (7). In a more recent report, these investigators rereviewed 78 open lung biopsies performed from 1978 to 1989 in patients with a clinical diagnosis of CFA (8). Biopsies were reclassified by two pulmonary pathologists as UIP in 37 (47%), NSIP in 28 (36%), RB-ILD/DIP in 13 (17%) (8). Most recently, these investigators described histological findings in 80 patients with fibrosing alveolitis associated with systemic sclerosis (44); NSIP was the predominant histological finding in 62 patients (fibrotic NSIP in 47; cellular NSIP in 15).

Travis and colleagues reexamined 101 surgical lung biopsies obtained at the Pulmonary Branch of the National Heart, Lung and Blood Institute (NHLBI) between 1970 and 1992 in patients with idiopathic ILD (5). Biopsies were reclassified as UIP in 56, NSIP in 29, and DIP in 16 (5). Among 29 patients with NSIP, 7 were classified as cellular NSIP and 22 as fibrotic NSIP. In cellular NSIP, mild to moderate chronic interstitial inflammation was evident but severe fibrosis was not found. Dense interstitial fibrosis was the hallmark of fibrotic NSIP, but the pattern differed from UIP (5).

Table 1 Clinical and Demographic Characteristics for Patients with NSIP

Series (Ref.)	No. of patients	Age in years Mean (range)	Sex	CTD n (%)	Current or previous smoking; n (%)	Symptom (%)	Symptom duration in months Mean (range)	Physical exam feature (%)
Katzenstein and Fiorelli (1)	64	46 (9–78)	26 male 38 female	10 (6%)	NA	Dyspnea Cough Chest pain (8) Weight loss (11)	8 (0.25–60.0)	Fever (22) Wheeze (6)
Park et al. (60)	7	56 (43–69)	1 male 6 female	NA	1 (14)	Dyspnea (100) Cough (57) Chest pain (28)	4	Fever (29) Crackles (100)
Cottin et al. (32)	12	53	6 male 6 female	3 (25%)	6 (50)	Dyspnea (100) Cough (67) Fatigue (58) Weight loss (42)	31 (1–64)	Crackles (100)
Nagai et al. (4)	31	58 (40–72)	10 male 16 female	Excluded	18 (58)	Dyspnea Cough	2 (0.25–32)	Fever (32)* Clubbing (10)
Bjoraker et al. (3)	14	57 (40–73)*	8 male 6 female	Excluded	8 (57)	Dyspnea (100) Cough (85)	15 15	Crackles (79) Clubbing (21)
Daniil et al. (7)	15	43 (31–66)	7 male 8 female	Excluded	9 (60)	Dyspnea (100) Cough (60)	18 (7–84)	Crackles (80) Clubbing (40)*

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Fujita et al. (43)	24	Median 60 (44–74)	7 male 17 female	8 (33)	NA	Cough (87) Dyspnea (71)	3 (1–8)	Crackles (100) Clubbing (0) Fever (29)
Travis et al. (5)	22	50 (30–71)	15 male 7 female	Excluded	15 (68)	Dyspnea (100) Cough (100)	NA	NA
Nicholson et al. (8)	28	53	20 male 8 female	NA	19 (68)	Dyspnea	11 median (0–180)	NA
Douglas et al. (36)	18	NA	NA	Polymyositis/ dermato-myositis in all	NA	NA	NA	NA
Flaherty et al. (6)	28 Fibrotic 5 Cellular	56 50	16 male/ 12 female 3 male/ 2 female	Excluded	20 (71) 2 (40)	NA	26 22	NA
Riha et al. (9)	7	49 (39–67)	2 male/ 5 female	Excluded	3 (43)	Dyspnea (100) Cough (57)	28 (12–36)	Crackles (71) Clubbing (57)
Bouros et al. (44)	62	46 (23–69)	13 male/ 61 female	Scleroderma in all	25 (33)	Dyspnea (89) Cough (35)	13 (0–60)	Crackles (85) Clubbing (3)

Source: Adapted from Ref. 30.

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Fujita et al. identified 24 cases of NSIP (16 idiopathic; 8 associated with CVD) between March 1990 and November 1997 (43). A mixture of cellular and fibrotic infiltration (Katzenstein/Fiorelli Group II) was observed in all cases. More recently, these investigators described three fatal cases of idiopathic NSIP (54) and a series of NSIP complicating systemic sclerosis (55).

Investigators from the University of Michigan recently rereviewed surgical lung biopsies from 168 patients with IIPs seen between October 1989 and February 2000 (6). The most common histological feature was UIP, which was observed in 106 patients; other histological patterns included NSIP in 33 patients (fibrotic in 28; cellular in 5), RB-ILD (n = 22), and hypersensitivity pneumonia (n = 5). Most recently, Australian investigators reviewed surgical lung biopsies from 70 patients with clinical, radiographic, and physiological features consistent with IPF from a single institution between 1990 and 2000 (9). Histological diagnoses included UIP (n = 59), NSIP (n = 7), AIP (n = 1), and DIP (n = 3).

In summary, these various retrospective reviews of surgical lung biopsies from patients with presumed IPF or CFA identified NSIP in 12–36% of cases (3,5–9). Although the prevalence and incidence of NSIP is not known, estimated prevalence of IPF ranges from 3 to 20 cases per 100,000 in the general population (56–58). By extrapolation, the prevalence of NSIP may range between 0.4 and 7.0 cases per 100,000.

IV. Histological Differences: NSIP Versus UIP

By definition, NSIP and UIP are distinguished by histopathological criteria. As has been discussed, temporal homogeneity is a cardinal feature of NSIP (2). In NSIP, the alveolar architecture is preserved and extensive fibrosis is lacking (2). The key and pertinent negative histological features of NSIP were summarized in the recent classification of IIPs adopted by the ATS and ERS (Table 2) (12). There is a spectrum from cellular (Fig. 1) to fibrosing (Fig. 2) or mixed cellular and fibrosing patterns (Fig. 3) (1,2,4,5,8). In the original description of 64 cases of NSIP, the following histological features were cited: patchy distribution (55%), foci of bronchiolitis obliterans organizing pneumonia (48%), collections of intra-alveolar macrophages (30%), bronchiolocentricity (28%), germinal centers (27%), fibroblastic foci (20%), granulomas (8%) (1). Other concomitant features observed in subsequent reports include bronchiolar fibrosis or inflammation, loosely formed granulomata, and lymphoid aggregates (5,32). A histological diagnosis of NSIP should prompt the consideration of hypersensitivity pneumonitis, especially if there is a bronchiolocentric pattern on lung biopsy (12,46).

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Table 2 Histological Features of NSIP

Key Features
Cellular Pattern
Mild to moderate interstitial chronic inflammation
Type II pneumocytic hyperplasia in areas of inflammation
Fibrosing Pattern[a]
Dense or loose interstitial fibrosis lacking the temporal heterogeneity pattern and/or patchy features of UIP
Lung architecture often appears lost by examination of H&E-stained sections, but relatively preserved on elastic stains
Interstitial chronic inflammation—mild or moderate
Pertinent Negative Findings
Cellular Pattern
Dense interstitial fibrosis: absent
Organizing pneumonia is not the prominent feature
Lack of diffuse severe alveolar septal inflammation
Fibrosing Pattern
Temporal heterogeneity pattern: fibroblastic foci with dense fibrosis are inconspicuous or absent—this is especially important in cases with patchy involvement and subpleural or paraseptal distribution
Both Patterns
Acute lung injury pattern, especially hyaline membranes: absent
Eosinophils: inconspicuous or absent
Granulomas: inconspicuous or absent
Lack of viral inclusions and organisms on special stains for organisms

[a]There is a spectrum from cellular to fibrosing patterns with some cases showing a combination of cellular and fibrosing features.
Source: Ref. 12.

In the original description, NSIP was subdivided into three histological patterns; (1) cellular interstitial infiltration with minimal or no fibrosis, (2) lymphoplasmacytic infiltrates with significant admixed fibrosis, (3) dense fibrosis (1). Subsequent studies support segregating NSIP into cellular (group 1) and fibrotic (group 2 or 3) subtypes because of differing prognosis between these histological entities (4–6,8,32).

The difficulties inherent in the histological diagnosis of NSIP has been highlighted by recent work. Distinguishing UIP from fibrotic NSIP (grade III) is difficult. Even with experienced pulmonary histopathologists, interobserver variation has been significant (kappa value for distinguishing UIP and NSIP3 was only 0.26) (8). A more recent study from the same group, with a more homogeneous group of NSIP complicating systemic sclerosis suggests an improved interobserver agreement between two histopathologists (kappa 0.72)

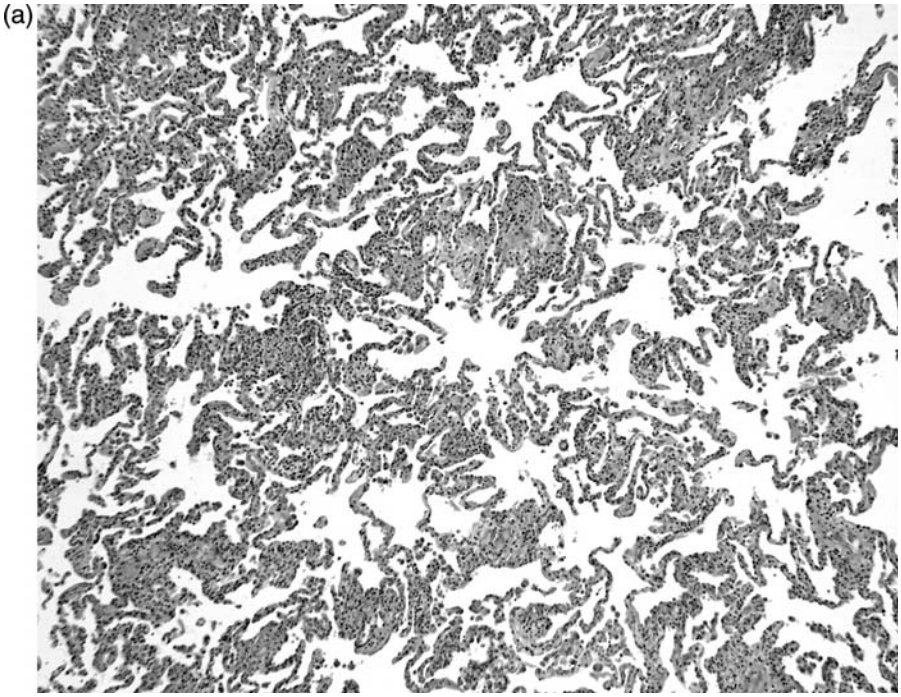

Figure 1 Photomicrograph (open lung biopsy): Nonspecific interstitial pneumonia (NSIP): cellular pattern. (a) There is mild patchy interstitial chronic inflammation. (b) The inflammation consists mostly of lymphocytes with a few plasma cells. No interstitial fibrosis is present but there is focal pneumocyte hyperplasia. (H&E).

(44). Flaherty and colleagues confirmed the frequent occurrence of histopathological variability (intra- and interlobar) in IIPs (30). Surgical lung biopsies sampling two or more lobes were obtained from 109 patients with a clinical syndrome consistent with IPF and either UIP or NSIP on biopsies. UIP was observed in all lobes (concordant UIP) in 51 patients, 28 exhibited UIP in one lobe, and NSIP in at least one lobe (discordant UIP); 33 had NSIP in all lobes biopsies (fibrotic in 28; cellular in 5). Importantly, patients with concordant UIP were older than all other categories and exhibited a lower total lung capacity (TLC) and greater extent of fibrosis on high-resolution computed tomographic (HRCT) scans. The survival characteristic of the cohort is illustrated in Fig. 4; NSIP patients experienced the best survival, whereas patients with UIP in any lobe demonstrated a similar, worse survival. The fact that UIP and NSIP were present concomitantly in some patients raises the possibility that NSIP may represent an early phase of UIP. Alternatively, NSIP and UIP may represent different histological responses to (nonspecific) injury.

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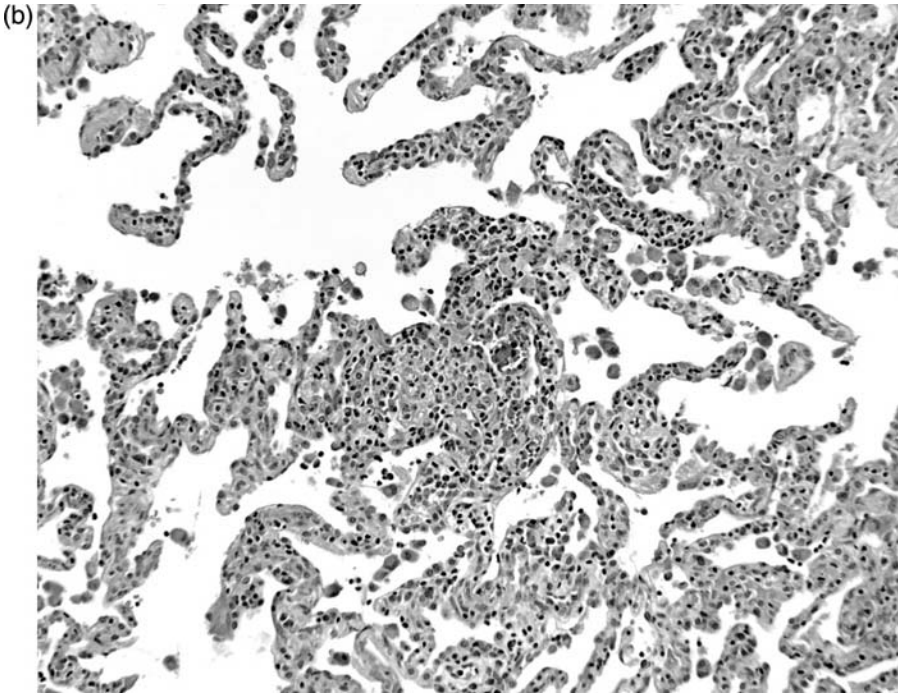

Figure 1 Continued

A recent study of whole lung explants from a cohort of patients with chronic ILD undergoing lung transplantation affirmed that both UIP and NSIP may be present in individual patients (59). In that study, surgical lung biopsies and subsequent lung explants were examined to study the possible relationship of UIP and NSIP (59). UIP was diagnosed in 20 explants; NSIP, in one. Previous surgical biopsies from the 20 UIP cases demonstrated UIP; 5 showed nonspecific changes. A constant feature in all UIP cases was a "patchwork pattern" (i.e., heterogeneity) with areas of interstitial abnormalities and normal lung. In contrast, the single NSIP case was characterized by uniform involvement of lung parenchyma and only small foci of honeycomb change. However, areas resembling NSIP were common in the background of otherwise typical UIP. NSIP-like areas were noted in 12 of 15 biopsy and 16 of 20 explant specimens from patients with UIP, and generally comprised $< 10\%$ of interstitial areas. Further, foci of DIP-like reactions were noted in 12 of 15 biopsy and 19 of 20 explant specimens from patients with UIP. The investigators argued that the dominant background pattern determined the histological diagnosis. Specifically, the presence of patchwork parenchymal involvement and/or significant architectural distortion excludes a diagnosis of NSIP or DIP.

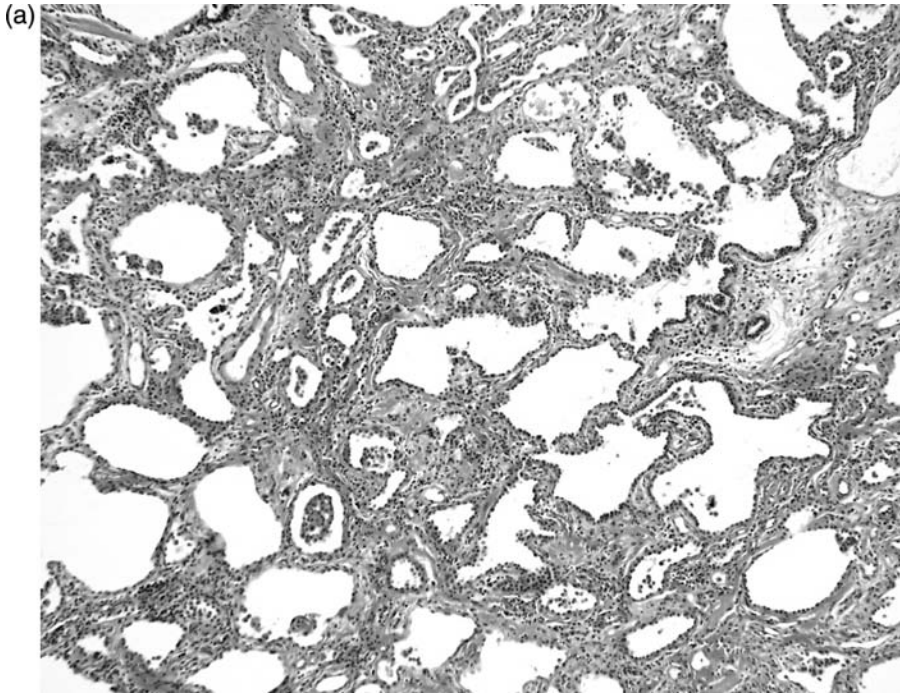

Figure 2 Photomicrograph (open lung biopsy): Nonspecific interstitial pneumonia (NSIP): fibrosing pattern. (a) There is uniform interstitial fibrosis causing thickening of the alveolar walls. Fibroblastic foci are not seen. (b) Dense collagen is present within the alveolar walls. Interstitial chronic inflammation is mild. Focal pneumocyte hyperplasia is present. (H&E).

V. Clinical and Demographic Features

The clinical and demographic features in clinical series of NSIP are enumerated in Table 1. The median age among these series is 53 years (range 43–60 years), which is on average 5–10 years younger than patients with UIP (3–8,32,54,60). Although studies from Japan (4,54) and Korea (60) cited a female predominance, investigators from North America (3,5,6) and Europe (7,8) reported a slightly higher proportion of males (see Table 1). Among patients with CVD, a striking female predominance has been noted (44). A history of previous or current smoking is elicited in 33–68% of patients with idiopathic NSIP (3–8,32,54,60). Presenting symptoms are primarily cough and dyspnea; duration of symptoms ranges from a few weeks to >1 year (30). In contrast to UIP, fever is sometimes noted in

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(b)

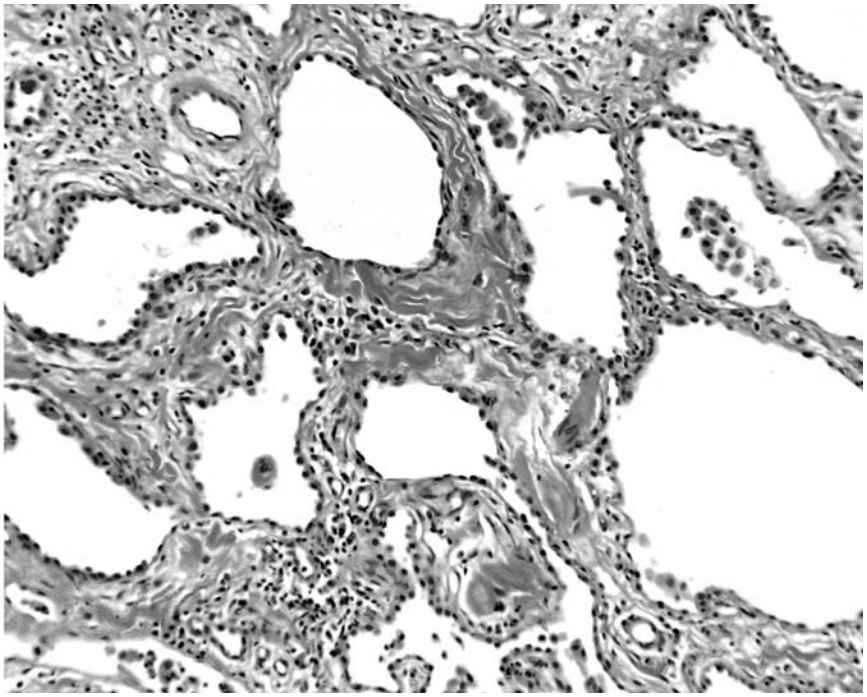

Figure 2 Continued

NSIP (up to 32% in one series) (4). Crackles are the most common physical examination finding (cited in 71–100%); clubbing is relatively infrequent (3–8,30,32,54,60).

VI. Physiological Findings

Pulmonary physiological aberrations are generally similar to UIP. Pulmonary function tests (PFTs) cannot discriminate NSIP from UIP (4,6–8). Typically, PFTs in NSIP demonstrate reduced lung volumes and decreased gas transfer, as enumerated in Table 3 (3,4,6,7,32,54,60). Gas transfer (i.e., diffusing capacity of the lung for carbon monoxide [DLCO]) is disproportionately reduced in NSIP compared to lung volumes (DLCO range 39–50% predicted) compared to the range 59–72% predicted for TLC) (3,4,6–8,60). Only one series examined oxygen saturation at rest and following exercise; the degree of desaturation was similar in patients with UIP (92% rest vs 85% exercise) and NSIP (93% vs 83%) (3).

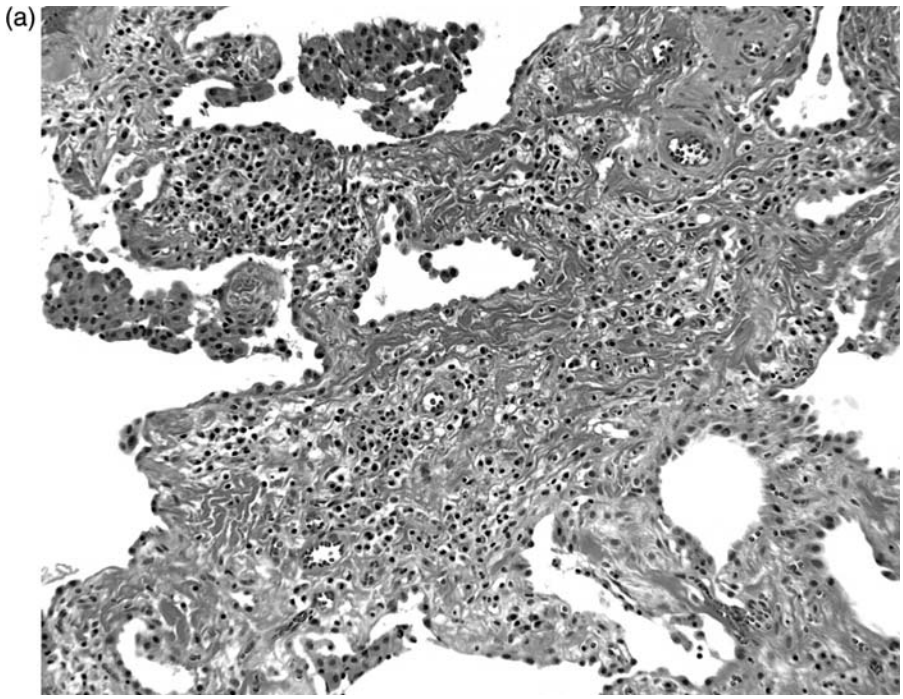

Figure 3 Photomicrograph (open lung biopsy): Nonspecific interstitial pneumonia (NSIP): cellular and fibrosing pattern. (a) The alveolar septa are markedly thickened by dense interstitial fibrosis and moderate interstitial chronic inflammation. (b) The inflammation consists mostly of lymphocytes with a few plasma cells. A few alveolar macrophages are also present. (H&E).

VII. Radiographic Characteristics

Numerous publications have characterized the salient radiographic features of NSIP (summarized in Table 4). Typically, chest radiographs demonstrate bilateral patchy alveolar and interstitial infiltrates (1,3,4,32,60,61). Characteristic features on HRCT scans include ground-glass opacities (GGOs), with or without consolidation with varying degrees of interstitial changes; honeycomb change (HC), a cardinal feature of UIP, is uncommon in NSIP (0–26%) (4,7,32,43,60–64). In addition, predominant GGOs are rarely (if ever) seen in UIP, but may be found in NSIP. Similar to UIP, a bibasilar predominance is the most common distribution in NSIP (42). These features are illustrated in Figs. 5–7 (4,7,32,43,60–64). CT features appear to be similar in idiopathic or CVD-associated NSIP (61). In one radiological-pathological correlative study,

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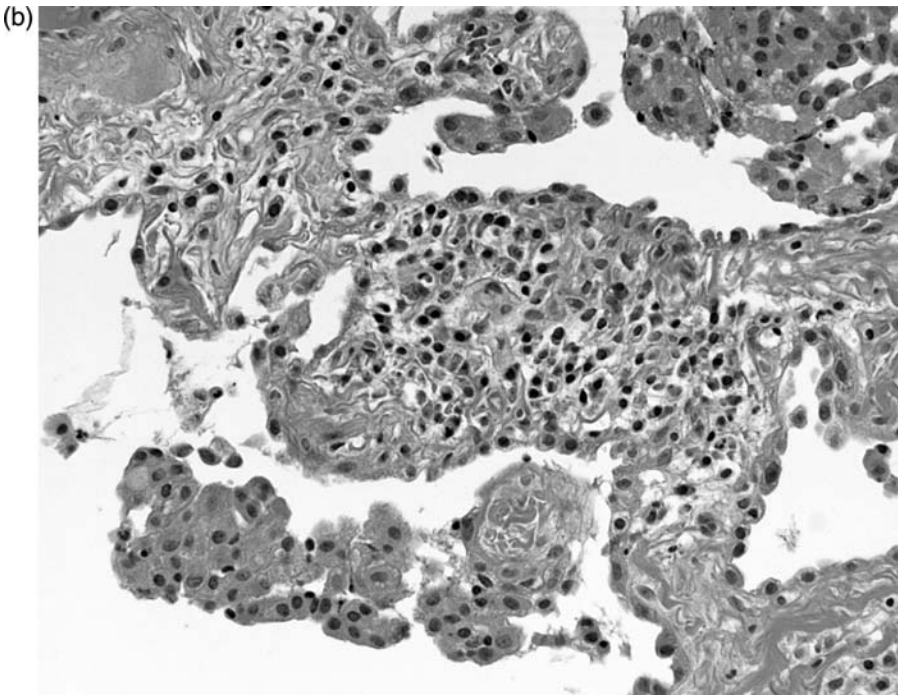

Figure 3 Continued.

GGOs with or without irregular linear opacities corresponded to varying degrees of interstitial inflammation or fibrosis (65). Areas of consolidation corresponded with histological BOOP, foamy cell collections in alveolar spaces, or microscopic HC with mucostasis.

Early studies suggested a low diagnostic accuracy for HRCT in identifying NSIP. Johkoh et al. assessed the diagnostic accuracy of HRCT, interpreted by two radiologists, in a cohort of patients with IIPs (UIP in 35; BOOP in 24; DIP in 23; AIP in 20; NSIP in 27) (66). A correct diagnosis was made in only 9% of NSIP cases. NSIP cases were most frequently misdiagnosed as DIP, BOOP, and UIP. Hartman et al. assessed HRCT scans from 50 patients with NSIP (64). In 32% of these patients, HRCT scans were ''typical'' for UIP as opposed to NSIP (64). Recently, investigators at the Royal Brompton Hospital examined HRCT scans in 21 patients with histological NSIP and 32 with histological UIP (67). Thin-section CT demonstrated moderate sensitivity (70%), specificity (63%), and diagnostic accuracy (66%) in separating NSIP from UIP. NSIP was associated with a finer reticular pattern and a higher proportion of GGOs (47 vs 27%) than UIP.

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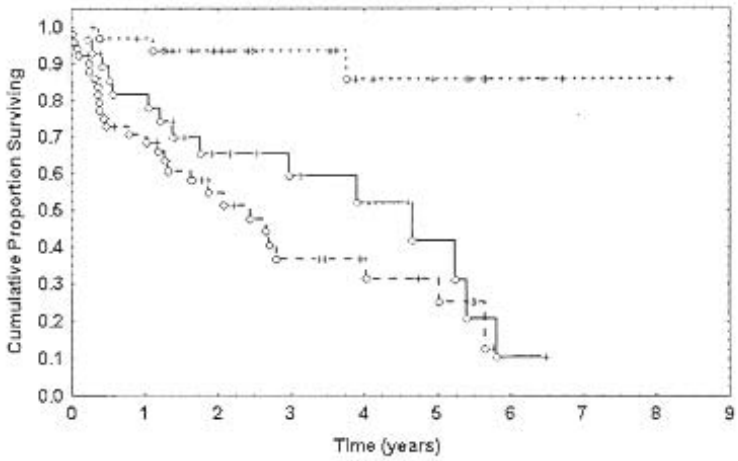

Figure 4 Kaplan-Meier survival curves for patients with concordant UIP (n = 51), discordant UIP (n = 28), and NSIP (n = 30), grouped by histological classification ($P > .0003$). (Dotted line, NSIP; solid line, discordant UIP; +, last follow-up visit; °, death.) (From Ref. 30.)

Table 3 Physiological Pattern of NIP

Study	FVC (% pred)	TLC (% pred)	DLCO (% pred)	Pa_{O_2}
Nagai et al. (4)	74	–	56	70 mm Hg
Cottin et al. (32)	59	61	52	9.3 kPa
Bjoraker et al. (3)	80	76	50	–
Daniil et al. (7)	73	72	44	74 mm Hg
Fujita et al. (43)	66	–	–	71 mm Hg
Nicholson et al. (8)	71	–	39	10.6 kPa
Flaherty et al. (6)	73[a]	78[a]	51[a]	–
	72[b]	78[b]	69[b]	–
Riha et al. (9)	63	92	39	–

[a]Fibrotic NSIP.
[b]Cellular NSIP.

The only CT feature independently associated with a histological diagnosis of NSIP was an increased proportion of ground-glass attenuation (OR 1.04 for each 1% increase in proportion of ground glass). We examined the ability of HRCT to discriminate UIP from NSIP in a cohort of 96 patients who had surgical lung biopsy (68). Two thoracic radiologists independently reviewed HRCT scans and assessed the probability of either UIP or NSIP. All 27

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patients with probable or definite UIP by CT criteria had histological UIP on surgical lung biopsy. The remaining 63% of UIP cases had CT scans interpreted as being indeterminate or probable or definite NSIP. Only 18 of 44 patients with HRCT suggestive of probable or definite NSIP had histological NSIP. Thus, the radiologists demonstrated high specificity (100%) but a low sensitivity (37%) in identifying UIP and sensitivity of 78% and specificity of 64% for identifying NSIP.

Published data about serial HRCT scans in NSIP are summarized in Table 5. In 1996, Park et al. described serial CT scans in six patients with NSIP (60). Abnormalities on CT resolved completely in three patients within 13 months; in the remaining three patients, partial improvement in the parenchymal opacities was noted (60). In another study, serial CT scans were performed in 13 patients with NSIP. GGOs were present in all patient on initial scans; HC was present in only one patient (62). CT scans were repeated at a mean interval of 11 months. Follow-up HRCT scans showed a decrease in the amount of GGOs (mean decrease $13.5 \pm 10.5\%$; $P = .003$) and an insignificant change in the amount of linear opacities (mean decrease $4.2\% \pm 5.2\%$; $P > .05$); no patient developed HC (62). The decrease in amount of GGOs correlated with the improvement in forced vital capacity (FVC) ($r = -0.7$; $P = .007$) and DLCO ($r = 0.6$; $P = .3$) (62). Nishiyama et al. evaluated serial HRCT scans in 14 patients with NSIP treated with a combination of corticosteroids and cyclophosphamide. An associated CVD was present in seven patients (61). Abnormalities seen on the initial HRCT improved in 12 of 14 patients (complete in 3; partial in 9) (61). Akira et al. evaluated serial HRCT scans from nine patients with NSIP over a mean time of 3.1 years (range 1–8 years) (63). In four patients with GGOs on initial CT, abnormalities resolved completely. Two patients with initial consolidation developed bronchiectasis between 1 and 2 years of follow-up. In one of these patients, the consolidation progressed to HC after 9 years of follow-up (63). These various studies suggest that HRCT scans in NSIP often improve with therapy, and this improvement correlates with improvement in pulmonary physiology.

VIII. Bronchoalveolar Lavage

Although data are limited, most studies suggest that NSIP is associated with lymphocytosis in bronchoalveolar lavage (BAL) fluid (mean 28–47%) (4,32,43,60) (Table 6). However, one study failed to detect lymphocytosis among eight patients with NSIP (mean 9.3%) (see Table 4) (7). This series also noted no difference in the cell profiles between the patients with NSIP and eight patients with UIP (7). In another study, BAL cell profiles from 6 patients with NSIP were compared with BAL findings in 50 normal control subjects (60).

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Table 4 Radiological Findings in Patients with NSIP

Series	n	CXR (%)	HRCT (%)	
			Features	Distribution
Katzenstein and Fiorelli (1)	64	Bilateral interstitial infiltrates (most) Diffuse alveolar or mixed alveolar/interstitial infiltrates (11) Normal (6)	NA	NA
Park et al. (74)	7	Parenchymal opacification (86)	Ground-glass opacity (100) Irregular lines (29) Consolidation (71)	Ground-glass opacity Upper lobe predominant (100) Lower lobe predominant (100) Irregular lines Upper lobe predominant (100) Lower lobe predominant (100) Consolidation Upper lobe predominant (60) Lower lobe predominant (100)
Park et al. (60)	7	Patchy opacification (86) Normal (14)	Bilateral patchy ground-glass or alveolar consolidation (71) Irregular lines (29) Honeycombing (0)	
Kim et al. (65)	23	NA	Ground-glass opacity (100) Consolidation (65) Irregular lines (87) Honeycombing (0)	Subpleural (100)
Nagai et al. (4)	31	Patchy bilateral infiltrates (77) Reticular nodular shadows (22)[a]	Honeycombing (26)[a] Ground-glass opacity (74)[a]	NA

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Cottin et al. (32)	12	Diffuse infiltrate (100)	Honeycombing (8) Ground-glass opacity (82) Septal thickening (45)	Parenchymal opacities Lower lobe predominant (73) Diffuse (27) Honeycombing Subpleural (100)
Bjoraker et al. (3)	14	Radiographic infiltrates (93)	NA	NA
Fujita et al. (43)	24	NA	Bilateral interstitial and patchy parenchymal opacification, predominant in middle and lower lung zones[b] Honeycombing (0)	Middle/lower lobe predominance
Kim et al. (62)	13	NA	Ground-glass opacity (100) Interstitial opacity (100) Honeycombing (8) Bronchiectasis (100)	NA
Daniil et al. (7)	15	NA	Typical of CFA(2)[a] Not typical of CFA(13)[a]	NA
Johkoh et al. (66)	27	NA	Ground-glass opacity (100) Interstitial opacity (93) Honeycombing (26)	Upper lobe predominant (4) Lower lobe predominant (74) Random (22) Peripheral (85)
Akira et al. (63)	9	NA	Bilateral disease (100) Ground-glass opacity (100) Consolidation (78) Intralobular lines (78) Bronchiectasis (78) Honeycombing (0)	Central (100) Peripheral (100)
Nishiyama et al. (61)	15	Bilateral infiltrates (100) Consolidation (27) Reticular density (13) Consolidation + reticular density (60)	Ground-glass opacity (13) Interstitial thickening (37) Honeycombing (0) Traction bronchiectasis (87)	Upper lobe predominant (0) Lower lobe predominant (80) Peripheral (33) Diffuse (60)

(*continued*)

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Table 4 Continued

Series	*n*	CXR (%)	HRCT (%)	
			Features	Distribution
Hartman et al. (64)	50	NA	Ground-glass opacity (76) Irregular linear opacities (46) Honeycombing (30) Consolidation (16) Nodular opacities (14) Enphysema (12) Traction bronchiectasis (36)	Ground-glass opacity Upper lobe predominant (8) Lower lobe predominant (59) Random (14) Subpleural (68) Random (21) Irregular linear opacities Lower lobe predominant (87) Random (13) Subpleural (96) Honeycombing Upper lobe predominant (20) Lower lobe predominant (67) Subpleural (93)
MacDonald et al. (67)	21	NA	Ground-glass opacity	Basal distribution (62) Subpleural distribution (60)
Riha et al. (9)	7	Fine reticular markings (71) Ill-defined patchy infiltrates (43) Honeycombing (14)	NA	NA

[a]$P > .05$ UIP compared to NSIP within series. C × *R*, chest radiography; HRCT, high-resolution computed tomography; CFA, cryptogenic fibrosing alveolitis.
[b]Number of patients not quantified.
Source: Ref. 30.

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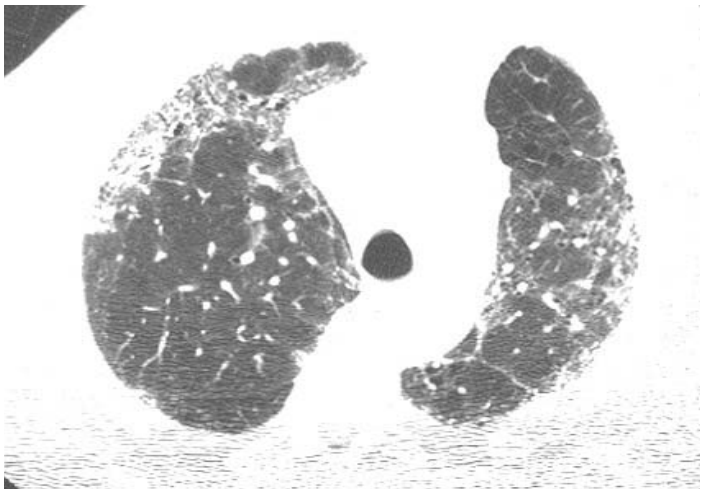

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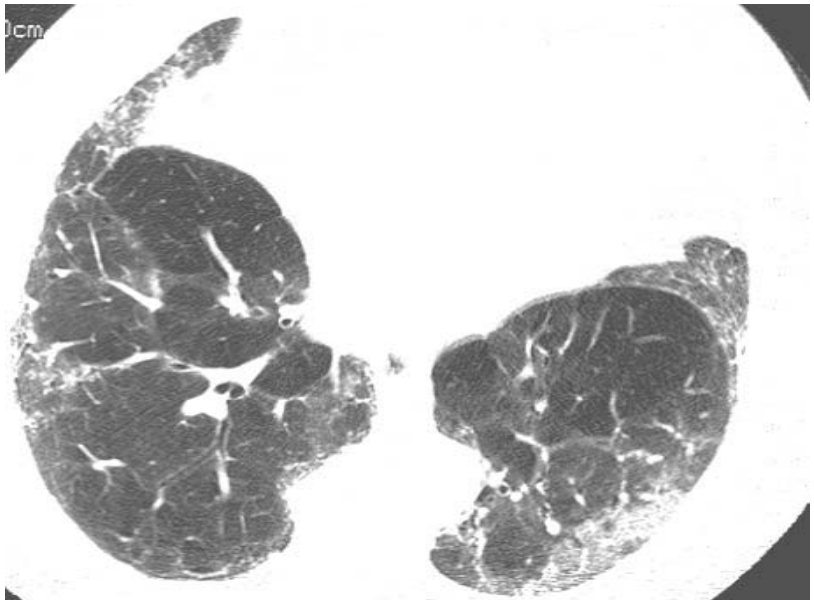

(b)

Figure 5 (a) NISP: cellular. HRCT (at level of aortic arch) from a 65-year-old woman reveals patchy ground-glass opacities, thickened interlobular septa, and early honeycomb change. (b) NSIP: cellular. HRCT from the same patient (lower lobes) reveals focal GGO, with a subpleural predominance. No honeycomb change is evident. (c) NSIP: cellular. HRCT from the same patient (at lung bases) reveals focal GGO, with a zone of consolidation with air bronchograms.

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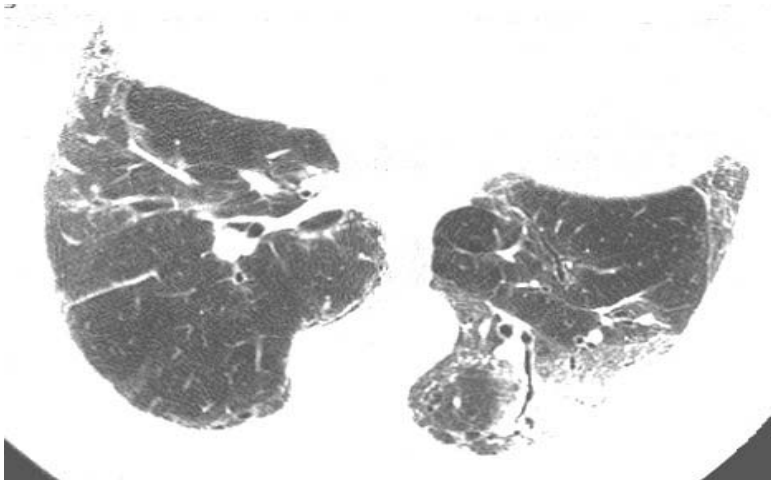

Figure 5 Continued

Patients with NSIP had a higher percentage of lymphocytes (36 vs 8; $P = .01$), neutrophils (24 vs 2; $P = .01$); and eosinophils (5 vs 0.3; $P = .05$) and a lower percentage of macrophages (34 vs 91; $P = .01$) (60). Cottin et al. reported BAL lymphocytosis in 8 of 12 patients with NSIP (mean 47%); BAL neutrophil counts were increased ($\geq 10\%$) in 6 patients (32). In two patients, BAL neutrophilia was pronounced (50 and 60% neutrophils). Four patients had a mixed pattern (elevations in both lymphocytes and neutrophils on BAL). Two other series also demonstrated BAL lymphocytosis in patients with NSIP (28 and 37%, respectively) (4,43). The CD4/CD8 ratio was also decreased (0.86 [43] and 0.63 [4], respectively).

IX. Natural History and Prognosis

Although prospective studies have not been done, the prognosis of NSIP and responsiveness to therapy is better than UIP. Early retrospective studies of NSIP cited generally favorable prognosis, with short-term (2- to 5-year) survival rates of 70–100% (1,3–5,32). Data on long-term (>10 year) survival are lacking. However, recent reports of fibrotic NSIP are less sanguine, with 5-year survival rates of 45% (8) and 35%, respectively (5). In the initial cohort of 64 patients with NSIP, follow-up data were available in only 48 patients (1). Five of 48 patients (11%) died of respiratory failure; 42% remained stable or improved; 45% recovered (1). The presence of fibrosis on surgical lung biopsy was associated with a worse prognosis. Five of 26 patients (19%) with fibrosis

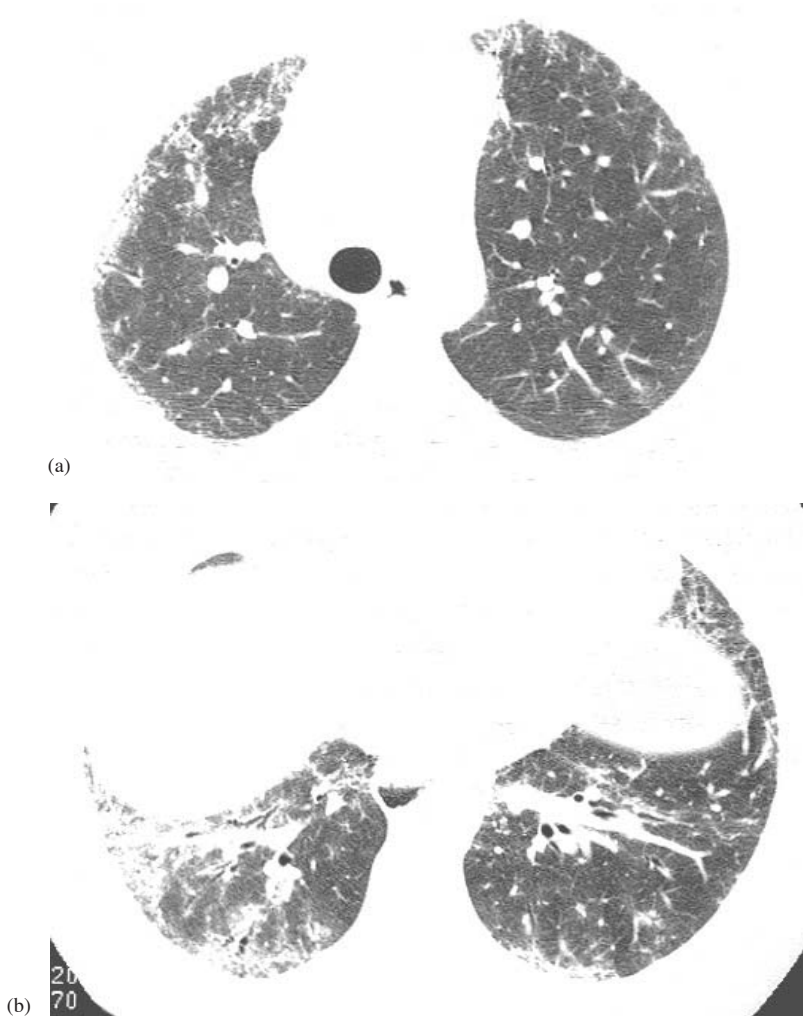

Figure 6 (a) NSIP: fibrotic. HRCT (level of the aortic arch) from a 41-year-old woman reveals thickened alveolar septa, some reticulation, and minimal GGO in the subpleural regions. (b) NSIP: fibrotic. HRCT from the same patient (at lung bases) from reveals slightly more prominent GGO and thickened interlobular septa. No honeycomb change is evident.

(grade II or III) died, whereas all 22 patients with inflammation alone (grade I) survived (1). One retrospective review of 31 patients with NSIP from Japan cited a 2-year mortality of only 6% (2 of 31) (4). A retrospective analysis from the Mayo Clinic cited 4-year survival rates of approximately 70% among

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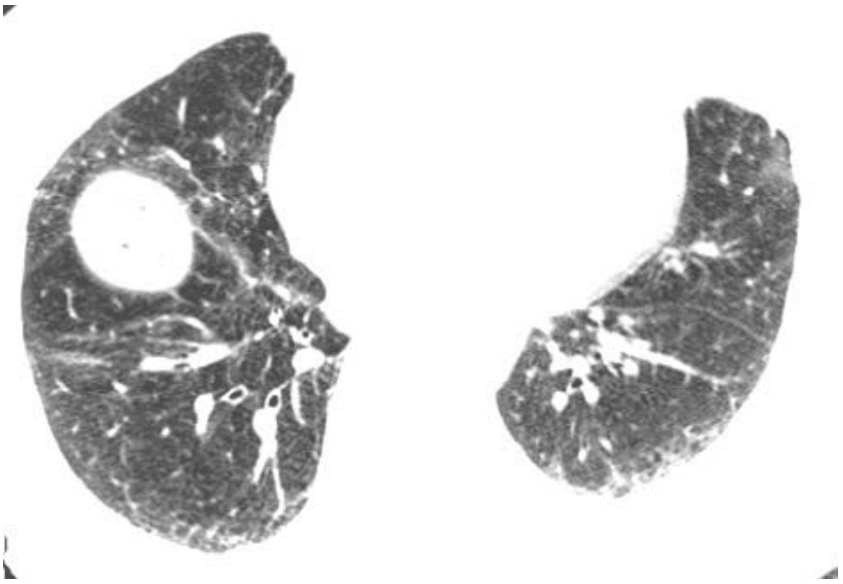

Figure 7 NSIP. HRCT (bases) from a 68-year-old woman reveals minimal GGOs in the subpleural regions. Most of the lung parenchyma appears normal. Neither honeycomb change nor reticular opacities are evident. Despite the mild changes on CT, VATS lung biopsy demonstrated fibrotic NSIP.

Table 5 Studies of Serial CT in Patients with NSIP

Series	N	Follow-up	Results
Park et al. (74)	6	13 mo	3 complete resolution 3 improvement
Kim et al. (62)	13	11 mo	Improved ground-glass opacity > irregular linear opacity
Nishiyama et al. (61)	15	15.6 mo	3 complete resolution 9 improvement 1 persistent 1 worsened
Akira et al. (63)	9	3.1 yrs	4 complete resolution 1 improvement 2 persistent 2 worsened

Table 6 Bronchoalveolar Lavage Findings from Patients with NSIP

Series	Total WBC/mm^3	Macrophages (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
Park et al. (60)	575	34	36	24	5
Cottin et al.[a] (32)	423	32	47	20	1
Fujita et al. (43)	NA	NA	28	NA	NA
Daniil et al. (7)	NA	79	9	8	3
Nagai et al. (4)	441	47	37	8	1
Nishiyama et al.[b] (61)	370	56	36	4	3
Park et al. (73)	473	13	25	12	2
Suga et al. (71)	137	70	21	7	3
Bouros et al. (44)	NA	78	8	5	4

[a]Data limited to idiopathic NSIP cases.
[b]Idiopathic and collagen vascular–associated NSIP.

patients with NSIP compared to 20% with UIP (3). Although the impact of therapy was difficult to ascertain, 13 of 14 patients with NSIP in that study received corticosteroids (3). French investigators described 12 patients with NSIP, all of whom were treated with CS (7 received concomitant immunosuppressive agents) (32). All patients were alive at follow-up (at a mean of 50 months). Travis et al. cited improved survival with NSIP compared to UIP (5). Five- and 10-year survival rates, respectively, were 100% and 90% with cellular NSIP; 100% and 35% with fibrotic NSIP; and 43% and 15% with UIP (5). Data regarding therapy were not available. A study of 168 patients with IIP from the University of Michigan cited the histological diagnosis of UIP as the most important predictor of mortality (6). Five-year survival among patients with NSIP was 90% (9 of 10) compared to approximately 40% survival for UIP. These investigators also found that a significant proportion (26%) of patients with IIP had histological features of both NSIP and UIP in the same lung biopsy specimen (i.e., discordant UIP) (30). Survival was better among patients with NSIP in *all* lobar samples compared to UIP (either concordant or discordant). The survival advantage of NSIP persisted even after age, gender, smoking history, physiological variables, and onset of symptoms were controlled. In a related article comprising many of these same patients, the histological diagnosis of UIP was associated with a poorer survival (32 deaths among 73 patients) compared to patients with a histological diagnosis of NSIP (2 deaths among 23 patients) (69). Median follow-up was 3.1 years for UIP and 3.3 years for NSIP, respectively. British investigators at the Royal Brompton Hospital cited 7-year survival rates of 71% (10 of 14) with NSIP and 7% (2 of 15) for UIP (7). In a more recent study from the same

institution, 67 patients with lone CFA were followed to death or 10 years after lung biopsy (8). Overall survival rates were 11% (4 of 37) with UIP and 39% (11 of 28) with NSIP. Median survivals were 24 months for UIP and 52 months for NSIP. Five-year mortality for fibrotic NSIP (grades II and III) was 45%. Older age and reduced DLCO were independently associated with heightened mortality (8). At last follow-up, only 11 of 28 (32%) NSIP were still alive. These data are sobering, and suggest that earlier projections of survival in NSIP were overly optimistic. Whether improved survival rates observed with NSIP are due to a different disease process or the same disease with lead-time bias remains unclear. Assessment of the impact of therapy is difficult, as not all NSIP patients have been treated, treatment regimens have not been uniform, and the duration of follow-up has been highly variable. The results of follow-up and treatment for the reported clinical series are highlighted in Table 7.

The impact of therapy is difficult to ascertain. French investigators reported a cohort of 12 patients with NSIP, all of whom were treated with corticosteroids; 7 patients received immunosuppressive drugs (IS) concomitantly (32). Ten patients (83%) improved and 2 worsened. However, all but one patient had persistent functional or radiological sequelae. Relapses occurred in four patients following cessation or taper of corticosteroid therapy (32). In three patients, multiple relapses occurred, suggesting the need for prolonged therapy in a subset of patients. In a retrospective review from Japan, 19 of 31 patients with NSIP were treated with corticosteroids (alone or combined with immunosuppressive therapy); the remaining 12 patients were not treated (4). In the untreated cohort, seven patients improved (complete remissions were achieved in 5 patients improved) and two patients remained stable. Nineteen patients were treated with corticosteroids as initial therapy; immunosuppressive agents were added in eight patients with suboptimal response to corticosteroids (4). Of 11 patients treated with corticosteroids alone, 8 improved, 2 worsened, 1 remained stable. Immunosuppressive or cytotoxic agents were added to corticosteroids in eight patients with a suboptimal response to corticosteroids alone. In this cohort, five patients improved, two died, and one deteriorated. Long-term survival was not examined. Prognosis (with or without therapy) was better for patients with ''cellular'' NSIP (4). In that study, 14 of 16 patients with cellular NSIP improved and 2 remained stable. By comparison, among 15 patients with ''fibrotic'' NSIP, 9 improved; 1 stabilized, 3 worsened, and 2 died. Others have confirmed that cellular NSIP has a better prognosis and responsiveness to therapy than fibrotic NSIP (5,6). British investigators reported 15 patients with idiopathic NSIP and 15 patients with idiopathic UIP seen at the Brompton Hospital from 1990 to 1995 (7). Thirteen of 15 patients with UIP were treated with corticosteroids (with or without concomitant immunosuppressive agents). Only one patient (7%) improved; two remained stable, and the rest deteriorated or died. The prognosis of NSIP was distinctly better. Two patients with NSIP had normal

lung function and were not treated. One patient was lost to follow-up immediately after lung biopsy. Twelve NSIP patients were treated with corticosteroids and/or immunosuppressive agents. One patient treated with high-dose prednisolone alone improved. Eight patients received azathioprine (100–150 mg/day) plus alternate day prednisolone (20 mg on alternate days) (7). Only one patient improved; three remained stable, two worsened; and two died (at 4 and 6 months). Two patients received oral cyclophosphamide (100–150 mg/day) plus prednisolone (20 mg alternate days). One patient deteriorated and one remained stable. One patient was treated with cyclosporine (5 mg/kg/day) plus prednisolone (10 mg on alternate days) and improved. Overall, 3 of 12 treated patients (25%) improved and 4 (33%) remained stable. In a subsequent study from the same institution, 78 patients with a prior diagnosis of CFA who had lung biopsies between 1978 and 1989 were reanalyzed (8). Reclassification identified 37 cases of UIP (47%), 28 cases of NSIP and (36%), 13 cases of RB-ILD (17%). Treatment regimens were variable. Overall favorable responses (assessed at 6 months) were achieved in 3 of 28 (10%) patients with UIP; 6 of 21 (30%) with NSIP, and 8 of 10 (80%) with DIP/RB-ILD. Among 28 patients with NSIP, 7 patients were not treated. The remaining 21 patients were treated with high-dose prednisolone alone ($n = 12$) or oral cyclophosphamide (100–120 mg/day) plus alternate-day prednisolone (20 mg on alternate days) ($n = 9$). Given the limited number of patients, optimal therapy for idiopathic NSIP is not clear. We prospectively studied 10 patients with NSIP and 29 patients with UIP treated with high-dose corticosteroids (1 mg/kg/day for 1–3 months with taper) (6). Clinical improvement (based upon clinical-radiological-physiological [CRP] scores) was documented with this regimen in 4 of 10 patients with NSIP (40%); 5 remained stable and one deteriorated (6). In contrast, only 5 of 29 (17%) of UIP patients treated with a similar treatment regimen improved (see Fig. 7).

Some studies have shown improvement in HRCT scans with therapy, but data on functional improvement are limited. In a short-term study (mean follow-up of only 7.5 months), HRCT scans improved with corticosteroid therapy in six of seven patients with NSIP (60). In another retrospective study, nine patients with idiopathic NSIP were treated with corticosteroids; two also received immunosuppressive agents (azathioprine [AZA] or cyclophosphamide [CP]) because of incomplete recovery with cortiosteroids (63). Chest CT scans normalized in four patients and improved in another three; in two patients, consolidation evolved into bronchiectasis. Changes in PFTs were less impressive. Forced vital capacity (FVC) improved in only three of eight patients and did not change in the remaining five. One patient later deteriorated after initial improvement. One patient died with severe pulmonary fibrosis after a 9-year follow-up (63). In another retrospective study, 24 patients with NSIP were treated with corticosteroids; 3 received corticosteroids plus cyclophosphamide (43). A ''good response'' was cited in 20 patients

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Table 7 Survival and Response to Treatment in Clinical Series of NSIP

Series	N	Survival	Treatment	Follow-up time	Follow-up response
Katzenstein and Fiorelli (1)	Group 1–31	NA	NA	61 mo	Alive & well (13/22)
				60 mo	Alive with disease (9/22)
					Dead (0/22)
	Group 2–24			40 mo	Alive & well (7/20)
				7 mo	Alive with disease (7/20)
				18 mo	Dead of disease (3/20)
				3 mo	Dead of other (3/20)
	Group 3–9			8 mo	Alive & well (1/6)
				36 mo	Alive with disease (2/6)
				15 mo	Dead of disease (2/6)
				17 mo	Dead of other (1/6)
Nagai et al. (4)	Cellular NSIP–16	NA	None (8/16)	NA	Improved (10/16)
			CS (6/16)		Remission (4/16)
			CS + IS (2/16)		No change (2/16)
					Worse (0/16)
					Dead (0/16)
	Fibrotic NSIP–15		None (4/15)		Improved (8/15)
			CS (5/15)		Remission (1/15)
			CS + IS (6/15)		No change (1/156)
					Worse (3/156)
					Dead (2/15)
Cottin et al. (32)	12	NA	CS (5/12)	50 mo	Improved (10/12)
			CS + IS (7/12)		Worse (2/12)
				Dead (0/12)	

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Bjoraker et al. (3)	14	Median > 13 yrs	NA	NA	NA
Douglas et al. (36)	70	Median > 7 yrs	CS (67/70)	NA	NA
Daniil et al. (7)	15	Median > 7 yrs	None (2/14) CS (1/14) CS + IS (11/14)	NA	Improved (2/12) Stable (4/12) Worse (3/12) Dead (1/12)
Nicholson et al. (8)	28	Median 52 mo	CS (12/28) CS + IS (9/28)	NA	NA
Bouros et al. (44)	62	Median > 10 yrs 5 yrs—91%	NA	NA	Dead (16/62)
Fujita et al. (43)	24	NA	CS (21/24) CS + IS (3/24)	NA Dead of other (2/24)	Improved (17/24) Worse (1/24) Dead of disease (4/24)
Travis et al. (5)	Cellular NSIP—7 Fibrotic NSIP—22	10 yrs—100% 10 yrs—35%	NA	NA	NA
Flaherty et al. (6)	33	Median > 9 yrs	None (2/33) CS (18/33) CS + IS (4/33) Other (9/33)	NA	Improved (4/10)[a] Stable (4/10) Worse (1/10)
Riha et al. (9)	7	Median 178 mo Other (1/7)	None (2/7) CS (2/7) CS + IS (2/7)	NA	NA

CS, corticosteroids; IS, immunosuppressive therapy.
[a]After 3-month trial of high-dose steroids in subset of NSIP patients.

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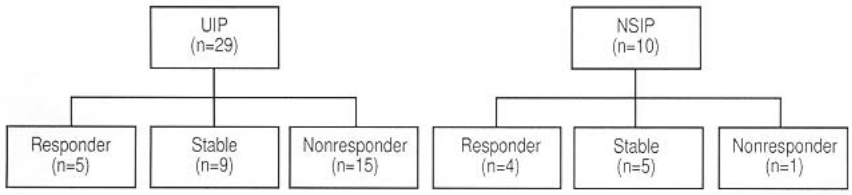

Figure 8 Results of high-dose steroid therapy in 29 patients with usual interstitial pneumonia (UIP) and 10 with nonspecific interstitial pneumonia (NSIP). Response to therapy was assessed by change in clinical, radiological, and physiological (CRP) score after 3 months of therapy. Responders were defined as having a >10-point drop in CRP score, stable a ± 10 change in CRP score, and nonresponders a >10 point rise in CRP score. A difference in the distribution of response was seen between UIP and NSIP patients ($p = .05$, Fisher exact test). (From Ref. 6.)

(83%), but details or PFTs were not reported. Four patients (17%) died of respiratory failure (within 1–91 months); two patients died of other causes. Japanese investigators performed serial HRCT scans in 15 patients with NSIP (7 of whom had CVD) (61). Fourteen patients were treated with cyclophosphamide plus low-dose prednisolone. Follow-up CT scans improved in 12 of 14 patients (86%), but pulmonary functional data were not provided.

In summary, these diverse retrospective studies (1,3–8,32,64) affirm that NSIP has a better prognosis and higher rate of response to therapy than UIP, but optimal therapy remains uncertain. Optimal treatment regimen(s) needs to be elucidated in randomized, controlled trials Further, none of these studies resolves the question of late sequelae. Additional prospective studies are required to assess long-term prognosis.

X. Biology of Disease

Considerable controversy exists regarding the nature and natural history of NSIP. Is NSIP a distinct disease entity with different pathogenetic mechanisms from UIP? Is it possibly an earlier stage of the same disease, or is it a response to limited injury in the lung? Although data are limited, recent studies have cited differences in biological characteristics between NSIP and UIP (43,45,60,70–72). These findings highlight potential differences in pathogenic mechanism between NSIP and UIP.

Korean investigators examined BAL and lung biopsies from seven patients with NSIP and 16 patients with UIP (73). Marked increases in BAL lymphocytes and interleukin-6 (IL-6) levels were noted in NSIP compared to UIP or normal controls; in NSIP, the number of BAL lymphocytes correlated

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with IL-6 concentrations. Interestingly, a subset of patients with UIP also had increased BAL lymphocytes and IL-6 levels. Immunohistochemical techniques localized IL-6 to bronchial epithelial cells and alveolar macrophages. Interleukin-6 may limit the fibroproliferative response, as IL-6 is known to downregulate inflammatory responses by preventing release of proinflammatory cytokines (e.g., IL-1, tumor necrosis factor-α [TNF-α]) from alveolar macrophages.

Suga et al. contrasted the activity of metalloproteinases (MMPs) in the lung parenchyma of 11 patients with NSIP and 26 patients with UIP (71). MMP-9 levels were elevated in UIP, whereas MMP-2 was increased in NSIP. Furthermore, the increased MMP-2 activity correlated with the increase in BAL lymphocytes. Immunohistochemical stains suggested that MMP-2 was expressed predominantly by regenerated cuboidal epithelial cells in NSIP. These patterns were distinctly different from the expression of MMP-9 and associated neutrophil-predominant BAL findings in UIP. In a preliminary report, Takehara et al. examined serum and BAL intercellular adhesion molecule-1 (ICAM-1) in 4 patients with NSIP, 4 with BOOP, 4 with UIP, and 18 healthy controls (72). ICAM-1 levels were increased in IIPs compared to healthy controls. The absolute level of BAL ICAM-1 was higher in patients with NSIP (83.0 ± 48.9 ng/mL) than in UIP (53.1 ± 19.1 ng/mL) or BOOP (25.4 ± 15.7 ng/mL); the small sample size limited statistical analysis. Interestingly, BAL ICAM-1 levels were higher in patients with long-standing IIP. Immunohistochemical analyses suggested expression of ICAM-1 in type II alveolar epithelial cells in IIP patients compared with control lung tissue. The investigators suggested that increased ICAM-1 expression may play an important role in fibrogenesis in IIPs . The role of epithelial cell injury in the pathogenesis of IIP has been supported by the work of Fujita and colleagues, who confirmed elevated serum levels of cytokeratin 19 in patients with NSIP complicating polymyositis/dermatomyositis (43).

NSIP patients may exhibit differences in fibroblast phenotype compared to UIP. Japanese investigators demonstrated increased contractility of fibroblasts isolated from surgical biopsies from patients with UIP compared to NSIP or surgically resected lungs from controls (70). This increased contractility associated with UIP fibroblasts was associated with enhanced F-actin content in fibroblasts. Conditioned media from UIP fibroblast cultures enhanced control fibroblast contractility, whereas media from NSIP or controls did not. Contractility correlated with fibronectin and transforming growth factor-β1 (TGF-β1) concentrations in UIP conditioned–media (70). The reason for these differences in fibroblast phenotype between UIP and NSIP are not known, but may in part reflect difference in disease duration between groups (duration of symptoms 77 months with UIP and 4 months with NSIP) (70).

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XI. Conclusions

The natural history and long-term prognosis of NSIP and its relationship to UIP remain to be clarified. From a practical standpoint, the histological diagnosis of NSIP suggests a more favorable prognosis than UIP and supports a more aggressive approach to therapy with corticosteroids or immunosuppressive agents. It is plausible, albeit unproven, that early institution of treatment favorably alters the natural history of NSIP, and may avert late sequelae. Does NSIP evolve to UIP? Observations supporting an evolution from NSIP to UIP are: NSIP occurs at an earlier age than UIP (30); both NSIP and UIP may be present concomitantly in individual patients (30); clinical, radiographic, and physiological features of NSIP overlap extensively with UIP (30); and foci of NSIP may be found in explanted lungs from patients with UIP undergoing lung transplantation (59). However, recent data (70–73) (discussed above) raise the possibility that idiopathic NSIP is a distinct clinical-pathological entity with unique biological features. Long-term prospective studies using standardized diagnostic and therapeutic criteria are required to define better the prognosis and optimal therapeutic options in this evolving field.

Acknowledgment

Supported in part by National Institutes of Health Grant 1P50HL46487.

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6

Role of Physiological Assessment in Usual Interstitial Pneumonia

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I. Introduction

Usual interstitial pneumonia (UIP), the distinct histopathological lesion observed in idiopathic pulmonary fibrosis (IPF) (1), exhibits stereotypic physiological aberrations which include reduced lung volumes; normal or increased expiratory flow rates; increased forced expiratory volume in 1 s/forced vital capacity (FEV_1/FVC) ratio; reduced diffusing capacity for carbon monoxide (D_{LCO}) (2–5); hypoxemia or a widened alveolar-arterial P_{O_2} gradient [$P(A\text{-}a_{O_2})$] which is accentuated by exercise (2–4); reduced lung compliance (6) downward and rightward shift of the static expiratory pressure-volume curve (7); and abnormalities on cardiopulmonary exercise tests (CPETs) (3,7–9) (discussed below). Impairment in gas exchange (D_{LCO}) and oxygenation may be evident early in the course of the disease even when spirometry and lung volumes are normal (5). A restrictive defect is characteristic of IPF/UIP, but lung volumes may be normal if emphysema coexists (2,11,74). In this context, D_{LCO} and oxygenation are disproportionately reduced (2,10,11). Cardiopulmonary exercise testing (CPET) demonstrates hypoxemia, widened $A\text{-}a_{O_2}$ gradient, reduced oxygen consumption (V_{O_2}), high respiratory frequency, low tidal volume (V_T) breathing pattern, increased dead space (V_D/V_T), increased minute ventilation for the level of oxygen consumption, and a low O_2 pulse (Table 1) (3,7,9).

In this chapter, we will first discuss the pathophysiological basis for the physiological aberrations observed in IPF/UIP. Many of the seminal physiological studies in the 1970s and 1980s included a variety of interstitial lung diseases (ILDs) in addition to UIP/IPF (12–16). In addition, most

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Table 1 Typical CPET Responses in IPF

Symptoms: Dyspnea, leg discomfort, generalized fatigue
↓ Aerobic capacity (V_{O_2}) peak, ↓ work
↓ Anaerobic threshold (AT)
Abnormal Pulmonary Gas Exchange
↓ Pao ↑ $P_{AO_2}-P_{aO_2}$, ↓ Sa_{O_2}
↑/↔V_D/V_T responses
↔ Pa_{CO_2} and ↔ P_{ETCO_2}
Abnormal Cardiovascular Responses
↓ Peak heart rate, ↑ HR at submaximal V_{O_2} (abnormal HR-V_{O_2}) relationship, $\Delta HR/\Delta O_2 > 50$
↓ O_2 pulse
ECG abnormalities
Abnormal Ventilatory Responses
↓ $V_E$peak, ↑ submaximal V_E (abnormal V_E vs V_{O_2} and V_E vs V_{CO_2} relationships)
Normal or ↑ V_E/MVV
↑ V_E/V_{CO_2} and ↑ V_E/V_{O_2} responses
↓ V_T, ↑ fb (or blunted V_T response)
↓ IC, ↑V_T/IC, ↔ EELV, ↑ EILV/TLC

↑, increase; ↓, decrease ↔, remain the same.
Source: Ref. 86.

published series of IPF or cryptogenic fibrosing alveolitis (CFA) failed to classify patients according to histological entities (e.g., UIP, desquamative interstitial pneumonia [DIP], nonspecific interstitial pneumonia [NSIP] (17–21). Historical publications detailing physiological aberrations in IPF or CFA undoubtedly were ''contaminated'' with other histological entities (e.g., NSIP and other idiopathic interstitial pneumonias [17–21]). Recent retrospective reviews of surgical lung biopsies (SLBx) previously labeled as IPF or CFA identified UIP in only 47–71% of cases; NSIP was the next most common, comprising 13–36% of cases (22–27). Current consensus statements reserve the term IPF to refer to a specific clinical entity associated with the histopathological pattern of UIP (4,28,29). An epidemiological study in a New Mexico county found that pulmonary fibrosis and IPF together accounted for 46.2% of ILDs in males and 44.2% in females (30). Physiological derangements observed in IPF are similar to those observed in myriad other ILDs (31,32). Irrespective of etiology, progressive lung destruction leads to increasing ventilatory, circulatory, and skeletal muscle impairment, reduced structural and functional reserves, impaired exercise intolerance, and exertional dyspnea. The mechanisms for these various physiological aberrations are discussed below.

II. Pathophysiology

The majority of interstitial lung diseases (ILDs) share common physiological abnormalities (31,32). A restrictive ventilatory defect is reflected by a downward and rightward shift of the static expiratory pressure-volume curve (6,12,31). Total lung capacity (TLC) and vital capacity (VC) are reduced; the lung recoil is increased over the range of the inspiratory capacity (33–35). The coefficient of retraction (pleural pressure at TLC/lung volume at TLC) is elevated compared to normal subjects (6,33). The mechanisms for these changes in compliance are multifactorial and include loss of lung volume (35–38), reduced alveolar distensibility (36,39–43), reduced alveolar size (39,44), and increased surface tension due to abnormalities of surfactant (45,46).

A. Flows and Lung Volumes

Static lung volumes are typically reduced in IPF (3,12,31). In ILDs, the VC is reduced to a greater extent than the functional residual capacity (FRC) (31). A review of several published series of ILDs reported a mean reduction in FRC to 79% predicted compared with a reduction in VC to 63% and TLC to 72% predicted (47). In fibrotic lung disorders, V_T and inspiratory capacity (IC) are reduced and the ratio of V_T to IC ($V_{T/IC}$) is increased compared to normal subjects (31). The TLC, determined by the balance between lung and chest wall recoil and inspiratory muscle strength, is usually less severely affected owing to the normal, or near normal, chest wall recoil and preserved inspiratory muscle function (47,48). The residual volume (RV) is preserved in most patients with pulmonary fibrosis and the RV/TLC ratio is frequently increased (19,31,47,49–52). Airway function, defined by spirometric measurements, is usually normal in ILD, but airflow obstruction is common in sarcoidosis (54–67), rheumatoid arthritis (68–70), and lymphangioleiomyomatosis (71). Conflicting reports have been published regarding small airway function using more sophisticated testing (31,72,73). These abnormalities are not specific, and the magnitude of the changes varies widely from patient to patient.

Some patients with IPF and concomitant emphysema exhibit atypical physiological features with preserved lung volumes (11,74,75). In a series of patients with IPF, Cherniack and colleagues found that FVC and TLC were higher in smokers compared to never smokers (74). Similarly, British investigators reported 48 patients with CFA; VC was normal ($> 80\%$ predicted) in 21 patients (44%) (11). Patients with preserved VC were more likely to be male (76 vs 48%), to be current smokers (57 vs 22%), and to have a heavier lifetime history of cigarette smoking (38 vs 25 pack years). The TLC was significantly higher in the cohort with preserved VC (90.1 vs 64.7% predicted). Prognosis or treatment did not differ between the two groups, and high-resolution computed tomography (HRCT) scans confirmed a similar

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degree of pulmonary fibrosis in the two groups. However, concomitant emphysema was much more likely in the group with a preserved VC (86 vs 19%). In a retrospective study of 238 patients with IPF, lung volumes (FVC and TLC) were significantly lower among never smokers than former or current smokers (75). In never smokers, FVC and FEV_1 were significantly lower and the FEV_1/IFVC ratio was significantly higher than in former smokers. Among current smokers, the FVC and FEV_1 were significant higher and FEV_1/FVC ratio were significantly lower when compared with never and former smokers (75). In addition, the median coefficient of retraction was significantly higher in never and former smokers than in current smokers. Among current smokers, the median coefficient of retraction was in the normal range (i.e., 3–8 cm H_2O/L). In addition, the D_{LCO}/V_A was lower in current smokers compared to never and former smokers and was lower in former smokers compared to never smokers. These investigators had previously shown that smokers with IPF exhibited a shift upward and to the left of the volume-pressure curve compared to nonsmokers with IPF (76). Thus, superimposed smoking can alter the typical physiological presentation of IPF.

B. Gas Exchange

D_{Lco} is typically reduced in ILD to a greater extent than the lung volume at which it is measured (31). In addition, the D_{LCO} is decreased to a greater extent with IPF than in other ILDs (5,8). Dunn et al. noted a D_{LCO} of 45% predicted in 21 patients with IPF compared with 79% predicted in 20 patients with sarcoidosis despite similar lung volumes (5). A similar difference was noted in D_{LCO}/alveolar volume (V_A) (54 vs 97% predicted).

C. Exercise Capacity

Resting pulmonary and cardiac function testing cannot reliably predict exercise performance and functional capacity (V_{O_2} peak) in individual patients with ILD (8,77,78). Even though exertional dyspnea is common in patients with ILD, exercise often is stopped because of other factors (e.g., leg discomfort, chest pain, or fatigue) (7,79,80). Furthermore, exercise limitation in most patients is likely multifactorial (81–85).

Mechanical Limitation

Multiple factors contribute to an increased ventilatory requirement during exercise in patients with ILD (86). Typical CPET responses in patients with ILD include reduced maximal or peak aerobic capacity (V_{O_2} peak), maximal work rate (WR peak), and submaximal exercise endurance compared to age- and sex-matched normal subjects (77,87–91). The reduction in aerobic capacity (V_{O_2}max) is related to resting pulmonary function including FEV_1

(percent predicted), TLC (percent predicted), and DLCO (percent predicted) (87,92,93).

The impaired ventilatory mechanics in IPF worsen the demand-capacity relationship of the respiratory system during exercise (94–96). During exercise, a marked increase in submaximal minute ventilation (abnormal V_E-V_{O_2} relationship) is noted in ILD. Mechanical ventilatory limitation where the ventilation achieved (VEmax) approaches or exceeds ventilatory capacity may occur, especially in IPF (9,79,97,98). The ventilatory reserve, an index of ventilatory demand (VEmax) to ventilatory capacity (MVV), may be reduced (high V_E/MVV) or remain normal. Through the use of exercise tidal flow-volume loop analysis, a visual index of ventilatory constraint is obtained by comparing flow-volume loops obtained during exercise with maximal resting flow-volume loops (extFVL/MFVL) (99,100). The volume and flow rate differences between the exercise tidal flow volume loop and MFVL curve may aid in identifying ventilatory limitation or constraint during exercise. A recent study of seven patients with ILD used this technique to demonsrated that some ILD patients were not breathing near their maximal ventilatory capacity and that respiratory mechanics did not appear to limit exercise (79). Importantly, patients who ceased exercise because of dyspnea ($n = 4$) exhibited significant expiratory flow limitation, increased end-expiratory lung volume (EELV), abnormal inspiratory flow loops, and less arterial desaturation compared to those who stopped on account of leg fatigue ($n = 3$) (Fig. 1) (79). In contrast, a recent study utilizing the negative expiratory pressure (NEP) technique failed to show evidence of expiratory flow limitation in patients with restrictive lung disease (2 of 19 patients with pulmonary fibrosis) (101).

Because of constraints on V_T expansion, patients with ILD generally exhibit a rapid shallow breathing pattern during exercise, whereas an increase in V_E is achieved mostly by increases in respiratory frequency with low V_T and minimal changes in EELV; this varies compared to the normal response (7,79,88,102) (Fig. 2; see Fig. 3). In patients with ILD, expiratory reserve volume is reduced and a blunted V_T increase occurs through encroachment on inspiratory reserve volume (IRV). As a result, V_T represents a greater percentage of the blunted IC increase (V_T/IC) from rest and compared to normal subjects (7,79) (Fig. 2). The peak V_T/VC is usually, but not always, normal (103,104). Interestingly, the rapid shallow breathing strategy during exercise in patients with IPF correlates with disease severity (105,106), may contribute to exertional dyspnea, may minimize work of breathing (88), and does not appear to be affected by administration of morphine (107) or supplemental O_2 (9,97). Recently, investigators monitored the breathing pattern in 10 patients with restrictive lung disease over 1 h and in 7 controls (108). On a separate day, dyspnea was monitored (Borg scale) while all subjects copied different resting V_T and respiratory frequencies. Small variations from average resting V_T caused marked increases in dyspnea in patients, and the

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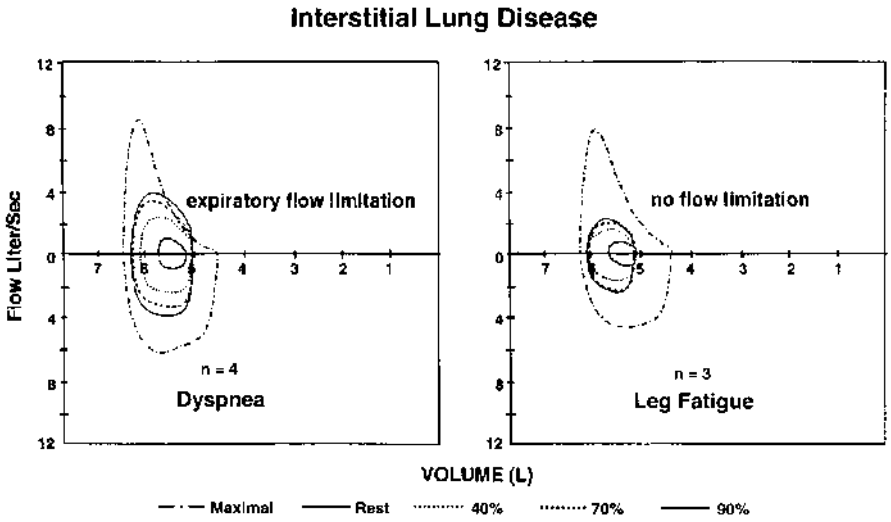

Figure 1 ILD: Maximal and extFVL in patients with ILD. Left: patients who stopped secondary to dyspnea. Right: patients who stopped due to leg fatigue. Minimal change was observed in EELV in either group, with the group complaining of dyspnea demonstrated significant expiratory flow limitation. (From Ref. 79.)

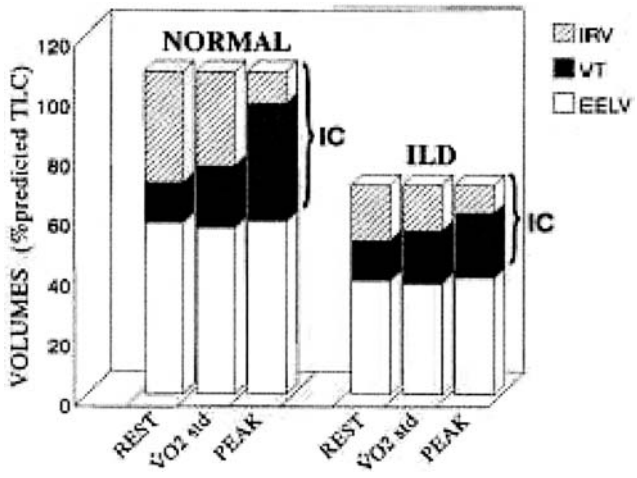

Figure 2 Operational lung volumes at rest, at a standardized V_{O_2} of 50% predicted maximum (V_{O_2}max, and at peak exercise in normal subjects and in patients with ILD. Values are means ± SE. TLC, total lung capacity; IRV, inspiratory reserve volume; EELV, end-expiratory lung volume. Inspiratory capacity (IC) is significantly reduced in patients with ILD ($P < .01$). (From Ref. 7.)

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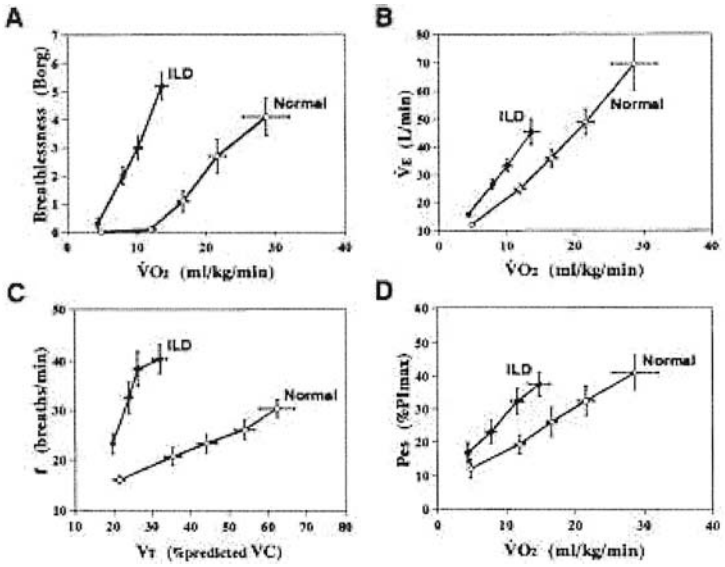

Figure 3 Responses to exercise in normal subjects and patients with ILD. *A*, dyspnea, *B*, minute ventilation (V_E). *C*, breathing pattern (breathing frequency [f]), *D*, esophageal pressure (Pes). Values are means 6 SE. Borg, Borg scale; uptake; V_T, tidal volume; PImax, maximal inspiratory pressure. Pes measurements were only obtained in 8 of 12 patients with ILD. All slopes are significantly greater ($P = .05$) in patients with ILD than in normal subjects. (From Ref. 7.)

relationship was parabolic ($r^2 = 0.97$; $P < .001$); demonstrating that patients with restrictive lung disease adapt a monotonous, tightly constrained breathing pattern, probably as a deliberate strategy to avoid dyspnea (108).

Gas Exchange Limitation

Patients with IPF usually exhibit arterial desaturation, reduced Pa_{O_2}, and abnormal increases in $P_{A}O_2$-Pa_{O_2} during exercise (8,77,87,109–111). Multiple mechanisms, including ventilation-perfusion (V/Q) mismatching, O_2 diffusion limitation, and low mixed venous P_{O_2} contribute to the abnormally increased P_{AO_2}-Pa_{O_2} (109,112,113). Exercise-induced hypoxemia may impair exercise performance by increasing ventilatory requirements with consequent abnormal ventilation and increased dyspnea and, potentially, by adversely affecting myocardial and circulatory function during exercise. Supplemental O_2 has been suggested to relieve myocardial ischemia and improve exercise performance in patients with exercise-induced hypoxemia (114). The impressive improvement in exercise performance with supplemental O_2 suggests that arterial hypoxemia and, in turn, reduced O_2 transport to exercising muscles are major contributing

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factors to exercise limitation in patients with ILD (9). Recent work suggests that hypoxemia is the predominant factor contributing to exercise limitation during incremental exercise (9,97). In one study, seven patients with ILD (four with IPF) and impaired room air exercise performance were treated with supplemental O_2 sufficient to prevent arterial desaturation; this resulted in improved exercise performance, which the investigators suggested most likely resulted from increasing O_2 delivery to exercising muscles (9). Despite an increase in mean VO_2 peak of approximately 20%, peak V_{O_2} remained only 67% of predicted. This suggests that other factors were involved and that exercise limitation was multifactorial. Subsequently supplemental O_2 (60)% and added dead space was studied in seven patients with ILD (four with IPF); the results confirmed that hypoxemia and not mechanics limited exercise in ILD (97).

V/Q mismatching resulting from pulmonary vascular involvement can present with high V/Q mismatch associated with capillary destruction resulting in increased V_D/V_T and inefficient ventilation ($\uparrow V_E/V_{CO_2}$); low V/Q mismatch resulting from rapid transit times through unaffected areas (''shuntlike effects'') can result in reduced Pa_{O_2} and widened P_{AO_2}-Pa_{O_2}. Although high V/Q abnormalities occur more frequently, most patients with ILD (65%) possess both high and low V/Q abnormalities (93). Hypoxemia in patients with ILD may also be a consequence of right-to-left intracardiac shunting through a patent foramen ovate (115).

The ventilatory equivalent for V_{CO_2} (V_E/V_{CO_2}) serves as a good noninvasive estimate of inefficient ventilation; inefficient ventilation is due primarily to increased V_D/V_T and excessive ventilation due to hypoxemia and mechanoreceptor stimulation (86). V_D/V_T usually remains the same or increases as a reflection of severe V/Q mismatching with exercise (87,95,109,115 –117). The Pa_{CO_2} usually remains unchanged from rest (87). Consequently, the acid-base status at peak exercise often reveals a mixed disturbance: metabolic acidosis without the appropriate respiratory compensation or metabolic acidosis and metabolic alkalosis.

Cardiovascular Limitation

Cardiovascular abnormalities during exercise are common in IPF patients and reflect predominantly pulmonary vascular and RV dysfunction but may also include left ventricular (LV) dysfunction. Pulmonary hypertension is common in advanced ILD (118,119), and may be present both at rest and during exercise. The elevation of pulmonary artery pressures with exercise can be impressive despite supplemental O_2 (120). Because of ventricular interdependence, patients with severe pulmonary disease and pulmonary hypertension manifest reduced RV ejection fraction and abnormal leftward bowing of the interventricular septum resulting in reduced LV ejection fraction; this may improve with transplantation (121,122).

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In patients with ILD, the heart rate (HR)–V_{O_2} relationship frequently reveals higher HR values at rest and at submaximal levels of V_{O_2} compared to normal subjects (88). In patients with more advanced disease, a hypercirculatory HR response pattern ($\Delta HR/\Delta V_{O_2} > 50$) is often noted (86). A low peak HR response is usually observed, especially in more severe disease as exercise terminates prematurely. The abnormal HR response is often accompanied by a reduced stroke volume (105,123,124), electrocardiographic abnormalities (125), and evidence of biventricular dysfunction (123,126). Cardiovascular dysfunction may result from both hypoxemia and/or the increased intrapleural pressures that are often seen as a result of the abnormal respiratory mechanics (94,96). Both preload (reduced LV filling) and increased LV afterload effects may result (127,128). A reduced O_2 pulse may reflect several factors including reduced stroke volume, hypoxemia, and deconditioning. A retrospective analysis of 42 patients with ILD (9 with idiopathic disease) suggests that exercise limitation may be primarily due to pulmonary circulatory pathophysiology rather than ventilatory mechanics (93). Reduced peak o_2 values were more often due to pulmonary vascular disease than impaired ventilatory mechanics measured during incremental exercise. The degree of circulatory dysfunction was proportional to the severity of underlying lung disease.

Symptom Perception

Abnormal symptom perception (especially breathlessness and leg/general fatigue) is an important cause of exercise cessation in patients with IPF. This may be associated with apparent, nonphysiological limitation to exercise ($V_E/MMV < 80\%$, peak $HR < 85\%$ or 15 beats). Deconditioning, peripheral muscle dysfunction, and nutritional status (129–131) are increasingly recognized to contribute to exercise limitation in patients with chronic lung disease including patients with IPF. Dyspnea is a prominent symptom contributing to premature exercise termination in many patients with ILD; leg fatigue and generalized fatigue are reported in others (81,132). The recent work of O'Donnell colleagues has expanded an understanding of the relationship between exertional dyspnea, mechanical derangement, and reduced exercise capacity (7). They compared dyspnea ratings with physiological variables during incremental and constant work exercise in 12 patients with ILD (IPF in 3 patients) and 12 age-matched normal subjects. The Borg-oxygen uptake (V_{O_2}) slope revealed significant differences between groups with an earlier onset and greater exertional dyspnea noted at submaximal values of V_{O_2} in patients with ILD (Fig. 3a). In addition, patients with ILD exhibit a rapid shallow breathing pattern (Fig. 3c) and a greater inspiratory effort during exercise compared to normal subjects. This was evidenced by an elevated slope of the relationship between esophageal pressure/maximal inspiratory pressure [(Pes)/PImax] and V_{O_2}. Both qualitative and quantitative differences in

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exertional dyspnea were observed between groups (7). The distinct qualitative differences between groups at symptom-limited peak exercise, when dyspnea intensity and inspiratory effort were similar, were attributed to differences in ventilatory demand and ventilatory mechanics. Importantly, different physiological factors appeared to contribute to exertional dyspnea intensity in each group. In patients with ILD, the best correlate of the Borg V_{O_2} slope was the resting V_T/IC ($r = 0.58$, $P < 0.05$); in normal subjects it was the slope of Pes/P_{Imax}-V_{O_2} ($r = 0.60$, $P < 0.05$) suggesting exertional dyspnea in ILD is more likely related to mechanical constraints on volume expansion rather than to indices of inspiratory effort.

III. Clinical Applications of Pulmonary Function Tests in IPF

Pulmonary function tests (PFTs) have important clinical applications in patients with IPF (133,134) including (1) aiding in diagnosis, (2) establishing disease severity, (3) defining prognosis, and (4) monitoring response to therapy and disease progression.

A. Differential Diagnosis

The physiological presentation of ILD is not specific. PFT data should be used in conjunction with clinical, radiographic, and histological information. In patients with appropriate symptoms, PFTs can identify early disease. In a report of 44 patients with dyspnea, normal chest radiographs, and biopsy-proven ILD, D_{LCO} was decreased in 73%; reductions in VC or TLC were noted in 57 and 16% of patients, respectively (135). Interestingly, PFTs may be abnormal even in patients with normal HRCT scan. We noted abnormal PFTs (mean FVC 72% and D_{LCO}/V_A 72% predicted) in three patients with normal HRCT scans and open lung biopsies demonstrating ILD (136). Others have shown that resting PFTs may be normal in the early phase of IPF (12,51). Risk and colleagues identified two patients with biopsy-proven IPF who had normal pulmonary mechanics and a $D_{LCO} > 70\%$ predicted; however, rest and exercise P_{AO_2}-Pa_{O_2} were abnormal (111).

Several investigators have attempted to differentiate ILDs based upon patterns of physiological aberrations (31). For example, RV is often elevated in asbestosis (137), silicosis (138), and hypersensitivity pneumonia (139), but is normal or reduced in IPF (31). In contrast, D_{LCO} is disproportionately reduced in IPF compared to sarcoidosis or asbestosis, even at comparable lung volumes (5,140). Unfortunately, there is considerable overlap in these findings which limits the practical clinical value of these differences. The physiological patterns are similar between UIP and NSIP (Table 2). In both UIP and NSIP, lung volumes and D_{LCO} are reduced (22–25,141). Investigators from the Mayo

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Clinic found no differences between spirometry or DLCO in patients with UIP or NSIP, but the TLC was lower in UIP (141); we confirmed similar findings (142). Several groups documented slightly greater impairment in DLCO in UIP compared to NSIP (23,25,143), whereas others noted no significant differences in TLC or DLCO between NSIP and UIP (24).

Differences in the CPET response may be noted between different specific ILD types (8,105). Patients with IPF experience a greater degree of arterial desaturation and abnormal pulmonary gas exchange during exercise than patients with sarcoidosis (5) and patients with fibrosing alveolitis complicating scleroderma (FA-SSc) (144,145). A similar degree of desaturation during 6-min walk testing in patients with UIP (92% rest vs 85% exercise) and NSIP (93 vs 83%) has been reported (25,141). Patients with IPF (124,146) may be more likely circulatory limited during exercise than patients with scleroderma (SSc) (147) and FA-SSc (144,145).

B. Diagnosing IPF

As has been discussed, IPF patients characteristically exhibit reduced lung volumes, normal or increased expiratory flow rates, increased FEV_1/FVC ratio; reduced DLCO; hypoxemia or a widened $P(A\text{-}aO_2)$ accentuated by exercise (2–4). In mild cases of UIP or in smokers, lung volumes may be preserved. In this context, exercise-induced hypoxemia or impairment gas exchange (DLCO) may be evident (3,5). As was previously discussed, lung volumes may be normal in IPF if emphysema coexists (2,11,74). As noted earlier, CPET reveals myriad abnormalities (see Table 1) (3, 7, 9). CPETs are invaluable in research

Table 2 Physiological Pattern of Usual Interstitial Pneumonitis and Nonspecific Interstitial Pneumonia

Reference		Number of patients	FVC patients	TLC (% pred)	DLCO	Pa_{O_2}
(23)	NSIP	31	74	–	56	70
	UIP	64	70	–	44	76
(24)	NSIP	15	73	72	44	74
	UIP	15	74	66	44	69
(141)	NSIP	14	80	76	50	–
	UIP	63	79	68	48	–
(25)	NSIP	28	71	–	39	10.6 P
	UIP	37	72	–	44	10.8 P
(142)	Fibrotic NSIP	28	73	78	51	–
	Cellular NSIP	5	72	78	69	–
	UIP	106	67	73	51	–

Source: Ref. 32.

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studies, but the practical value of formal CPET is debated. A six-min walk test, with oximetry, is a noninvasive, relatively inexpensive method to assess the severity of functional impairment, need for supplemental oxygen, and to monitor the course of the disease in patients with IPF (148). One group has confirmed a fall in saturation of $<2\%$ in 16 of 40 patients with varying interstitial lung diseases (19 IPF patients); 8 patients exhibited a drop of 2–5%, whereas 16 experienced desaturation $>5\%$ (149).

C. Do PFTs Assess Disease Severity in IPF?

IPF is a heterogeneous disorder with varying degrees of inflammation, fibrosis, destruction, and distortion of the lung parenchyma (20,52). Several investigators examined the potential for physiological measurements to discriminate fibrosis from inflammation (14,74,150–152). In a seminal study, Gaensler et al. noted a "fair correlation" between histological severity of IPF on surgical lung biopsies (SLBx) and physiological indices (15). Crystal and colleagues noted a "good" correlation between fibrosis and the coefficient of retraction and the change in exercise Pa_{O_2} and $P_{A}{}_{O_2}$-Pa_{O_2} but a "poor" correlation with spirometry, lung volumes, D_{LCO}, or resting gas exchange in 18 patients with IPF (12). Fulmer and colleagues described semiquantitative histological analysis of SLBx in 23 patients with IPF (6). Spirometry, lung volumes, D_{LCO}, static volume-pressure relationships and steady-state exercise gas exchange were measured. Most parameters of lung distensibility correlated with the extent of fibrosis but not with cellularity. Spirometry, lung volumes, and D_{LCO} correlated poorly with histological abnormality, but exercise gas exchange [Pa_{O_2} and $P_{A}{}_{O_2}$-Pa_{O_2}], corrected for achieved V_{O_2}, correlated best with histological fibrosis and, to a lesser extent, cellularity (6). In another study of 14 untreated patients with IPF and 7 with pneumoconioses, gas transfer and lung volumes correlated with the extent of fibrosis and cellular infiltration; however, physiological parameters could not discriminate alveolitis from fibrosis (153). Cherniack et al. used a complex semiquantitative histological scoring system in 96 patients with IPF (74). No significant correlations were found between histological fibrosis and any physiological parameter. However, the D_{LCO} correlated with "desquamation" of cells within the alveolar space. Significant differences were noted between morphological-physiological correlations in never smokers and ever smokers. Exercise gas exchange correlated with histological fibrosis only in never smokers.

Exercise $P_{A}{}_{O_2}$-Pa_{O_2} correlated best with histological index in patients with pneumoconiosis and or interstitial pneumonia (15) and correlated with cellularity and fibrosis in patients with IPF when resting and peak exercise Pa_{O_2} did not (6). The increase in $P_{A}{}_{O_2}$-Pa_{O_2} with exercise in IPF correlated with D_{LCO} and D_{LCO}/V_A (percent predicted) (116). In another study of 15 patients with IPF, D_{LCO}/V_A (percent predicted) correlated with extent of V/Q

mismatching, extent of O_2 diffusion limitation, and exercise P_{AO_2}-Pa_{O_2} (109). In that study, patients with higher vascular tone (as determined by supplemental o_2 breathing release from hypoxic vasoconstriction) demonstrated better overall V/Q matching, as was previously reported in patients with primary pulmonary hypertension (154). The investigators speculated that patients with early IPF and less anatomical derangement of the pulmonary vasculature would be more capable of adjusting V/Q matching to maintain normal/near normal Pa_{O_2}, whereas in more advanced disease, the pulmonary circulation progressively loses this ability. Furthermore, the impact of a worsening V/Q during exercise would be amplified because of the greater fall in mixed venous Pa_{O_2}, shorter transit times, and shorter residence time for O_2 equilibrium (109).

Exercise arterial desaturation correlates with resting DLCO measurements in patients with ILD (155). Although the prediction of exercise desaturation from resting DLCO measurements in patients with ILD may not be consistently reliable (111,156,157), patients with DLCO less than 70% are more likely to desaturate (111). When DLCO is less than 50% predicted, patients are more likely to develop pulmonary hypertension and an abnormally widened P_{AO_2}-Pa_{O_2} during exercise (19). Cor pulmonale usually occurs with DLCO < 30%.

Among pulmonary functional parameters, DLCO correlates better with extent of disease on HRCT scans than lung volumes or spirometry (144,158). British investigators analyzed HRCT scans in 68 patients with CFA; 14 had concomitant emphysema (144). Extent of fibrosing alveolitis and emphysema on CT were independent determinants of functional impairment. Among the 14 patients with emphysema, lung volumes (FVC and TLC) were preserved and DLCO and Pa_{O_2} were reduced. In patients without emphysema on CT, the extent of disease on CT correlated with percent predicted DLCO, $r = -0.68$), oxygenation desaturation with exercise ($r = 0.64$), and the physiological component of the CRP score ($r = -0.62$); spirometry or lung volumes were less helpful (144). Another study of 39 patients with IPF observed moderate correlations between global extent of lung involvement on HRCT with DLCO ($r = -0.40$, $P = .03$) and FVC ($r = -0.46$, $P = .003$) (158). The extent of ground-glass opacities (GGO) also correlated with FVC ($r = -0.58$, $P = .0001$) (158). Both the extent of GGOs and overall global extent of disease on CT correlated with Pa_{O_2} at peak exercise. Another study of 38 patients with pulmonary fibrosis associated with the Hermansky-Pudlak syndrome observed similar correlations between the extent of disease on HRCT and FVC ($r = -0.66$) and DLCO ($r = -0.66$) (159). Unfortunately, such global physiological correlations are of doubtful clinical value in individual patients.

Watters and colleagues developed a composite score incorporating clinical (dyspnea), radiographic (chest radiograph), and physiological parameters (i.e., the CRP score) as a means more objectively to monitor the course of IPF (152). An arbitrary scoring system was developed (maximal score of 100

points for the most severe disease) which incorporated seven variables including (1) grade of dyspnea (maximum 20 points): (2) chest radiographic features (maximum 10 points) (i.e., profusion of interstitial opacities, honeycomb change, and presence or absence of pulmonary hypertension); (3) FVC, FEV_1, and thoracic gas volume (Vtg) (maximum 25 points); (5) D_{LCO}/V_A (maximum 5 points); (6) resting P_{AO_2}-Pa_{O_2} gradient (maximum 10 points); (7) exercise Pa_{O_2} corrected for achieved V_{O_2} (maximum 30 points). This system weighted heavily measurements of gas exchange. In the original study, the composite CRP score correlated better with a semiquantitative histological pathology score on SLBx than any of the individual components. More recently, a modified CRP scoring system (total of 100 points) has been devised in 238 patients with biopsy-confirmed UIP (75). This modified CRP score incorporated additional clinical and radiological findings and gave less weight to physiological parameters. In this scoring system, FVC, FEV_1, Vtg, and resting gas exchange (D_{LCO} or Pa_{O_2}) were excluded; the only relevant physiological variables were percent predicted TLC (maximum 11.0 points) and Pa_{O_2} at maximal exercise (maximum 10.5 points). Nonphysiological variables included age (maximum 25.6 points), smoking history (maximum 13.6 points), clubbing (maximum 10.7 points), changes on chest radiograph (i.e., extent of profusion of interstitial opacities, and presence or absence of pulmonary hypertension) (maximum 28.6 points). In addition, since not all clinicians have access to exercise studies, an abbreviated CRP scoring system was developed, which excluded the Pa_{O_2} during maximal exercise. In 77 patients in whom semiquantitative analyses of pathology were performed, the three CRP scores were compared for their ability to predict histology. The original CRP score failed to correlate with any pathological feature. In contrast, the complete CRP score correlated with the extent of fibrosis ($P = .046$), cellularity ($P = .045$), the granulation/connective tissue ($P < .001$), and the total pathological derangement ($P = .003$). The abbreviated CRP score correlated with fibrosis ($P = .021$), the granulation/connective tissue ($P < .001$), and the total pathology score ($P < .001$).

D. Prognosis

The value of physiological studies in predicting prognosis or responsiveness to therapy is limited (31). Physiological parameters provide only a rough estimate of severity of disease, but cannot accurately discriminate inflammation from fibrosis (5,74,153). Severe derangements in physiological tests (e.g., PFTs, gas exchange, oxygenation) predict a worse prognosis and lower survival rates (20,158,160–165). Numerous studies cited higher mortality rates when FVC or D_{LCO} were severely impaired. However, the thresholds for predicting higher mortality vary. For FVC, values associated with higher mortality included $< 67\%$ predicted (160), $< 60\%$ predicted (51), and $> 10\%$ decrease in VC in

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1 year (163). Others studies cited worse survival when DLCO was severely impaired, but the cut-off points for DLCO included < 30% predicted (166), < 45% predicted (19,51), < 39% predicted (167), and > 20% decrease in 1 year (163). It is of interest that one group reported reductions in DLCO < 45% predicted to be associated with an increased incidence of pulmonary hypertension (168). Changes in TLC are less predictive prognosis or survival, but some studies cited higher mortality rates when TLC was < 78% predicted (10) or <80% predicted (169). In a study of 56 patients with IPF, parameters associated with worse survival included an initial FVC < 60% predicted, (2) DLCO < 40% predicted, (3) mean pulmonary artery pressure > 30 mm Hg, or (4) age at first symptoms over 40 years (51). A retrospective study of 244 patients with CFA noted that decreased FVC was an independent predictor of increased mortality (170). Another retrospective study of 99 patients with IPF treated with corticosteroids (with or without azathioprine) suggested that survival was worse in patients with a pretherapy TLC < 78% predicted or VC < 83% predicted (10). Interestingly, DLCO , Pao_2 at rest, PAO_2-Pao_2, and ΔPao_2 with exercise did not predict survival in that series (10). Increased FEV_1/FVC ratio has also been associated with diminished survival (171). Some investigators suggested that changes in oxygen saturation with exercise correlated with subsequent disease progression in IPF (2,116). Most recently, one group identified 115 patients with IPF referred for lung transplantation, and examined baseline clinical, physiological and radiological features that predicted 2-year survival (167). A DLCO < 39% predicted and increased fibrotic abnormality on HRCT were the best predictors of survival (167). In a separate study by these investigators, 106 nonsmoking patients with UIP were prospectively followed (167a). By univariate Cox regression analysis, the following parameters predicted survival: age, FEV_1, FVC, DLCO, Pao_2, O_2 saturation, HRCT fibrosis score, and clearance of inhaled technetium 99m–labeled diethylenetriamine penta-acetic acid (Tc 99m–DTPA) from the lungs ($t_{0.5}$) (167a). Multiple stepwise Cox regression analysis identified the fast component of $t_{0.5}$ ($P = .03$), percent predicted TLC ($P = .02$), percent predicted DLCO ($P = .003$), and age ($P = .003$) as independent predictors of survival. Inclusion of other pulmonary function or CT scores did not improve the model. A recent retrospective study of 43 patients with UIP found that mortality was independently linked to a high fibroblastic foci (FF) score on surgical lung biopsy ($P = .006$) and a low percent predicted DLCO ($P = .01$) (172). Multivariate analysis revealed that increasing FF scores were independently associated with greater declines in FVC and DLCO at both 6 and 12 months. King et al. prospectively studied 87 patients with UIP (173). An increased risk of death was associated with degree of dyspnea ($P = .003$); Vtg, percent predicted ($P = .0002$); TLC, percent predicted ($P = .0001$); FVC, percent predicted ($P = .003$); FEV_1, percent predicted ($P = .02$); DLCO, percent predicted ($P = .003$); coefficient of retraction (a measure of lung "stiffness")

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($P < .0001$); and exercise Pao_2, ($P = .006$). Resting Pao_2 or DLCO/VA did not correlate with mortality. Greater degrees of granulation tissue/connective tissue on SLBx and dyspnea at presentation were independent predictors of mortality (173). Another study by these investigators of 238 patients with UIP (75) found that exercise Pao_2 on CPET was predictive of survival, and accounted for as much as 10.5% of the maximal score in the final model. In contrast to previous reports, resting DLCO did not predict survival in that cohort.

In summary, prognosis is worse in patients with severe decreases in FVC, TLC, DLCO, or oxygenation, but the correlations are inexact and discrepancies exist. Recent studies suggest that HRCT may be superior to physiological parameters in ascertaining prognosis or responsiveness to therapy (162,174). A predominant ground-glass pattern on HRCT scan predicts a high rate of responsiveness to corticosteroid therapy, whereas "reticular" or "honeycomb" patterns predict a low rate of therapeutic response (144,158,162,175). Gay et al. prospectively examined 38 patients with biopsy-proven IPF (162). Using receiver operating curve (ROC) analysis, only semiquantitative estimates of HRCT and histological fibrosis predicted survival. Importantly, no physiological parameter was predictive or survival nor did they enhance the ability of HRCT to predict prognosis. A recent study confirmed the predictive value of semiquantitative HRCT estimates of fibrotic abnormality but noted that the DLCO percent predicted added predictive value to HRCT findings (167).

More recently, the modified CRP scoring system (total of 100 points) described earlier has been examined for prognostic value in 238 patients with biopsy-confirmed UIP (75). This modified CRP score incorporated additional clinical and radiological findings and gave less weight to physiological parameters. Both the complete modified CRP score and abbreviated CRP scoring systems (75) were superior to the original CRP score (152) in predicting survival. Notably, the complete CRP was superior to the abbreviated CRP score in this regard. Using CRP data to generate survival curves, 5-year survival could be predicted with surprising accuracy (75). For example, 5-year survival rates at CRP scores of 20, 40, 60, and 80 were 89, 53, 4, and <1%, respectively. Although the complete CRP was superior to the abbreviated CRP score in predicting survival, the abbreviated CRP system is easier and more adaptable to clinical practice, because it does not include CPET parameters. Additional studies using these or similar CRP scoring systems are worthy of further study.

E. Monitoring Response to Therapy and Disease Progression

Serial PFTs are invaluable in clinical practice to determine disease progression and/or response to therapy. Given the limited morphological-physiological

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correlations described earlier, and the need for simple, patient-friendly diagnostic studies, most clinicians rely on spirometry, lung volumes, DLCO, and measurement of arterial oxygenation. To understand the threshold of change in these parameters which may be clinically significant, one needs first to appreciate the variability of these physiological indices.

The American Thoracic Society has published detailed guidelines standardizing spirometric measurement (176). The variability in FVC is well defined among normal subjects and patients with pulmonary diseases (176). Recent guidelines provided standardization for DLCO (177). Despite these standards, significant variability remains. Kangalee and Abboud reported interlaboratory and intralaboratory variability in testing of a single, normal subject during a 13-year period (178). The coefficient of variation was lowest for spirometry and for intralaboratory testing. Variability was significant greater for TLC and DLCO, particularly in the interlaboratory measurement of DLCO. Care must be taken in interpreting serial physiological studies, particularly the DLCO (179). Most investigators define clinically significant changes in spirometry for patients with IPF as a change in FVC $\geq$10–15% (20,160,163,166,169,180–182) and $\geq$ 20% for DLCO (53,163,166,180,181). Data on the reproducibility of exercise testing in IPF patients are sparse. Marciniuk et al. studied six patients (three with IPF) during three maximal exercise studies over a 28-day period (183). The coefficient of variation was acceptable for maximal V_{O_2} (5.3%) and oxygen saturation (2.5%).

Changes in PFTs over time are prognostically useful in patients with IPF. In a seminal study, Stack et al. noted improved survival in IPF patients demonstrating an early improvement in FVC $>$ 10% with corticosteroid therapy (182). Augusti and colleagues performed serial PFTs in 19 patients with IPF (184); a decrease in FVC, TLC or DLCO by 1.5 years was noted in patients who continued to progress during the three years of follow-up. Van Oortegem et al. retrospectively reviewed 25 patients with IPF; improvement in FVC or DLCO after three months of therapy predicted stability or improvement during long-term follow-up (185). Hanson et al. described 58 IPF patients who survived at least 1 year from the initiation of therapy and had serial spirometry (163). Survival was better in patients with an improved or unchanged FVC at 1 year compared to patients exhibiting a $\geq$10% reduction in FVC. Survival was worse among patients experiencing $\geq$20% decline in DLCO after 1 year of therapy. The concordance between both of these studies was good; no patient with improved FVC had a fall in DLCO. Interestingly, measurement of resting or exercise oxygenation provided no incremental value. Xaubet et al. followed 23 patients with IPF for a mean of 7.5 months after initial assessment (158). The overall extent of abnormality in HRCT correlated with changes in DLCO, ($r = -0.57$) and FVC ($r = -0.51$).

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As has been mentioned, the CRP score represents a potentially more accurate method for assessing response to therapy in IPF. Response is defined as $\geq$ 10-point drop in CRP, stability, $<$ 10-point change, nonresponse (NR), and $\geq$ 10-point rise in CRP (152). We found that responders or stable patients (based on CRP score) after 3 months of corticosteroid therapy had an improved long-term survival compared to non responders (186). Direct comparisons between CRP scoring and simpler pulmonary function testing are required.

IV. Conclusions

Sequential physiological studies are critical to assess the severity of disease and monitor response to therapy. Optimal parameters to follow the course of IPF have not been validated. The American Thoracic Society (4) recommends the following physiological tests to monitor the clinical course of IPF: lung volumes, DLCO, resting arterial blood gases, and CPET with measurements of gas exchange. In that statement, improvement was defined as $\geq$ 10% increase in TLC or VC, $\geq$ 15% increase in DLCO and $\geq$ 4 mm Hg increase in O_2 saturation or $>$ 4 mm increase in Pao_2 during a formal CPET (4). Although these recommendations are reasonable, the cost effectiveness of formal CPET has not been proven. A simpler approach may be to utilize serial spirometry (i.e., FVC, FEVI), DLCO, and 6-min walk testing with oximetry to assess evolution of the disease. Although optimal frequency of testing has not been elucidated, we believe serial studies at 3 to 4-month intervals are usually adequate. More frequent studies may be appropriate in the setting of clinical deterioration or worsening symptoms.

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7

Role of High-Resolution Thin Section Computed Tomographic Scanning

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I. Introduction

Within the past decade, high-resolution computed tomographic (HRCT) scans, using 1 to 2 mm–thick slices and a reconstruction algorithm that maximizes spatial resolution, have been increasingly used to diagnose, stage, and monitor diverse interstitial lung diseases (ILDs) (1–3). HRCT is noninvasive and can be performed without contrast (4,5). HRCT is far superior to conventional chest radiographs in assessing parenchymal details and demarcating the extent and distribution of the disease (6–10). HRCT can assess the *nature and extent* of the pulmonary parenchymal abnormalities, narrow the differential diagnosis, and *in some cases, substantiate a diagnosis even without a surgical lung biopsy*. HRCT has been utilized not only to establish a diagnosis of idiopathic pulmonary fibrosis (IPF) (11, 12) but also to provide prognostic information in this disorder (6,7,13). Current recommendations from international consensus statements restrict the term *IPF* to patients with idiopathic usual interstitial pneumonia (UIP) (1,2). Historically, surgical lung biopsy (SLBx) has been considered the ''gold standard'' for the diagnosis of UIP (14). However, given the expense and potential morbidity associated with SLBx (15), many clinicians rely on

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HRCT and clinical features to corroborate the diagnosis of IPF/UIP (without histological confirmation) (16,17). In clinical practice, surgical lung biopsies were performed in fewer than 15% of patients with IPF in the United Kingdom (18,19) and in fewer than 30% of patients with suspected IPF in the United States (4,16,20). Although indications for SLBx remain controversial, several recent studies affirm that HRCT may obviate the need for surgical lung biopsy provided the CT findings are *classic* for UIP (11,21–27). In fact, an international consensus statement on IPF proposed that "bibasilar reticular abnormalities with minimal ground glass opacities on HRCT scans" was one of four major criteria for the diagnosis of UIP (2). Further, HRCT may be comparable or superior to surgical lung biopsy in assessing the prognosis and potential responsiveness to therapy (7,13,28).

In this chapter, we will address the following questions: What is the value of HRCT scans in the evaluation of ILD? What type of computed tomographic (CT) scan and algorithm should be used in the assessment of ILD? What are the salient CT features of IPF/UIP? How accurate is HRCT in diagnosing UIP and distinguishing this disorder from other ILDs? Are features on CT sufficiently specific to obviate the need for surgical lung biopsy? Among patients with IPF/UIP, does HTCT offer an advantage over other parameters to predict prognosis or potential responsiveness to therapy? Should serial CT scans be performed in patients with IPF/UIP?

II. HRCT Technique Aspects

Although a thorough explanation of the operations of a CT scanner is complex, a familiarity with the basic principles and terminology is necessary to understand the CT material that follows. Simply stated, the cross-sectional depiction of the body on CT eliminates the superimposition of structures that occurs with chest radiography. Furthermore, CT detects differences in radiographic density of only 0.5% compared to 10% difference required for conventional radiography. Conventional CT refers to an axial CT scanning mode in which an individual image or a cluster of a few individual images are obtained in a single breath hold. The patient is then allowed to breathe before another individual image or cluster of images is obtained. Owing to variation in the size of each breath, inevitably areas of the lung may be missed or scanned twice. Collimation refers to the slice thickness used or the thickness of the body that is scanned to generate an image. Most conventional CT scans use 5 to 10 mm slices. Images are obtained in contiguous fashion (e.g., from lung apices through lung bases); there are no intentional skip areas in conventional CT. Each CT image is a 512×512 matrix of numbers or pixels in two dimensions (x and y). Voxel refers to the added z dimension when a volume of data is

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discussed, as in helical or spiral CT. Each number is assigned a value of −1000 (air) to +1000 (metal, mineral) Hounsfield units (HU), with zero representing water. CT scanners undergo regular quality control and calibration with water phantoms and calibration to air to make sure that there is no drift in CT numbers. It is not possible to display the full range of CT numbers on a single image because of the limited number of shades of gray on an electronic display. CT images are therefore viewed on different window and level combinations, each designed for the depiction of specific structures. Thoracic CT images are usually viewed on at least two window and level setting combinations: one for evaluating the lung parenchyma and the other for evaluating soft tissues that make up the mediastinum and chest wall. *Level* and *window* each refer to the Hounsfield unit attenuation values. The level is best set midway between the attenuation value of the structure of interest, and that of the surrounding tissue, whereas the width is chosen to include the entire range of attenuation values around the level that are present within the scanned tissue. For example, when viewing the soft tissues, a window level of 20 HU and width of 450 HU may be used. The gray scale seen represents the pixel values that are 225 HU on either side of 20, or −205 to +240 HU. All pixels with a value of less than −205 HU will appear black and all pixels greater than 240 HU will appear white. When viewing the lungs, a level of −700 HU and width of 1000 HU may be used; all pixels greater than −200 HU will appear white.

HRCT is a powerful technique for evaluating diffuse ILD and bronchiectasis. Several studies have shown that HRCT more accurately and confidently yields a correct diagnosis when compared to both chest radiography and conventional CT (29). HRCT is particularly useful in the clinical setting of suspected ILD or bronchiectasis when the chest radiograph is normal or equivocally abnormal. The abnormal CT in these patients may be sufficient evidence to proceed with lung biopsy when the chest radiograph was not convincing. In some patients, the HRCT features are *disease specific*, making tissue confirmation unnecessary.

Full details of the HRCT technique are described in Table 1. HRCT refers to scanning in an axial mode with the thinnest possible slice thickness available, usually 1.0–1.5 mm combined with the use of a high spatial frequency reconstruction an algorithm that enhances the ability to see fine lines and interfaces. This is in contrast to conventional chest CT that uses a reconstruction algorithm that smoothes interfaces, reducing image noise and creating a more pleasing image. The thin collimation further reduces partial volume averaging from adjacent structures, and makes subtle ground-glass opacity and thickened septa more conspicuous. The downside is an increase in visible image noise. This is usually not a problem, and in larger patients, can be countered by increasing the kilovolt peak (kVp) and milliamperes (mAs) used for scanning. The so-called "low-dose" HRCT (120 kVp, 20 mA, 2 s scan time)

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Table 1 HRCT Technique

Thin-collimation: 1.0–1.5 mm
High-spatial frequency reconstruction algorithm (''bone algo'')
Imaging spacing: varies from every 1 cm throughout lungs to 3–6 evenly spaced images or cluster of images at aortic arch/carina/just above diaphragm
Typical scan parameters: 140 kVp, 170 mA, 1–2 sec scan time
Patient position: supine; prone images to avoid confusion with dependent atelectasis
Lung inflation: inspiration; expiratory images for evaluation of air trapping and small airway disease
May photograph 6 or 9 images on one 14×17 in. sheet of film (fewer than standard CT)
Optional retrospective targeted image reconstruction to reduces image pixel size

may be used in the follow-up of patients with known disease, but typically has poorer image quality than the standard HRCT technique, particularly in obese patients (30).

HRCT is inherently a sampling examination of the lungs in which thin sections are taken at staggered intervals, revealing both the pattern and distribution of abnormality, so that a differential diagnosis or sometimes a single diagnosis can be rendered (31). There is a tendency to think that the greater the sampling (i.e., the more images), the more information provided; however, there is little uniformity in the number of images obtained across centers. Sampling ranges from one to two images at set levels (aortic arch, carina, 1 cm above the diaphragm) to six to eight images evenly spaced throughout the lungs to images at 1-cm intervals throughout the entire lungs (32). Using HRCT as a sampling tool, the pattern and anatomical distribution of abnormality are determined and used to narrow the differential diagnosis or even substantiate a specific diagnosis (e.g., in classic cases of UIP, Langerhans cell granulomatosis (LCG), lymphangioleiomyomatosis (LAM) or emphysema). HRCT is the test of choice for identifying and characterizing bronchiectasis, having replaced the more invasive bronchography.

Most HRCT images are obtained with the patient in the supine position. When the lung abnormality is diffuse in distribution and/or severe in profusion, inspiratory images alone may be sufficient. However, when the only abnormality is in the dependent portion of the lungs, on supine images it is difficult to determine if the findings represent true lung disease or dependent atelectasis. The latter occurs more often in current and exsmokers (34–43%) than nonsmokers (12%) (33) and with increasing age. Since dependent atelectasis occurs in the most dependent portion of the lungs, when a patient is placed prone, atelectasis shifts from the anatomically anterior lung to the anatomically posterior aspect of the lungs which is now the most dependent lung. In contrast, with true lung disease, the abnormal opacities persist; note the opacity may be less than when the patient is supine, as some, but not all, of

the opacity may have been dependent atelectasis. If HRCT scans are done according to a protocol and not checked routinely before the patient leaves the radiology department, prone images should be included in the scanning protocol.

Dynamic, or ultrafast, HRCT can be performed throughout the respiratory cycle on an electron beam CT scanner. Some of the same information can be obtained from end-inspiratory and end-expiratory HRCT images on a conventional CT or helical CT scanner (34). Normal lung will increase in attenuation at end-expiration, similar to the increased lung opacity seen on end-expiratory chest radiographs. Failure of the lung parenchyma to increase in attenuation on expiration indicates air trapping, and suggests small airway disease. In some disease processes, such as bronchiolitis obliterans, a mosaic attenuation pattern of air trapping on expiratory HRCT images may be the only evidence of abnormality, as the lungs may appear entirely normal on inspiratory images (35–38).

No discussion of HRCT is complete without a discussion of the anatomy on which HRCT interpretation is based (39) (Fig. 1). The smallest anatomical unit visible on HRCT is the secondary pulmonary lobule. The walls of the lobules, the interlobular septa are below the resolution limit of HRCT and are visualized only if abnormal. The interlobular septa correspond to the Kerley-B lines on chest X-radiographs. The occasional visible septum may be normal. The lower limit of resolution on HRCT is approximately 0.1 mm, which is the

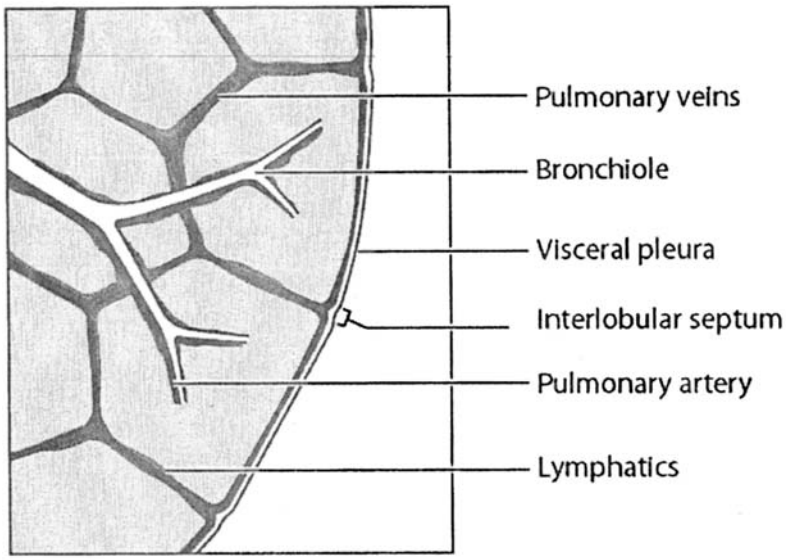

Figure 1 Anatomy of secondary pulmonary lobules.

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width of the interlobular septa. When they are perpendicular to the plane of imaging, 0.2–0.3 mm structures can be routinely identified on HRCT. The diameter of the pulmonary artery supplying each lobule is 1 mm, and the diameter of the intralobular acinar arteries is 0.5 mm; both are therefore readily seen on HRCT. Bronchi are visible based on the thickness of their walls. The 1-mm diameter bronchiole supplying the lobule has an approximately 0.15 mm wall, just at the limit of HRCT resolution, and is barely, if at all, visible on HRCT images.

Helical CT refers to scanning that acquires a continuous volume of data, with the detector rotating continuously as the patient moves through the scanner gantry. For each gantry rotation, one "image" is generated. Since the gantry rotates like an unwound coil around and around the patient, a single volume of data is acquired that can more easily be viewed in additional planes, such as the coronal or sagittal plane. Pitch refers to the tightness of the coil. When a scan is obtained with a pitch of 1, it means that the detector rotated 360 degrees as the patient moved the distance set as the collimation. At a pitch of 2, the patient has moved twice the collimation distance through the scanner gantry for one detector revolution. The larger the pitch, the larger the effective slice thickness; in other words, the actual images generated are thicker than the collimation set. These original helical CT scanners are now known as single-detector–row CT scanners, as they have one detector-row. Multidetector CT scanners have since been developed, with multiple detector-rows. Initially two– and four–detector-row scanners, these acquired two or four images per rotation of the gantry around the patient. Currently, 16–detector-row scanners that use 16 data channels are available, permitting isotropic resolution of each voxel, such that reconstructions in any plane can be generated from the data with no loss of information; something not previously possible. With 16 images of very thin collimation, less than 1-mm slice thickness is possible. Together with electrocardiographic gating, they can produce in seconds a thoracic CT study capable of seeing not only small pulmonary arteries to evaluate for pulmonary embolism, but hold promise in the diagnosis of atherosclerotic coronary artery disease by performing a noninvasive CT coronary angiogram.

III. What Are the Salient Features of UIP on HRCT Scans?

Although its specificity is controversial, we believe HRCT can obviate the need for surgical biopsies in some patients with UIP provided the radiographic features are classic (13). Idiopathic interstitial pneumonias (IIPs) represent a number of clinicopathological entities, including usual interstitial pneumonia (UIP) (also termed idiopathic pulmonary fibrosis), acute interstitial pneumonia (AIP), respiratory bronchiolitis-associated interstitial lung disease (RB-ILD),

desquamative interstitial pneumonia (DIP), nonspecific interstitial pneumonia (NSIP), lymphoid interstitial pneumonia (LIP), and cryptogenic organizing pneumonia (COP) (40). Of these entities, UIP is the most common, and is associated with the poorest prognosis (14,41–46). The histopathological pattern from surgical lung biopsy (SLB) specimens provides the basis for a final clinical-radiological-pathological diagnosis (40).

Several studies have affirmed that a "confident diagnosis" of UIP by HRCT criteria by experienced radiologists is highly specific (> 95%) for UIP (11,21–27). However, CT features are classic for UIP (i.e., enabling a confident diagnosis) in fewer than two-thirds of patients with histological UIP. In addition, the accuracy of CT diagnoses may be substantially less among less experienced radiologists or clinicians. CT is most useful when the disease is extensive and a predominantly reticular pattern is evident.

Evaluation of HRCT includes not only overall (global) extent of deranged lung parenchyma but also the predominant "pattern" (13). Three major patterns include (1) ground-glass opacities (ill-defined, hazy areas of increased alveolar attenuation that do not obscure pulmonary vessels), (2) reticular pattern (intersecting fine or coarse lines) and (3): honeycomb change (small cystic lucencies). As will be discussed later, the predominant pattern on CT may predict potential responsiveness to therapy.

The salient HRCT features of UIP include patchy involvement, basal and peripheral predominance, coarse reticular or linear opacities (intralobular and interlobular septal lines), large areas of spared lung parenchyma, honeycomb change (HC), traction bronchiectasis or bronchiolectasis, and minimal or no ground-glass opacities (GGO) (Table 2) (8,9,13,22,23,26,29,46–49) (Figs. 2–9). With advanced IPF/UIP, severe volume loss, anatomical distortion, and dilated pulmonary arteries may be observed (8). Zones of emphysema (typically in the upper lobes) may be present in smokers (10,13,50) (Fig. 6).

Table 2 High-Resolution Thin-Section CT Scans in UIP

Cardinal HRCT Features of UIP
Predilection for basilar and peripheral (subpleural regions) of the lungs
Patchy involvement with areas of spared lung parenchyma
Course reticular or linear opacities (intralobular or interlobular septal lines)
Honeycomb cysts
Traction bronchiectasis or bronchioloectasis
Ground-glass opacities absent or inconspicuous
Volume loss, anatomical distortion, enlarged pulmonary arteries (late findings)
Zones of emphysema may coexist (in smokers)

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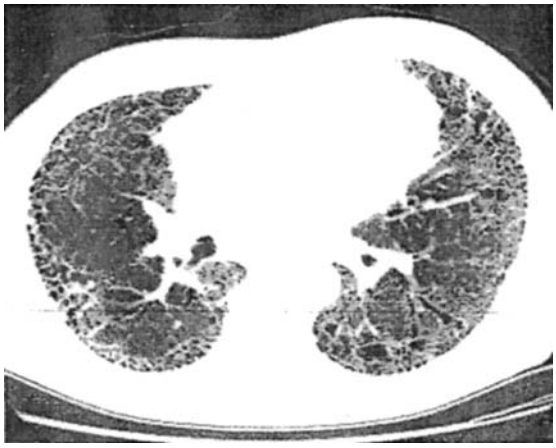

Figure 2 Usual Interstitial Pneumonia (UIP). HRCT scan reveals extensive subpleural reticular opacities, honeycomb cysts, and traction bronchiectasis. No significant ground glass opacities are present.

Honeycomb change is a cardinal feature of UIP (13,51) (Figs. 9 and 10), but is rarely present in IIPs (44,51,52). Cystic changes may be observed in other lung disorders (e.g., LCG (53,54), LAM (55,56), and emphysema), but the nature of cystic lesions in those disorders differs from the classic honeycomb cysts observed in IPF/UIP (13) The cysts in LAM are well-defined and lack the subpleural, basilar predominance typical of IPF/UIP (56). In LCG, the cysts preferentially involve the upper and mid lung zones (in contrast to UIP), and are usually associated with a nodular component (53,54). Emphysema is distinguished from HC by the lack of well-defined walls (6). Patchy, focal GGOs may be present in UIP (Figs. 11 and 12), but *extensive* or *predominant* GGO suggests an alternative diagnosis (e.g., DIP (57), AIP [58–60], NSIP [61], hypersensitivity pneumonia (HP) [62–64], COP [65], LIP [40, 66], pulmonary alveolar proteinosis [67]). Each of these entities will be briefly discussed later in this manuscript.

IV. How Accurate (Specific) Is HRCT to Diagnose UIP?

Several studies over the past 15 years evaluated the accuracy of a CT diagnosis of UIP (assessed by thoracic radiologists). The correctness of the first choice CT diagnosis of UIP is between 67 and 100% (11,22–27). When one restricts the analysis to those cases in which the radiologist made a ''confident'' first choice diagnosis of UIP, the accuracy is extremely high (88–100%) (11,22–27). However, the percentage of cases in which a confident diagnosis is made ranges

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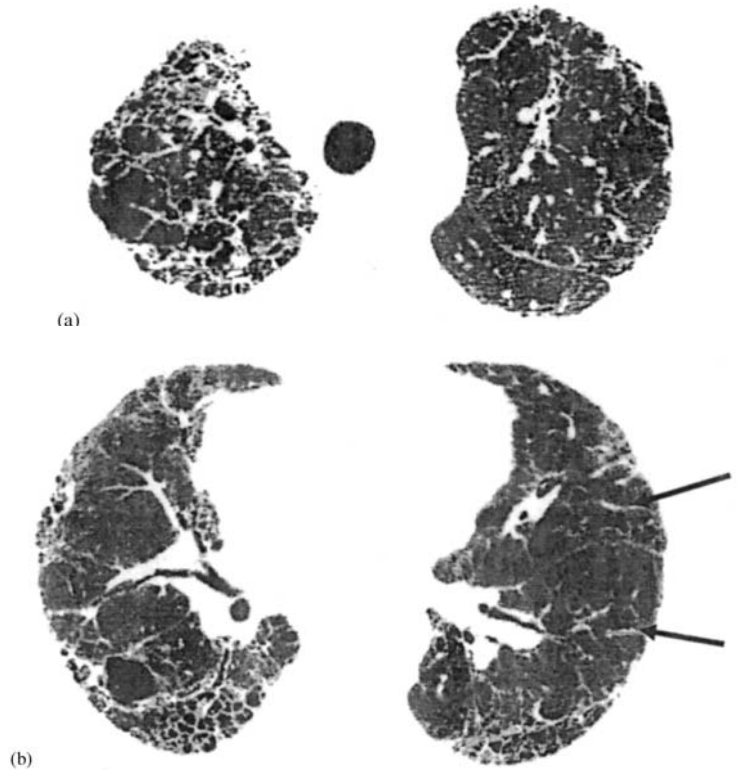

Figure 3 (a) Usual Interstitial Pneumonia. HRCT (upper lobes) demonstrates thickened interlobular septa, and a reticular pattern. Note the subpleural predominance. No significant ground glass opacities are present. (b) UIP. HRCT scan from the same patient (lower lobes) showing subpleural honeycomb change, traction bronchiectasis, and thickened interlobular septa (arrows).

from 37 to 75% (11,22,24,25,27). Therefore, one can expect that between 25 to 63% of cases with histological UIP will not receive a confident first-choice diagnosis of UIP and may require surgical biopsy to confirm the diagnosis. The sensitivity of a stereotypical HRCT in predicting histological UIP ranges from 63 to 90%, the specificity ranges from 70 to 100%, and the positive predictive value (how likely one is to be correct if one makes a CT diagnosis of UIP) from 88 to 92% (11,23,25,46,68). Several reasons may account for this variability in reported accuracy in the CT diagnosis of UIP. Some studies included more heterogeneous patient populations with conditions that were not limited to IIP. These studies included patients with sarcoidosis, COP, HP, silicosis, lymphangitic carcinomatosis, LCG, bronchoalveolar cell carcinoma, and other chronic infiltrative lung diseases (11,22–25,29,69). It is difficult to

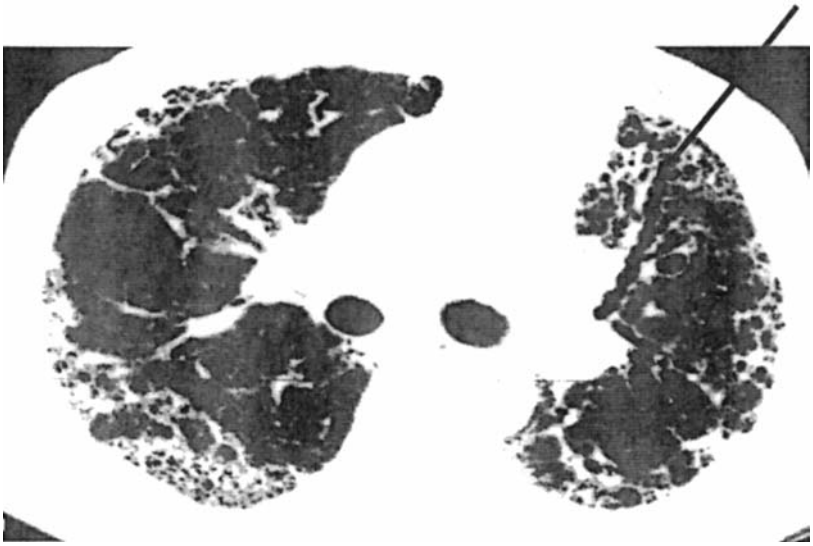

Figure 4 UIP. HRCT scan from lower lobes demonstrates patchy, subpleural honeycomb change, reticulation, thickened interlobular septa, and minimal ground glass opacities. Traction bronchiectasis is also present (arrow).

distinguish UIP from fibrotic NSIP, because these conditions share several CT features (68). Another potential source of variability is the inconsistent imaging criteria used by radiologists in the CT diagnosis of UIP.

V. Differential Diagnosis of ILDs

Several CT features including predominant pattern (i.e., alveolar opacification versus cystic radiolucencies), distribution of disease (e.g., basilar, peripheral, axial, bronchovascular), and concomitant features (e.g., presence of nodules) are invaluable to narrow the differential diagnosis. Extensive or predominant GGOs are not a feature of UIP, but may be observed in a host of disorders, including DIP (70,71), RB-ILD (52,70–73), HP (64,74,75), cellular NSIP (68,76,77), organizing pneumonia (OP) (78–80), LIP (66,81,82), and pulmonary alveolar proteinosis (83,67). By contrast, cystic radiolucencies, a cardinal feature of UIP, may be observed in myriad other fibrotic disorders such as LCG, LAM, emphysema, sarcoidosis, and pneumoconiosis (84,85). Certain CT features (e.g., distribution of lesions, other concomitant lesions) can usually differentiate these entities from UIP. In the sections which follow, we provide specific CT features of several disorders which may present with cough, dyspnea, a restrictive defect, and interstitial infiltrates on chest radiographs

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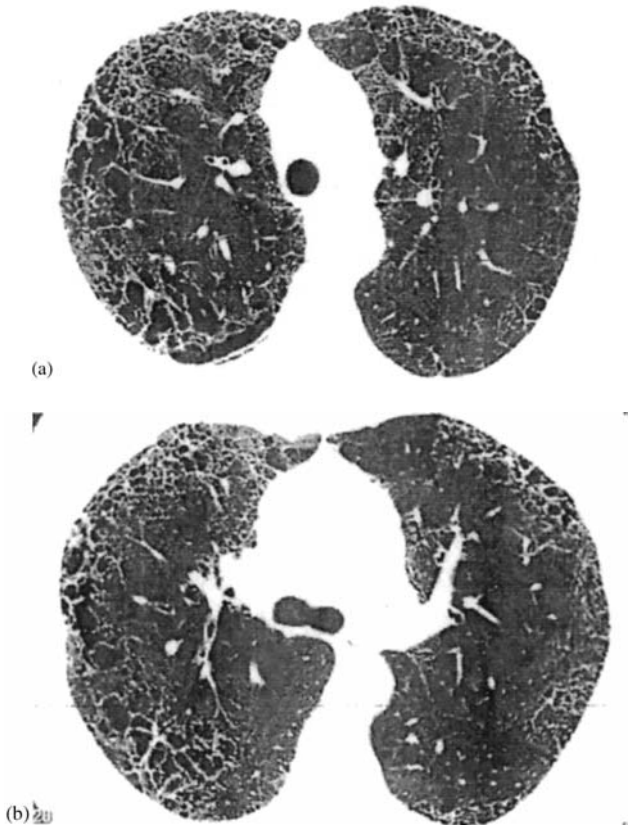

Figure 5 (a) UIP. HRCT (upper lobes) demonstrates peripheral honeycomb change and thickened interlobular septa. No significant GGO are present. (b) UIP. HRCT from the same patient at level of the carina. Peripheral honeycomb change, thickened interlobular septa, and a reticular pattern are evident.

(mimicking UIP). Recognizing the salient CT features of these other ILDs will facilitate discrimination from UIP.

A. DIP

DIP is a rare disease in smokers characterized by filling of alveolar spaces with macrophages containing finely granular yellow pigment derived from complex phagolysosomes (52,86). Interstitial inflammation or HC are absent or minimal (52,71). Clinical features overlap with IPF (e.g., cough, dyspnea, a restrictive ventilatory defect), but in contrast to UIP, the long-term prognosis of DIP is generally excellent (2,52,57,71,72).

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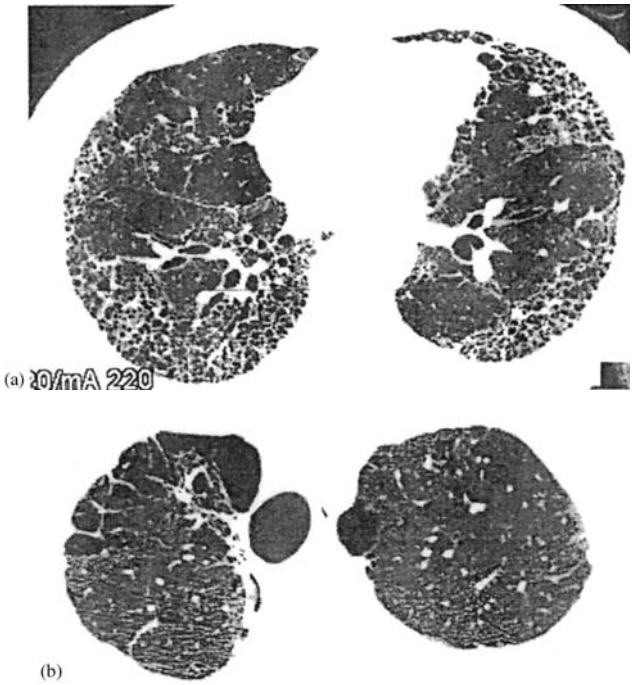

Figure 6 (a) UIP. HRCT (lower lobes) showing extensive reticulation and honeycomb change, with peripheral (subpleural) distribution. Temporal heterogeneity is present as demonstrated by areas of profound honeycombing adjacent to areas of relatively normal lung. (b) UIP with concomitant emphysema. HRCT scan (apices) from the same patient showing no honeycomb change and no definite evidence of UIP. Focal emphysematous changes are present in the right apex (consistent with his history of cigarette smoking).

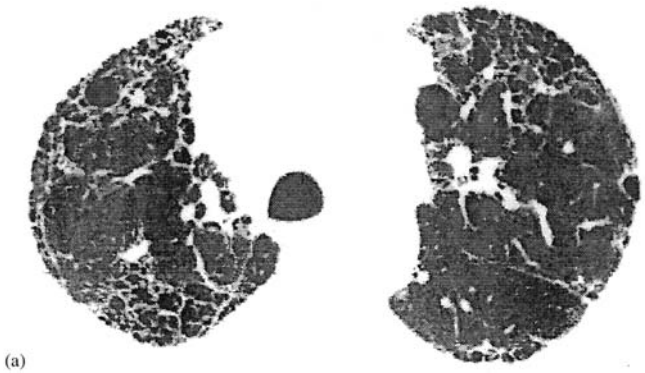

Figure 7.

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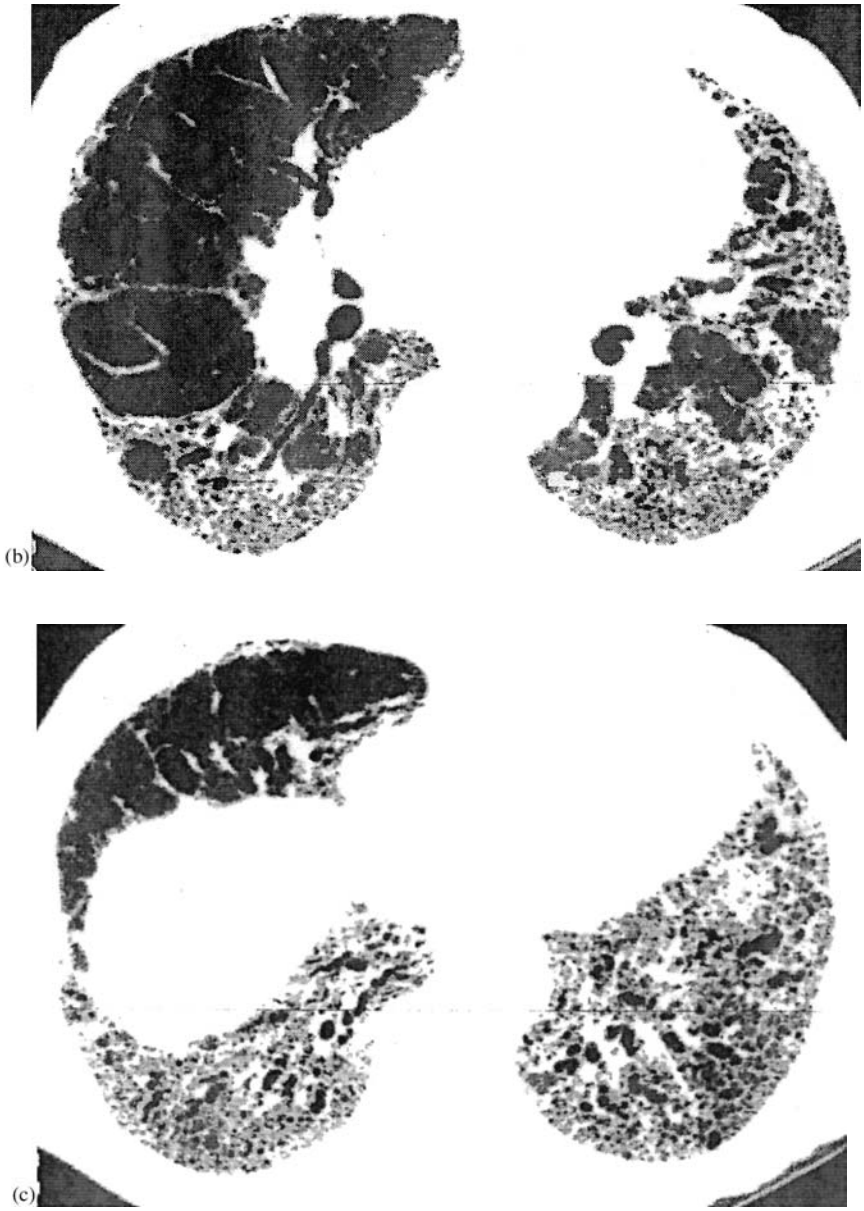

Figure 7 (a) UIP. HRCT scan (upper lobes) demonstrates honeycomb change, thickened interlobular septa; no GGO. (b) UIP. HRCT scan from same patient (lower lobes) with extensive coarse GGO and honeycomb change. Note the patchy nature of the disease process. (c) UIIP. HRCT from same patient at lung bases. Extensive coarse GGO, honeycomb change, and traction bronchiectasis are present.

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The CT features of DIP differ strikingly from UIP. The cardinal feature of DIP on HRCT is extensive areas of GGOs, which reflect filling of the alveolar spaces with pigmented macrophages (Figs. 13 and 14). (52,57,87). The distribution of the process is similar to UIP, with a proclivity for lower lung zones and subpleural regions (26,57,70,71,87) (Fig. 13). Reticular abnormalities (e.g., interlobular or intralobular septal lines), traction bronchiectasis

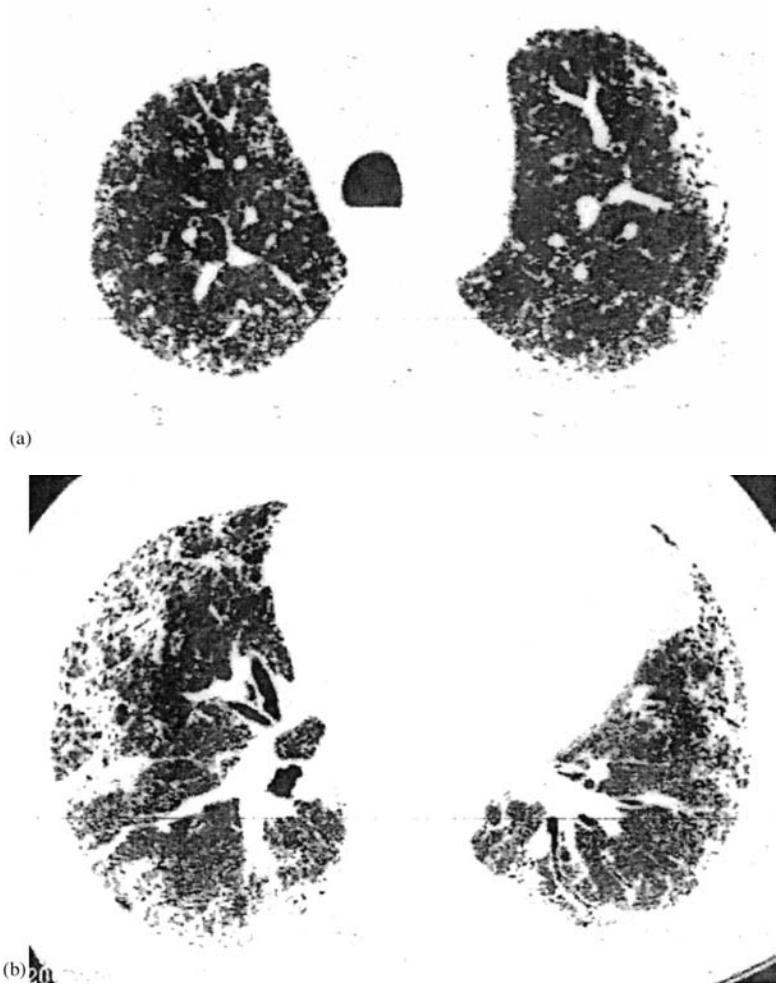

Figure 8 (a) UIP. HRCT (upper lobes) reveals subpleural reticulation and slight peripheral ground glass opacities. (b) UIP. HRCT from the same patient (lower lobes) showing peripheral distribution of disease. (c) UIP. HRCT from same patient (lung bases) showing patchy, ground glass and reticular opacities.

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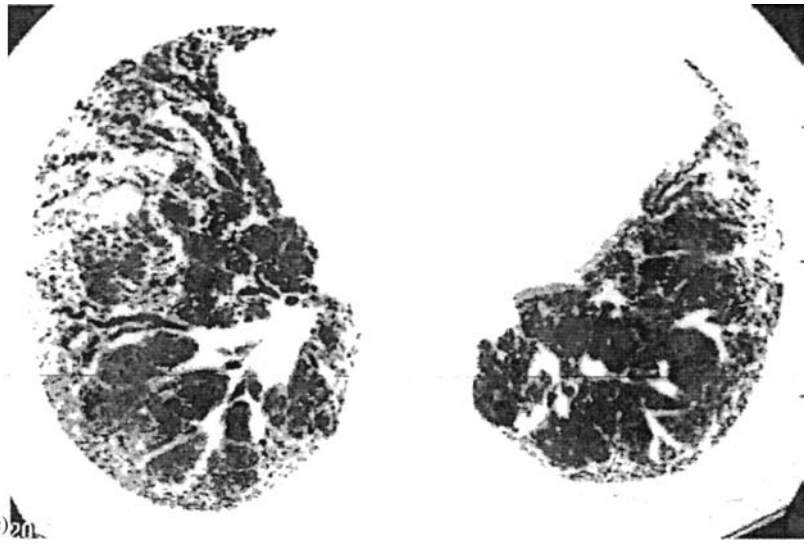

Figure 8 Continued.

(50%), and subpleural nodules are present in one-third to half of patients, but are not a dominant feature (26,57,70,87). Foci of HC may be noted in 9–33% of patients, but are not severe (26,57,70,87). Well-defined cysts within areas of GGO are observed in 41–50% of patients (57,87). Emphysema may be a concomitant feature (70).

Prognosis of DIP is generally excellent, with 5-year survival rates exceeding 90% (42,52,86,88). Improvement can occur spontaneously following cessation of cigarette smoking or with corticosteroid therapy (52,57,72). Ten to 20% of patients deteriorate despite therapy (57,71,86). With treatment, GGOs in DIP usually remain stable or improve; progression to a reticular pattern occurs in fewer than 20% of patients (87).

B. RB-ILD

RB-ILD is characterized by dense collections of pigmented alveolar macrophages within respiratory bronchioles; the distal lung parenchyma is spared (52,71,72,89). By contrast, the process in DIP is more uniform and extensive than RB-ILD and exhibits a striking intra-alveolar component (71,72). Fibrosis in RB-ILD is mild, and is restricted to peribronchiolar areas (89). HC is minimal or absent, but microscopic centrilobular emphysema is common (71,90). More than 90% of cases of RB-ILD occur in smokers (52,71,72,89,90). Most experts believe that RB-ILD and DIP share a common pathogenesis and are responses to constitutents in cigarette smoke or inhaled noxious agents (52,71,70,72). Patients with RB-ILD are relatively young (mean age less than

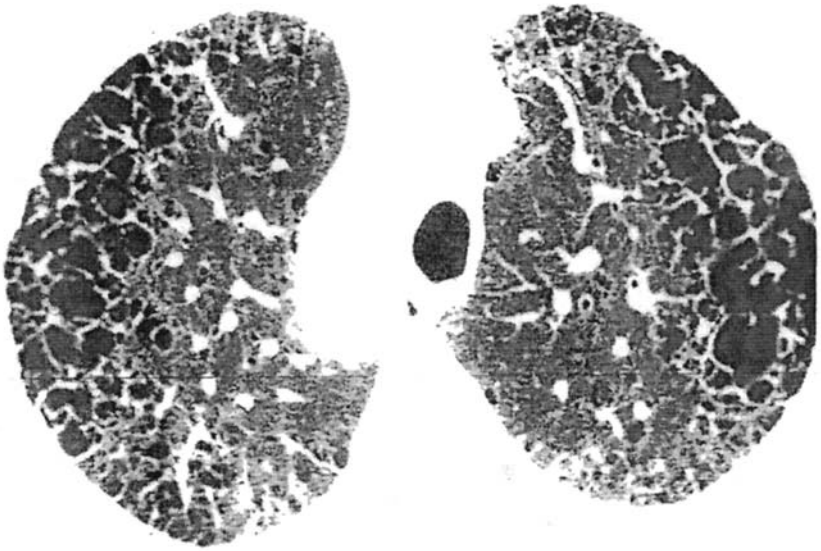

Figure 9 UIP. Extensive honeycomb change. Note predilection for sub-pleural regions. No significant GGO are present. (Reproduced with permission. From Ref. 84.)

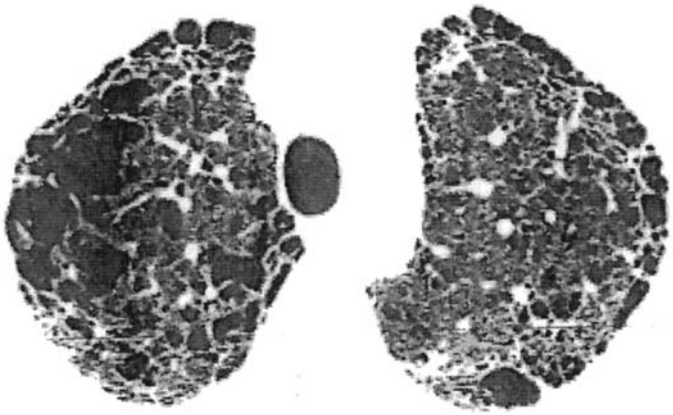

Figure 10 UIP. HRCT (upper lobes) showing extensive peripheral honeycomb change. No significant GGO.

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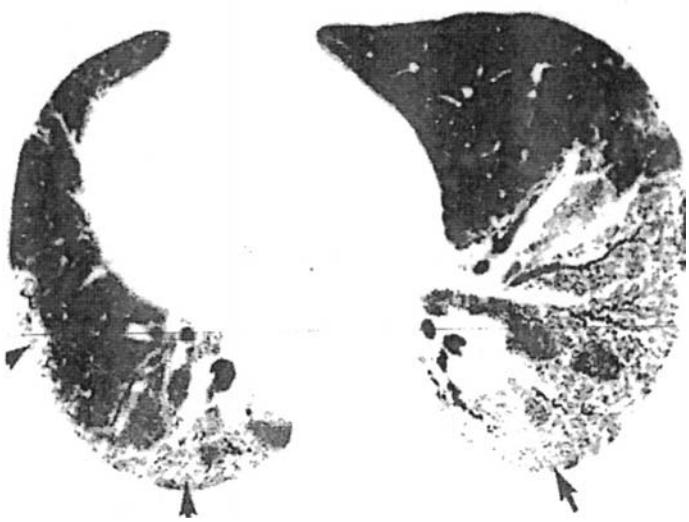

Figure 11 UIP. Coarse ground glass opacities are present (arrows). Note patchy nature of the disease, with areas of radiographically normal lung parenchyma.

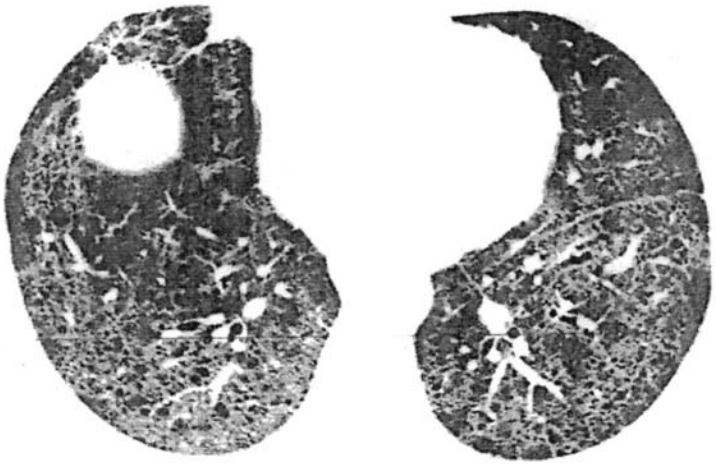

Figure 12 UIP. HRCT (lower lobes) demonstrates ground glass opacities, honeycomb change, and traction bronchiectasis.

40 years), and symptoms of cough, dyspnea, or sputum production are mild (71,89,90). Chest radiographs in RB-ILD demonstrate small irregular opacities (''dirty lungs'') (91) or reticulonodular infiltrates (89), but are normal in up to 28% of patients (71,72,92).

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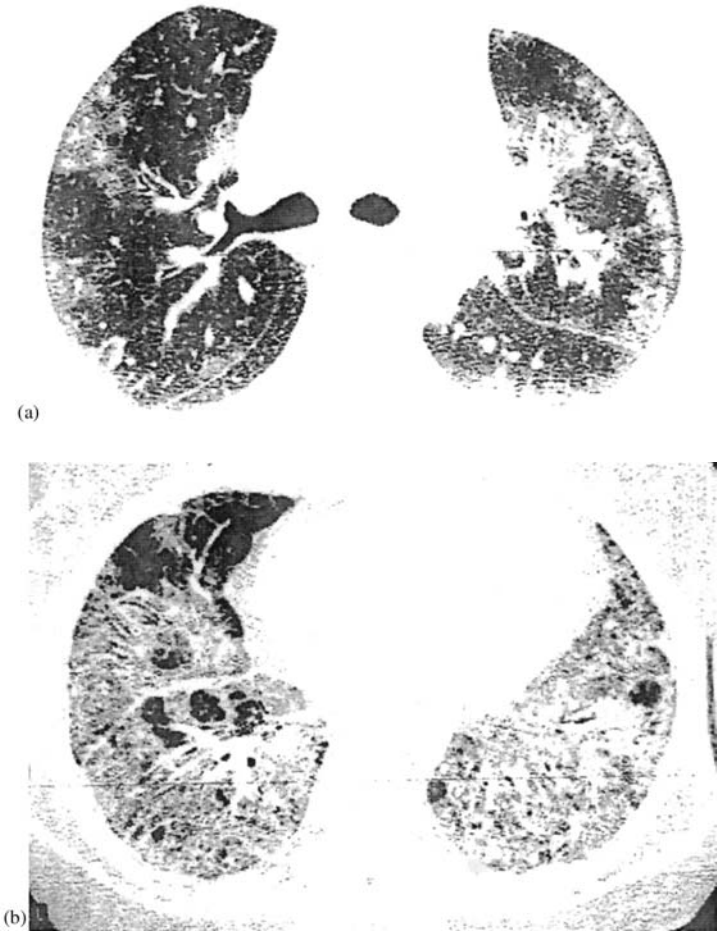

Figure 13 (a) Desquamative interstitial pneumonia (DIP). HRCT (level of carina) reveals focal ground glass opacities with a distinct predilection for peripheral (subpleural) regions. (b) DIP. HRCT scan from same patient (lower lobes) demonstrates dense GGO with consolidation. (Reproduced with permission. From Ref. 12.)

Cardinal findings on HRCT include 2 to 3-mm centrilobular nodules and thickening of the walls of central and peripheral airways (70,92,93) (Fig. 14). Patchy GGO is present in 50–75% of cases, and reflects macrophage accumulation in the alveolar spaces and alveolar ducts (70,92,93). The extent of centrilobular nodules correlates with the degree of macrophage accumulation and chronic inflammation in respiratory bronchioles (93). Additional features which may be observed include patchy areas of hypoattenuation (reflecting air trapping), intralobular lines, and basal and peripheral HCs (suggesting UIP)

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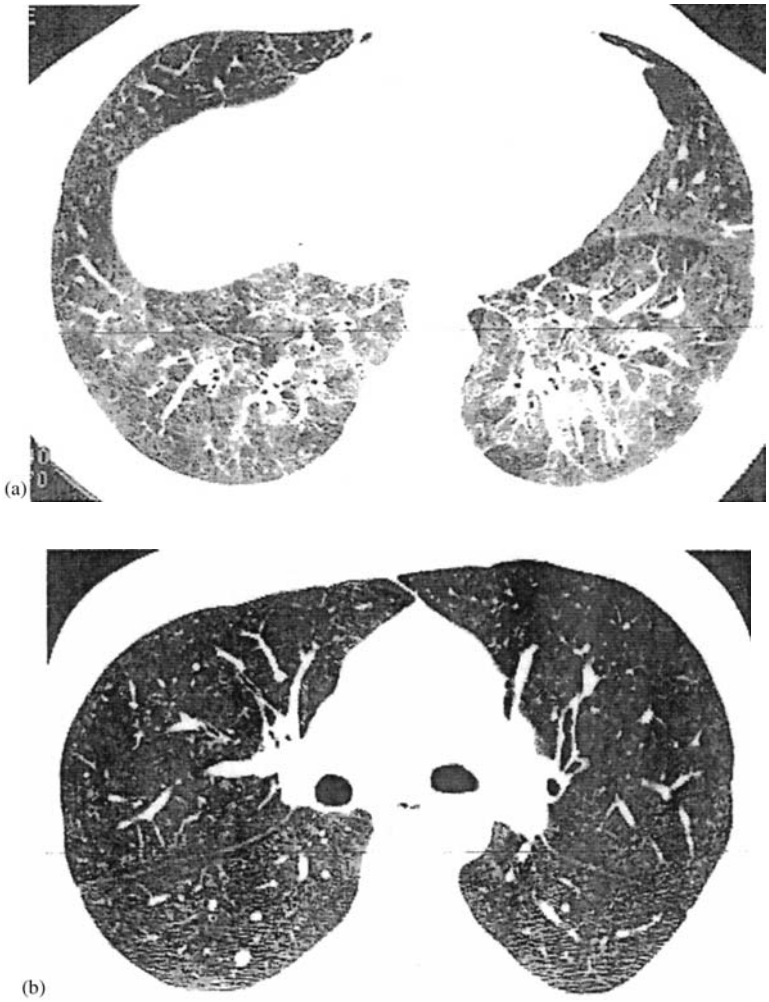

Figure 14 (a) Desquamative Interstitial Pneumonia (DIP). HRCT scan (lower lobes) showing patchy ground glass opacities; note the absence of honeycomb change. (b) Respiratory bronchiolitis interstitial lung disease (RBILD). HRCT showing diffuse centribulobular nodules.

(52,70–72,94). Most authorities believe that there is a continuum of the smoking-related diseases from respiratory bronchiolitis (RB) to RB-ILD to DIP (70) and the CT features similarly progress from predominantly centrilobular nodules in RB to a combined centrilobular and ground-glass pattern in RB-ILD and a more diffuse ground-glass pattern in DIP (70–72). It should also be emphasized that subtle abnormalities on CT, including GGOs,

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have been noted even in asymptomatic smokers with normal lung function (33,93).

The prognosis of RB-ILD is generally excellent. Smoking cessation is the mainstay of therapy. Following cessation of smoking, symptoms usually improve or resolve, and severe pulmonary fibrosis is rare (71,89–91). Ten-year survival exceeds 90% (42,52,88). However, some patients deteriorate despite treatment and cessation of smoking, suggesting that the spectrum of RB-ILD is broader than the original descriptions (89,90).

C. AIP

AIP (formerly termed Hamman-Rich syndrome), is the most fulminant of the IIPs, progressing to fatal respiratory failure within a few days to weeks (52,59,95,96). Histologically, AIP is characterized by acute and organizing diffuse alveolar damage (DAD) with hyaline membranes, fibrinous exudates, and epithelial cell necrosis (52,95). Because of its acute course, AIP generally is not confused with IPF/UIP. However, a subset of patients with IPF/UIP develop an accelerated course (the so-called IPF exacerbation), often as a terminal event, with features of DAD (in addition to UIP) on lung biopsy or necropsy (97–99). The factors responsible for this accelerated phase of UIP are unknown, but viral infections, high concentrations of oxygen, or drug reactions are plausible etiological factors (96).

HRCT scans in AIP reveal extensive, homogeneous GGOs (75–100%) with consolidation (25–92%); there is often a striking geographical and patchy distribution (Figs. 15A and B). In the early phases, HC is absent (26,58–60, 100). However, later phases may be associated with bronchial dilatation, reticulation, dilated, restructured airspaces (resembling HC), and architectural destruction and distortion (26,59,60,100).

Mortality rates in patients with AIP exceed 60%, with most deaths occurring within the first month (95,96). Mechanical ventilatory support with positive end-expiratory pressure (PEEP) is often required for severe AIP (58, 95, 96, 99). Although data regarding therapy are sparse, some patients respond dramatically to high-dose intravenous corticosteroids (95,96,99). Patients surviving the initial episode may heal with no sequelae or with variable degrees of fibrosis (95,96).

D. LIP

LIP is a rare benign lymphoproliferative disorder characterized by a diffuse interstitial proliferation of small lymphocytes and plasma cells (81,101,102). LIP occurs most commonly in patients with connective tissue disorders (particularly Sjögren's syndrome), chronic liver disease, and diverse autoimmune disorders or immunodeficiency states including human immunodeficiency virus (HIV) infection and common variable immune deficiency (CVID)

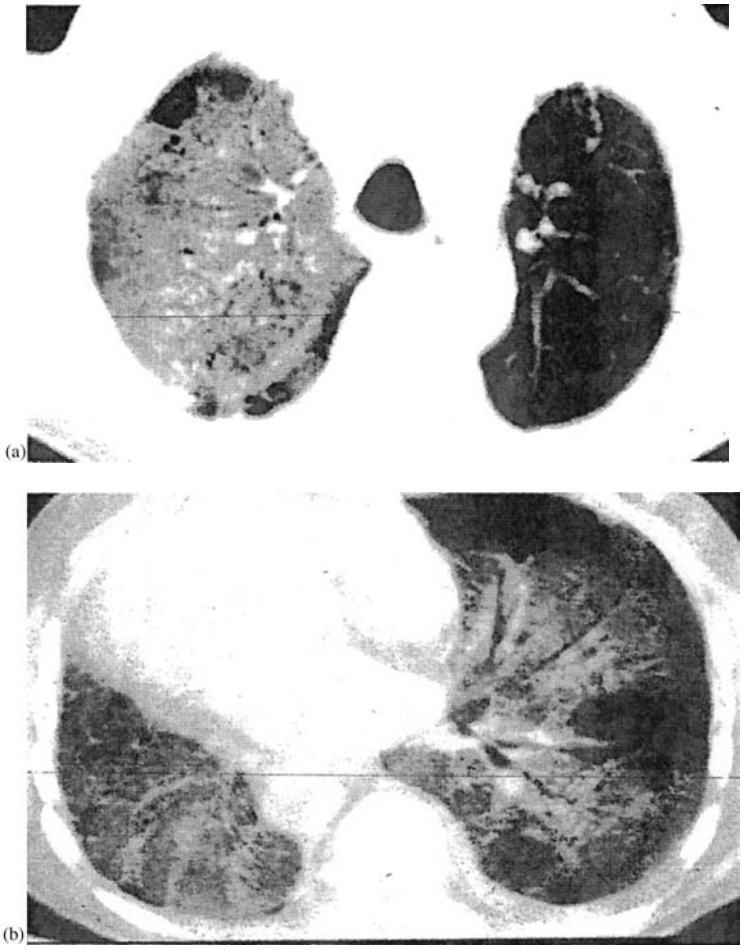

Figure 15 (a) Acute Interstitial Pneumonia (AIP). HRCT (upper lobes) showing dense consolidation right upper lobe in 74 year old male with a prior history of UIP. Open lung biopsy demonstrated diffuse alveolar damage (DAD) and AIP (consistent with an acute exacerbation of IPF). The process resolved with pulse intravenous methylprednisolone therapy. (Reproduced with permission. From Ref. 84.) (b) Acute Interstitial Pneumonia. HRCT from same patient (lower lobes) showing dense airspace consolidation bilaterally, with pronounced air-bronchograms in left lung. (Reproduced with permission. From Ref. 12.)

(66,81,82,101–104). Clinical presentation of LIP is indolent, with progressive cough, dyspnea, and pulmonary infiltrates over months or even years (81,101,102,104). In non–HIV-infected patients, extrapulmonary symptoms are rare (101,102).

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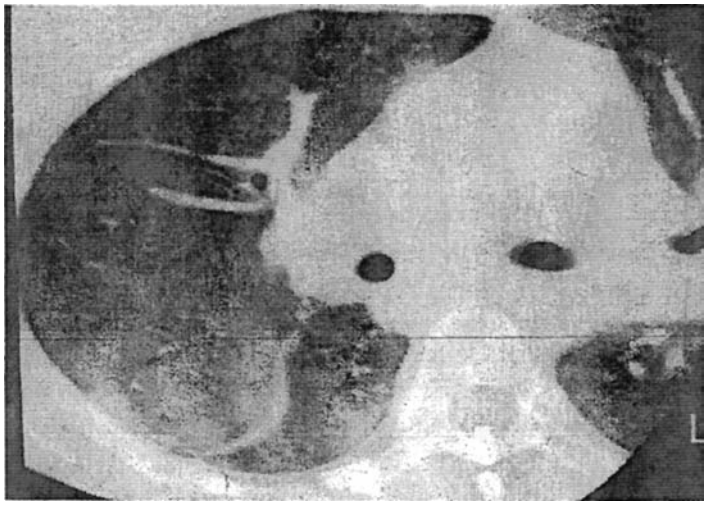

Figure 16 Lymphocytic Interstitial Pneumonia (LIP). Dense ground glass opacification and consolidation in a patient with systemic lupus erythematosus. Open lung biopsy confirmed LIP.

Chest radiographs in LIP may reveal bilateral reticular or reticulonodular infiltrates, dense alveolar infiltrates, or focal nodules (66,81,101,102). Cardinal features on HRCT scans include GGOs (100%), poorly defined centrilobular nodules (2–4 mm) (100%), and small subpleural nodules (86%) (66) (Fig. 16). Other common findings include thickening of bronchovascular bundles (86%), interlobular septal thickening (82%), mediastinal or hilar lymphadenopathy (68%), large nodules (41%), architectural distortion (36%), bronchiectasis (18%), pleural thickening (18%), honeycombing (5%) (66). Cystic airspaces may be observed in up to two-thirds of patients, and may progress over time (66,82). In contrast to the cysts of DIP, which are confined within the areas of GGO, the cysts in LIP tend to be predominantly peribronchovascular and subpleural (105) (Fig. 17).

The natural history of LIP ranges from spontaneous resolution to fatal respiratory failure (81,101,102,104). Given the rarity of LIP, optimal therapy is not known. Favorable responses to corticosteroids have been cited in both HIV-infected and non–HIV-infected patients (81,101,102), but controlled studies are lacking. Immunosuppressive or cytotoxic agents have been tried, but their efficacy is unproven.

E. NSIP

NSIP was recognized as a distinct IIP in 1994 (41), and is discussed in great detail in (Chapter 5). In this chapter, we limit our discussion to the CT features

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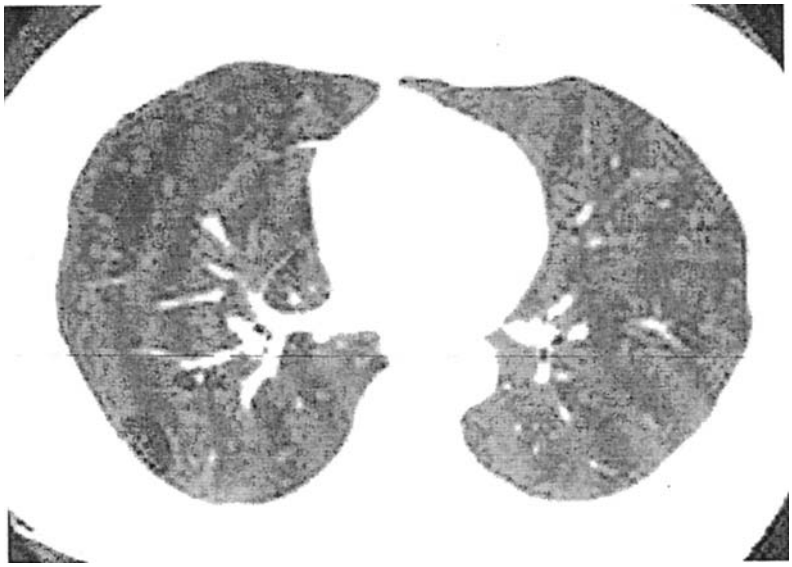

Figure 17 Lymphocytic Interstitial Pneumonia (LIP). HRCT demonstrates 2 well-circumscribed cystic lesions in right lower lobe in a patient with LIP.

of NSIP and underscore features which discriminate UIP from NSIP. Typically, HRCT in NSIP demonstrate patchy GGOs, with a basilar, subpleural predominance, often associated with a reticular pattern and traction bronchiectasis (26,51,68,76,77). HC may be present, but is not a dominant feature (26,51,61,68,76,77). In several publications, salient aberrations described in NSIP included GGOs in 76–100% of patients, reticular abnormalities (46–93%), honeycombing (0–30%), nodules (0–19%), and consolidation (16–80%) (26,61,77,106,107) (Figs. 18–19). The cardinal features discriminating NSIP from UIP are more pronounced GGO and less HC in NSIP (46,51,68) (Fig. 20). CT features of NSIP and variable, however, and the uncertainty remains in the imaging criteria for NSIP (76,40). The CT features between NSIP and UIP overlap considerably; a recent multicenter study found CT features consistent with UIP in 15–50 (30%) patients, with NSIP, being confirmed by surgical lung biopsy (SLB) (61). In addition, among patients with histological UIP, only 25–75% exhibit HRCT features that are ''typical for UIP'' (11,22,24,25,44,46,68). In a cohort of patients with UIP or NSIP, British investigators graded HRCT scans as either ''typical'' or ''atypical'' for cryptogenic fibrosing alveolitis (CFA) (44). Among 12 patients with histological UIP, HRCT scans were typical of CFA in 8 compared to 2 of 15 in patients with NSIP; $P = .0047$). Furthermore, patients with HRCT scans typical of CFA had more fibrosis and a poorer prognosis.

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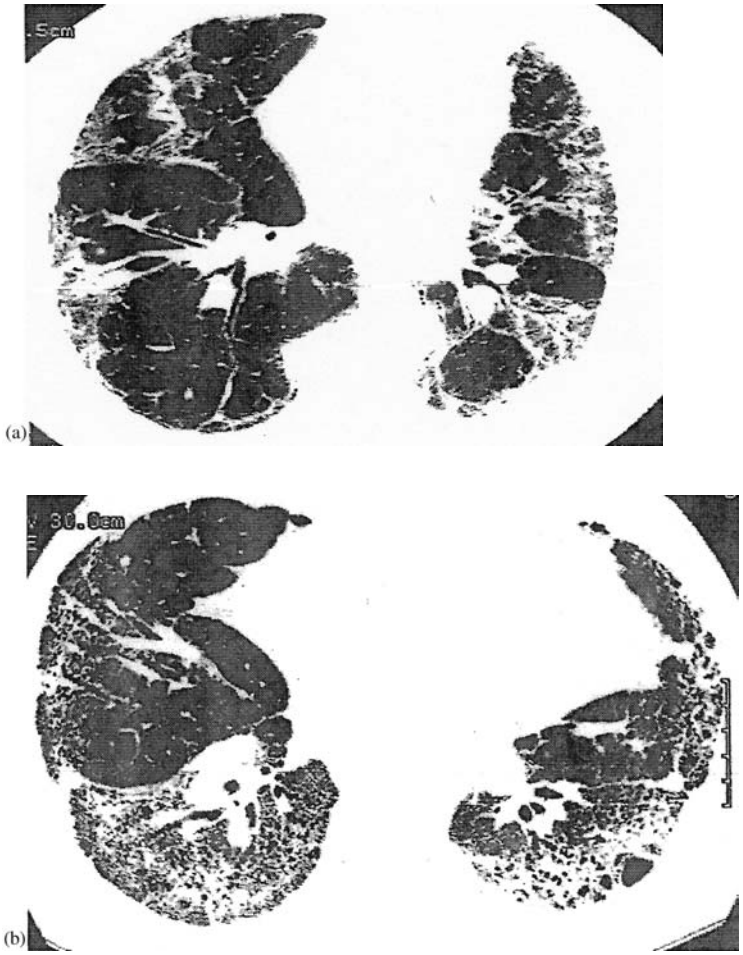

Figure 18 (a) Nonspecific Interstitial Pneumonia (NSIP). HRCT scan (lower lobes) reveals patchy, ground glass densities, with a sub-pleural predominance. Thickened interlobular septa are also present. (b) Nonspecific Interstitial Pneumonia (NSIP). HRCT scan from the same patient (basilar regions) showing patchy GGO and honeycomb change. Traction bronchiectasis is also evident.

CT features of NSIP are nonspecific and overlap with other ILDs. Early studies of a subset of patients with idiopathic interstitial pneumonias (IIPs) suggested a low diagnostic accuracy (9%) for HRCT in identifying NSIP (26). In that study, HRCT scans from 129 patients with *histologically confirmed* IIPs were evaluated by two chest radiologists (26). The radiologists had no access to clinical or histological data. Differential diagnosis was limited to five types of IIP (i.e., UIP, DIP, NSIP, AIP, COP).

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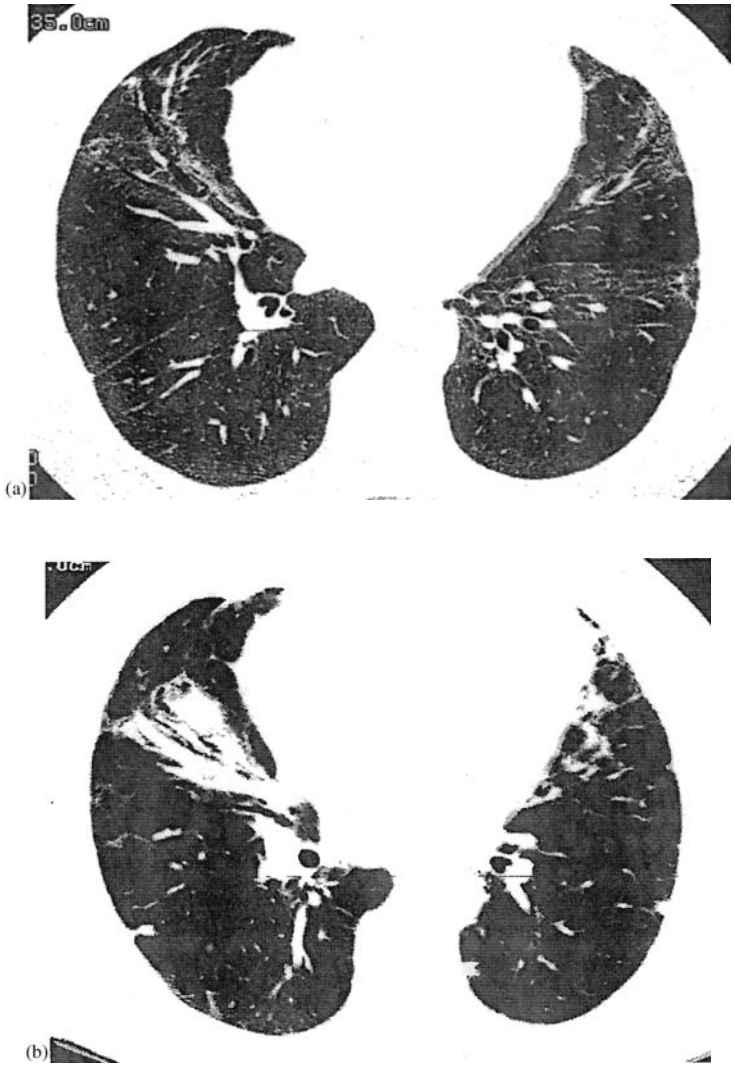

Figure 19 (a) Nonspecific interstitial pneumonia (NSIP). HRCT scan (lower lobes) in October 1996 demonstrates patchy GGO and mild bronchial dilatation; no honeycomb or reticular change is present. (b) Nonspecific interstitial pneumonia (NSIP). HRCT scan (lower lobes) from the same patient 5 years later in October 2001. He had previously been treated for 12 months with corticosteroids with symptomatic relief and stable pulmonary function. A dense focal consolidation is present in the right middle lobe; thickened interlobular septa are also present. (c) Nonspecific interstitial pneumonia (NSIP). HRCT scan (basilar regions) from the same patient in October 2001 shows scattered GGO and traction bronchiectasis. No definite honeycomb change is evident.

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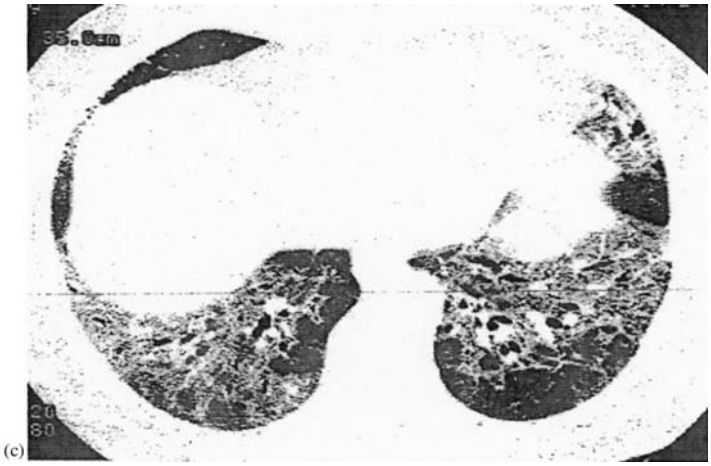

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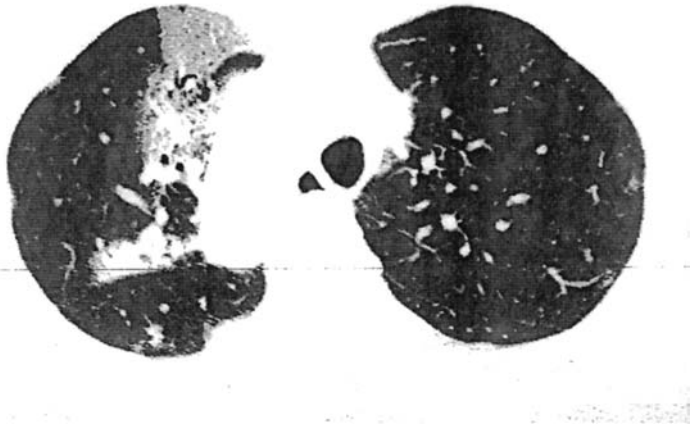

Figure 20 Nonspecific interstitial pneumonia (NSIP). Dense focal alveolar (ground glass) opacities in a patient with polymyositis. Video-assisted thoracoscopic lung biopsy (VATS) demonstrated features of cellular NSIP.

The percentage of correct diagnoses were: UIP (71%), COP (79%), DIP (63%), AIP (65%), NSIP (9%). Two subsequent studies assessed the ability of HRCT to discriminate NSIP from UIP; *other diagnoses were not evaluated* (46,68). In one study, HRCT were retrospectively reviewed by four observers in patients with NSIP (n = 21) or UIP (n = 32) confirmed by surgical lung biopsy (SLBx); all had a clinical course *consistent with* IPF (68). Overall, CT had an accuracy of 66% for discriminating NSIP and UIP. The sensitivity of CT for the diagnosis of NSIP was 70% with a

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specificity of 63%; for UIP, sensitivity was 63% and specificity 70%. NSIP was characterized by increased GGOs and finer fibrosis. By logistic regression analysis, the only CT features independently associated with a histological diagnosis of NSIP was the proportion of GGOs (odds ratio of 1.04 for each 1% increase in GGO). When GGO predominated, a diagnosis of NSIP was confirmed in 24 of 35 patients (69%). By contrast, NSIP was confirmed in 35 of 79 (44%) patients with a mixed pattern on CT and in only 25 of 98 (26%) patients with predominantly reticular disease (68). Overlap was noted between UIP and NSIP with respect to the coarseness of fibrosis. Of 17 patients with the coarsest reticular pattern, 4 (25%) had NSIP and 13 (76%) had UIP. Further, cellular NSIP was associated with finer fibrosis (less coarseness) on CT and a lower likelihood of subpleural distribution compared to fibrotic NSIP. Logistical regression analysis demonstrated that misdiagnosis of UIP by CT in patients with histological NSIP was associated with less GGOs ($P < .005$) and a subpleural distribution ($P = .02$) on CT. Conversely, a misdiagnosis of NSIP by CT in patients with histological UIP was independently associated with a lower likelihood of a subpleural distribution ($P < .005$), fine fibrosis ($P < .005$), and more GGOs ($P < .01$). More recently, investigators from the University of Michigan evaluated a cohort of 23 patients with histological NSIP and 73 patients with histological UIP (confirmed by SLBx) (46). Two thoracic radiologists independently reviewed each HRCT scan and recorded each case as either definite UIP, probable UIP, indeterminate (equal probability of UIP or NSIP), probable NSIP, or definite NSIP. HRCT criteria used to support the diagnosis of UIP was the presence of HC (8). The absence of HC, the presence of GGOs, and an apical or nonsubpleural distribution favored NSIP (68,108). Complete agreement was reached by the radiologists in 35 patients (36%) (kappa = 0.20, $P < .0001$). When *definite or probable* UIP were combined and *probable or definite* NSIP were combined, complete agreement was reached in 61 (64%) cases (kappa = 0.43, $P < .0001$). All 27 patients with "probable or definite" UIP by HRCT criteria had histological UIP on SLBx. In contrast, only 18 of 44 (41%) patients with "probable or definite" NSIP on HRCT had histological NSIP on SLBx; the remaining patients (59%) had UIP. Overall, the radiologists demonstrated a high specificity (100%) but low sensitivity (27%) in identifying UIP and a sensitivity of 78% and specificity of 64% for identifying NSIP (46). In addition, when HRCT features were interpreted as "definite" or "probable" UIP, mortality was higher compared to CT scans interpreted as "indeterminate" or "probable or definite" NSIP. These data suggest that HRCT "typical" of UIP likely represents more advanced disease than patients with histological UIP but CT scans that are atypical for UIP.

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F. COP

COP, also called idiopathic bronchiolitis obliterans organizing pneumonia (BOOP), is a rare disease of unknown cause characterized by a subacute course, cough, dyspnea, crackles, and focal infiltrates on chest radiographs (80,109,110). Secondary causes include connective tissue disorders, diverse immune disorders, drugs, radiation therapy, chemotherapy, infections, and bone marrow or lung transplantion (80,109,110). Pulmonary function tests demonstrate a restrictive defect with reduced the diffusing capacity of the lung for carbon monoxide (DL_{CO}); airflow obstruction is noted only in smokers (80,109,110). Rapidly progressive COP, with severe hypoxemia and acute respiratory distress syndrome, has been described, but is rare (111).

Although COP is usually not confused with UIP, clinical, physiological, and radiographic features overlap to some extent. Chest radiographs in COP reveal focal, alveolar opacities (mimicking pneumonia) in 67–85% of patients; a reticulonodular pattern is found in 15–30% (80,109,110). HRCT demonstrates focal peripheral alveolar infiltrates with striking air bronchograms (Figs. 21 and 22). Airspace consolidations are observed in 70–91% of patients, with predominantly basal and subpleural (Fig. 21) or peribronchial distribution (26,78–80,112,113). Associated features include GGOs (60%), small nodular opacities (30–63%), linear or reticular opacities (15–60%), bronchial dilatation (43–58%), and large focal nodules or masses (26,78,79,112–114). Bronchial wall thickening or dilatation is restricted to the areas of extensive consolidation (78). Mild subpleural HC is rare (< 5%) (26,78,79). Patients with a reticular pattern tend to be less responsive to corticosteroid therapy compared to patients with pure consolidation (109). Consolidation is a nonspecific CT pattern; the differential diagnosis includes infections, alveolar cell carcinoma or lymphoma, lipoid pneumonia, eosinophilic pneumonia, vasculitis, sarcoidosis, collagen vascular diseases, and cellular NSIP (109).

Corticosteroids are the mainstay of therapy for COP, and they are highly efficacious. Responses are often dramatic, and complete remissions are achieved in more than 75% of patients (80,109,110). Relapses may occur as the corticosteroid is tapered or discontinued (115).

G. Pulmonary Alveolar Proteinosis

Pulmonary alveolar proteinosis (PAP) is a rare diffuse lung disease of unknown etiology characterized by the accumulation of amorphous, periodic acid-Schiff (PAS)–positive lipoproteinaceous (surfactantlike) material in the distal airspaces (67). Clinical features include dyspnea, cough, and hypoxemia, which evolve over weeks to months (67,116). Chest radiographs reveal symmetrical, fluffy, perihilar infiltrates (a batwing appearance), but asymmetrical or even unilateral involvement occurs in 20% of patients (67,116). Compared to conventional chest radiographs, HRCT more clearly depicts the alveolar

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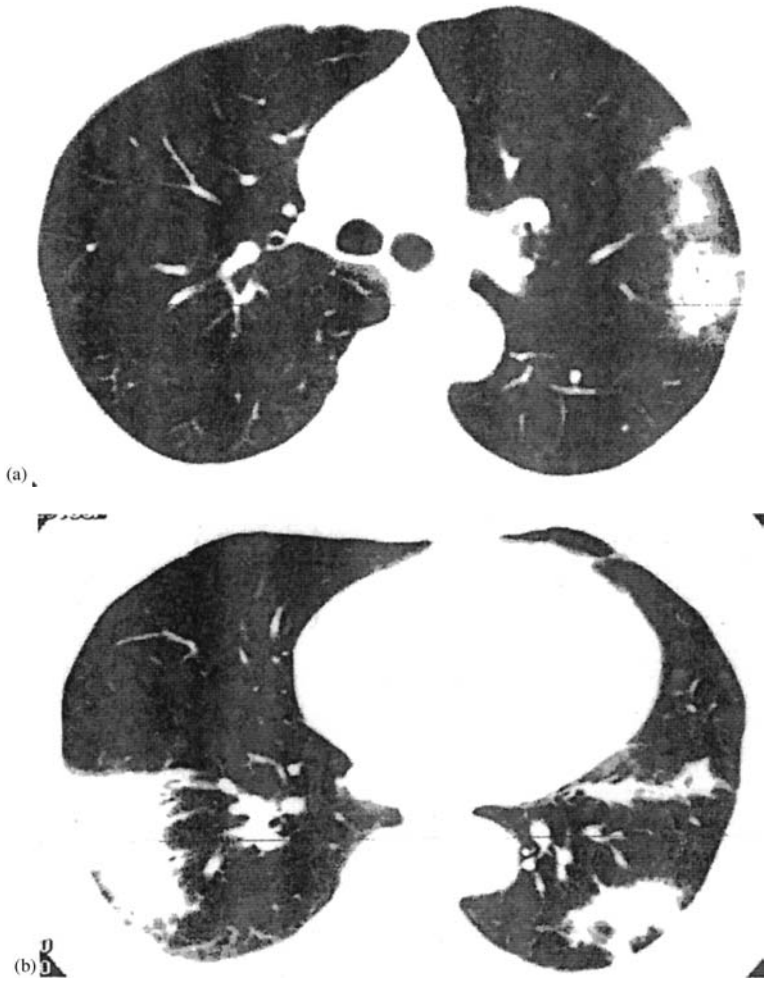

Figure 21 (a) Cryptogenic organizing pneumonia (COP). HRCT (at the level of the carina) demonstrates focal dense consolidations; note the peripheral location and the lack of reticulation or honeycomb change. (b) Cryptogenic organizing pneumonia (COP). HRCT from the same patient (lower lobes) demonstrates dense focal consolidations in the periphery of both lower lobes. Note the lack of reticulation or honeycomb change.

pattern, with air bronchograms sometimes revealing sharply demarcated areas of lung involvement (67,83) (Figs. 23 and 24). CT invariably demonstrates patchy or confluent GGOs, with airspace consolidation in >75% of patients (67,83,116). Reticular opacities or interlobular septal thickening may be found in abnormal lung regions displaying GGOs; significant fibrosis is uncommon

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(<10%) (67,83,116). The so-called "crazy paving" appearance on HRCT refers to a network of a smooth thickened interlobular septa superimposed on ground-glass attenuation (117) (Figs. 25A and B). The "crazy-paving" appearance may reflect accumulation of surfactantlike material in the airspaces

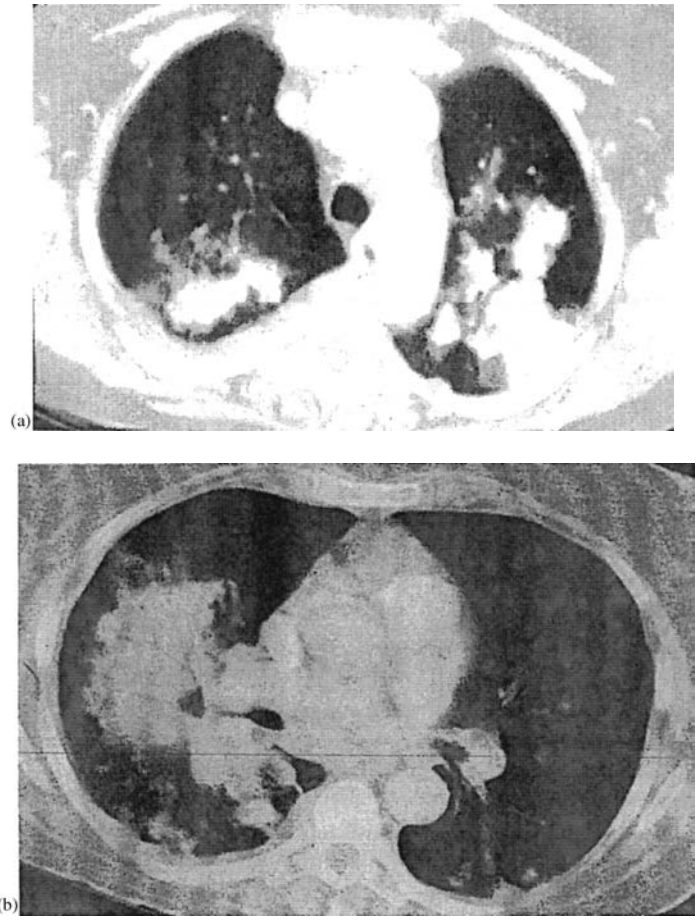

Figure 22 (a) Cryptogenic organizing pneumonia (COP). HRCT (upper lobes) demonstrates dense focal consolidations in the periphery of both lungs. Note the lack of reticulation or honeycomb change. (b) Cryptogenic organizing pneumonia (COP). HRCT from the same patient (lower lobes) demonstrates dense focal alveolar infiltrates with prominent air-bronchograms in the right lower lobe. A few patchy nodular densities are present in the sub-pleural region of the posterior segment of the left lower lobe. (c) Cryptogenic organizing pneumonia (COP). HRCT scan from the same patient shows dense consolidation right lung with air-bronchograms and patchy nodular infiltrates.

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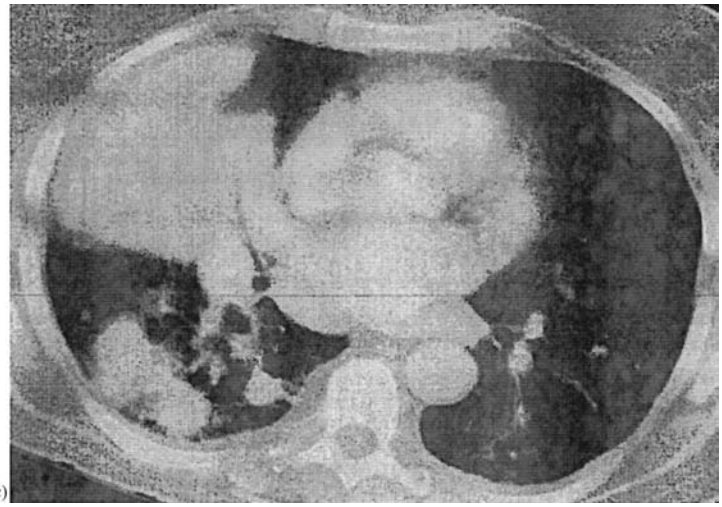

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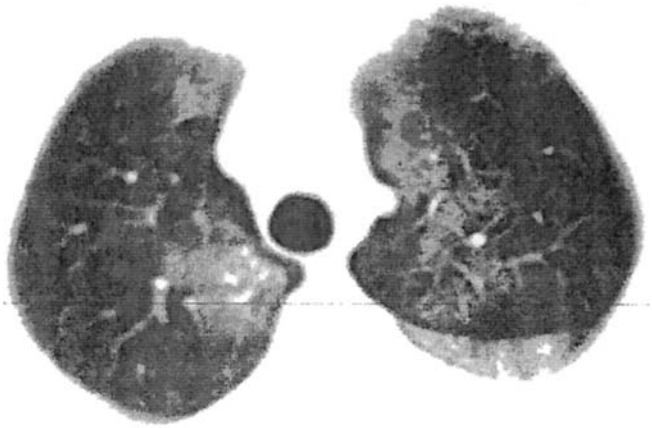

Figure 23 Pulmonary alveolar proteinosis (PAP). Focal, dense ground glass opacities which are well demarcated from adjacent radiographically normal lung parenchyma.

adjacent to the interlobular septa (118,119). However, this appearance is nonspecific and may be seen in a variety of conditions other than PAP (e.g., bronchoalveolar cell carcinoma, lipoid pneumonia, acute respiratory disease syndrome (ARDS), AIP, diffuse alveolar damage [DAD] superimposed on UIP, drug-induced pneumonia, and diverse alveolar and interstitial disorders) (67,119). Whole-lung lavage, to remove the viscid intra-alveolar

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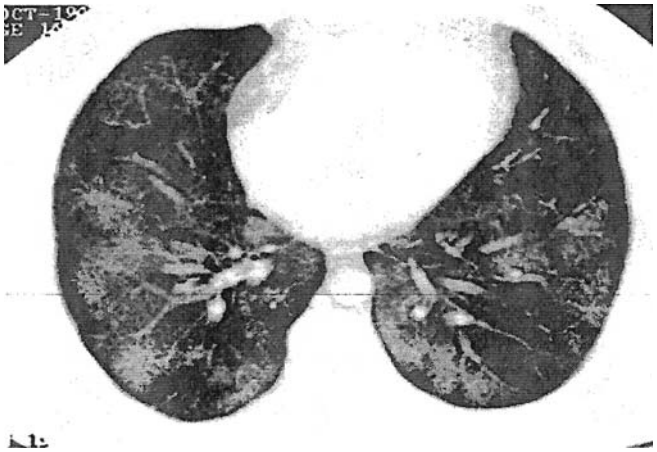

Figure 24 Pulmonary alveolar proteinosis (PAP). CT scan demonstrates multiple foci of ground glass opacities, interspersed with normal appearing lung parenchyma. Open lung biopsy demonstrated classic features of PAP. (Reproduced with permission. From Ref. 85.)

material physically, has been the standard therapy for more than 5 decades, and is usually efficacious (67,116). Chest CT scans obtained after whole-lung lavage characteristically show a reduction in GGOs and interlobular thickening (83,120), but reticular opacities may persist (83). Recently, physiological improvement has been noted following administration of granulocyte-monocyte colony-stimulating factor (GM-CSF) (121).

H. Hypersensitivity Pneumonia (HP)

Hypersensitivity pneumonia (HP) (also termed extrinsic allergic alveolitis) is a cell-mediated response to a variety of inhaled organic dusts or inorganic chemicals (122–124). Acute HP presents with fever, dyspnea, cough, peripheral blood leukocytosis, and pulmonary infiltrates 2–12 h following exposure to the offending antigen(s) (122,123). Following removal of the offending agent, symptoms abate or resolve within 12–48 h (122). Chest radiographs in acute HP usually reveal bilateral alveolar or interstitial infiltrates, but may be normal (64,123,125). Chronic, low-dose exposure to sensitizing antigens may cause chronic HP, which evolves over months to years (74,126). Cardinal features of chronic HP are progressive cough, dyspnea, crackles, a restrictive defect on pulmonary functions tests (PFTs), hypoxemia, and basilar interstitial infiltrates on chest radiographs (74,125). These features mimic IPF/UIP.

The CT appearance of HP is variable and correlates with the clinical course of the disease. *Acute* HP is characterized primarily by ground-glass attenuation and air-space consolidation (127) (Figs. 26–28). *Subacute* HP is

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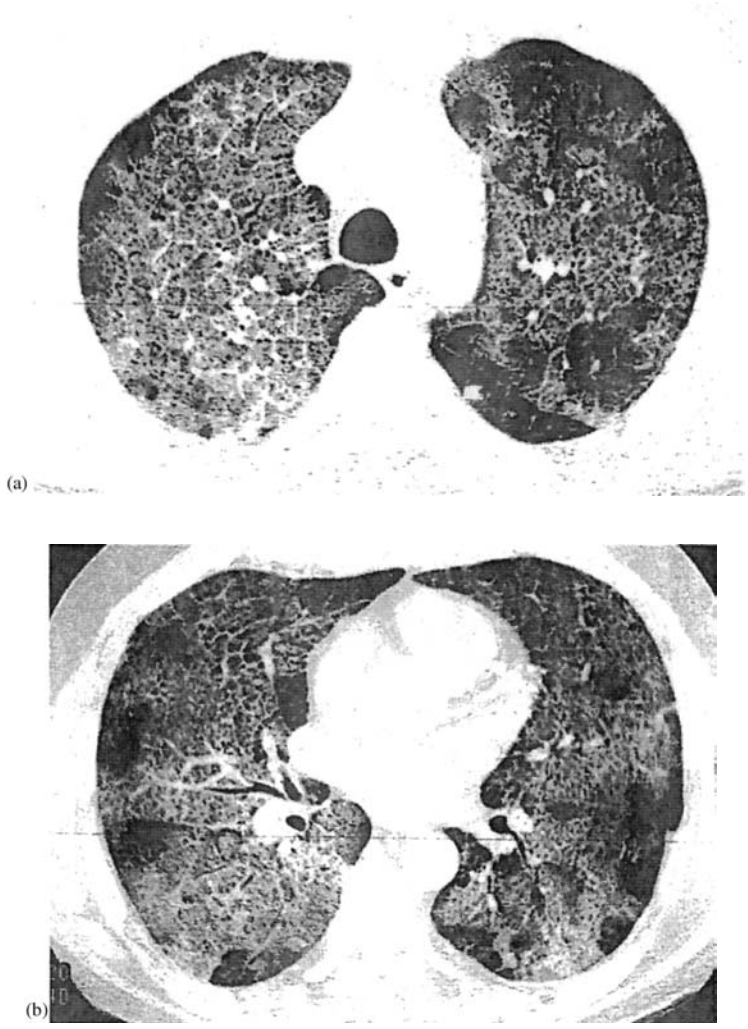

Figure 25 (a) Pulmonary alveolar proteinosis. HRCT (upper lobes) demonstrates the crazy-paving pattern, with a network of thickened interlobular septae superimposed on ground-glass attenuation. (b) Pulmonary Alveolar Proteinosis. HRCT from the same patient (lower lobes) demonstrates extensive GGO and a ''crazy-paving'' pattern.

distinguished by the presence of small, poorly defined centrilobular nodules, ground-glass attenuation, peribronchiolar distribution, a predilection for mid or upper lung zones, mosaic pattern of attenuation, and focal air trapping (65,125). Acute or subacute HP may resemble desquamative interstitial

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Figure 26 Acute hypersensitivity pneumonia (HP). HRCT scan from a 59 year-old woman with fever, cough, and dyspnea demonstrating dense focal alveolar (ground glass) opacities in a peribronchiolar distribution. Transbronchial lung biopsies demonstrated lymphocytic infiltrates, foamy macrophages, and non-caseating granulomas, consistent with HP. The disease cleared completely following institution of corticosteroids and avoidance of further exposure to molds. (Reproduced with permission. From Ref. 84.)

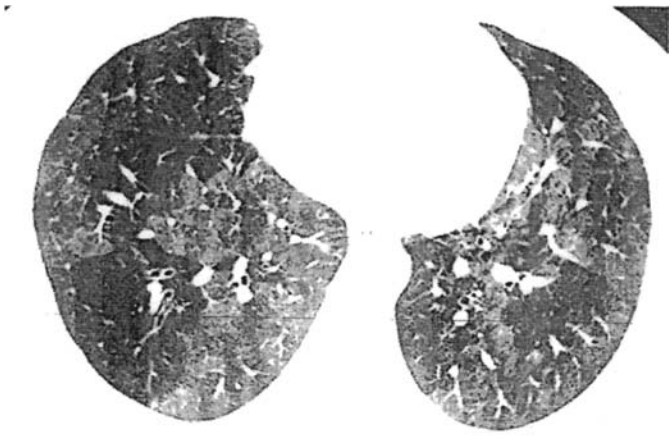

Figure 27 Acute hypersensitivity pneumonia. Patchy ground glass attenuation, in a peribronchiolar distribution. Note the lack of honeycomb change.

pneumonia (DIP) on CT (74,75). CT features of *chronic* HP include HC (50%), alone or in combination with GGOs (57–71%); micronodules (42–50%); and emphysema (40–50%) (62–64). With advanced disease, emphysema or fibrosis may be observed (even in nonsmokers) (62,74,123,124,126,127). Chronic HP

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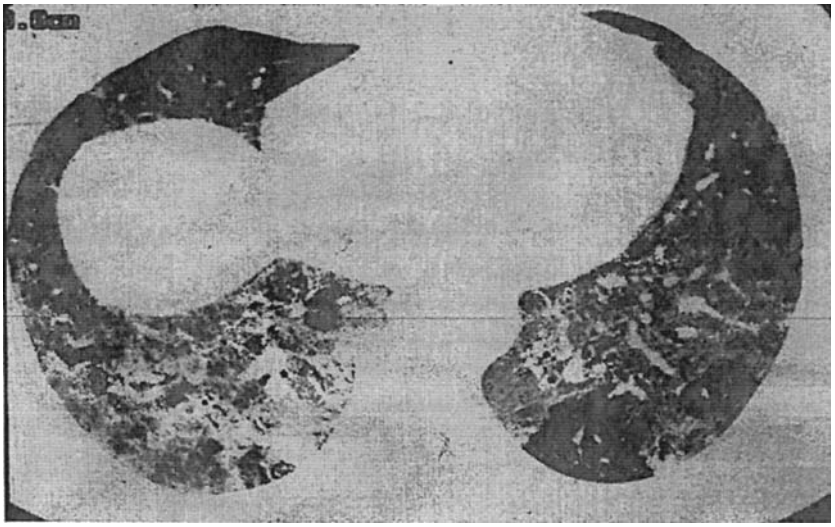

Figure 28 Acute hypersensitivity pneumonia. HRCT scan (lower lobes) demonstrates diffuse ground glass attenuation and bronchial dilatation. No honeycomb change.

may be difficult to distinguish from IPF/UIP (Fig. 29). However, in contrast to UIP, typical features of chronic HP on CT include upper and middle lung zone predominance, poorly defined micronodules, widespread GGOs, and air trapping (particularly on expiratory CT images) (63,75). Micronodules and extensive GGOs are present in 32–42% of patients with chronic HP but in only 6–12% of patients with idiopathic UIP (75). Lower lobe predominance, subpleural location, and HC are cardinal features of UIP (noted in $> 80\%$ of patients) but are evident in a minority of patients with chronic HP (75).

Identification and avoidance of the offending agent is the cornerstone of treatment for HP. The role of corticosteroids to treat HP is controversial, but short-term responses occur (122). We recommend corticosteroids for severe or progressive cases, but long-term benefit has not been established. Data on immunosuppressive or cytotoxic agents are limited to anecdotal cases; efficacy is unproven.

I. Sarcoidosis

Sarcoidosis, a multisystemic granulomatous disease of unknown etiology, involves the lung or intrathoracic lymph nodes in more than 90% of patients (128–130). The clinical spectrum of sarcoidosis is protean, but pulmonary manifestations dominate (128–130). Chest radiographs are abnormal in more than 90% of patients with sarcoidosis (130) and are usually adequate to

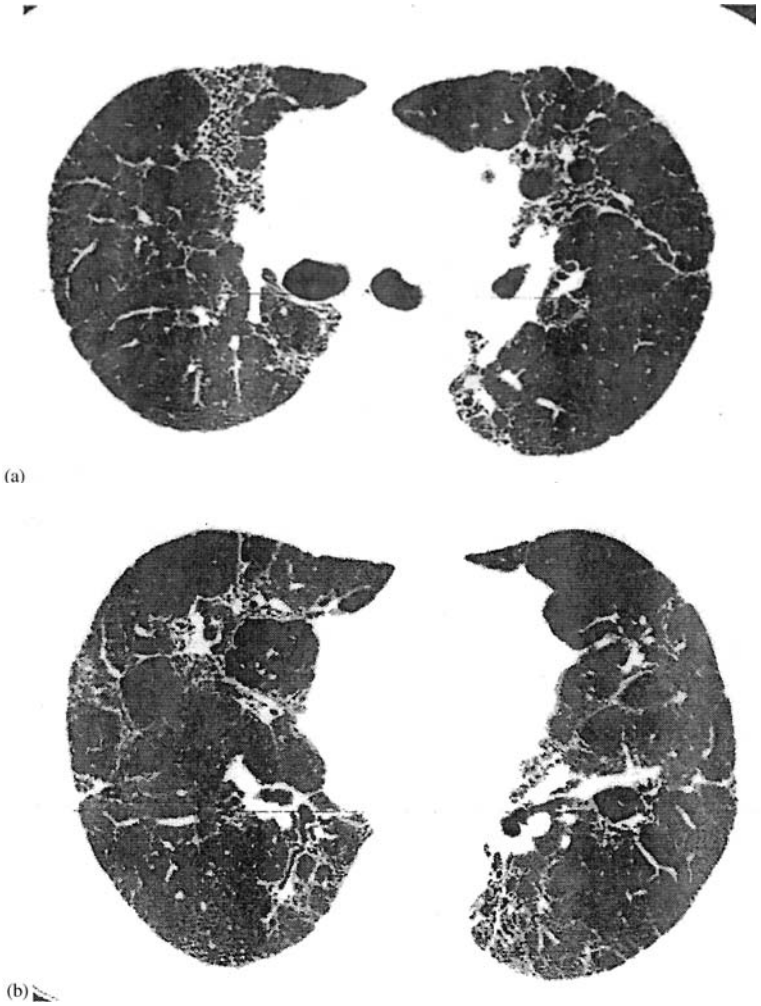

Figure 29 (a) Chronic hypersensitivity pneumonia. HRCT scan (level of carina) reveals thickened interlobular septae, distortion, and focal honeycomb cysts in a 58 year old male with chronic HP. (b) Chronic hypersensitivity pneumonia. HRCT scan (lower lobes) from the same patient demonstates thickened interlobular septae, focal areas of ground glass opacities, and faint peribronchiolar nodules.

delineate the extent of intrathoracic lymphadenopathy and parenchymal involvement (17).

Routine chest CT (either conventional or HRCT) is *not* necessary to diagnose or stage sarcoidosis, and is not cost effective (131). Lymph node enlargement (mediastinal or hilar) is noted in more than 75% of patients with

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sarcoidosis (17,131,132). In addition, conventional CT often demonstrates enlarged lymph nodes in subcarinal, paratracheal, pretracheal, or para-aortic areas (131) (Fig. 30). However, this additional information is rarely of clinical value. HRCT may be helpful in atypical cases or to discriminate alveolitis from fibrosis (17). Characteristic HRCT features of sarcoidosis include distribution along bronchovascular bundles and lymphatics (Figs. 31–32), middle or upper

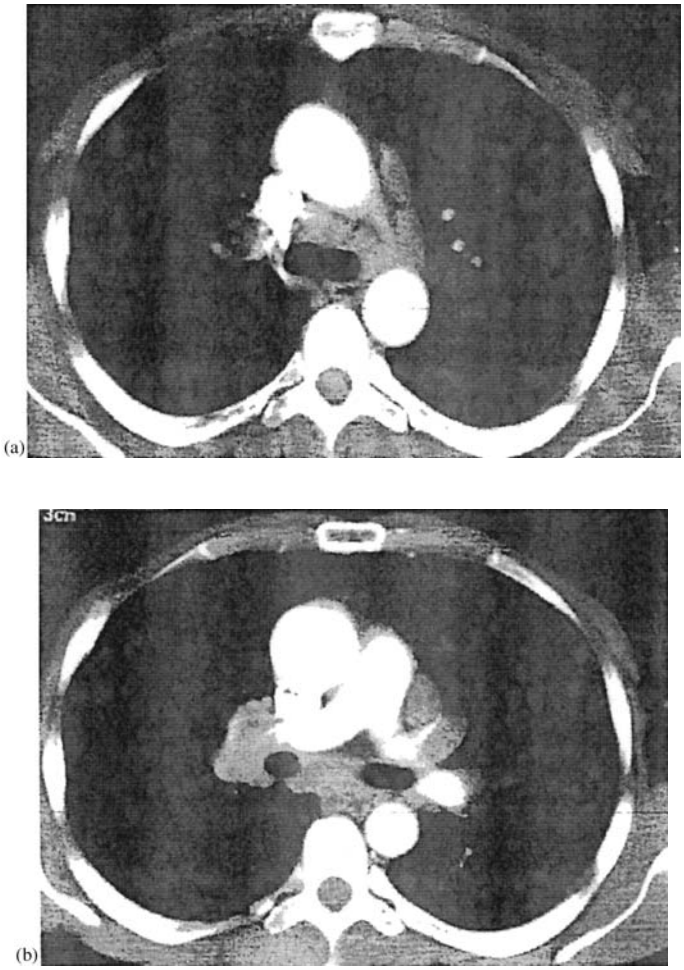

Figure 30 (a) Sarcoidosis. Conventional CT scan at the level of the aortic arch demonstrates pre-tracheal and para-aortic lymph node enlargement. (b) Sarcoidosis. Conventional CT scan from the same patient at the level of the carina demonstrates bilateral hilar lymphadenopathy, marked widening of the carina, and subcarinal lymph node enlargement.

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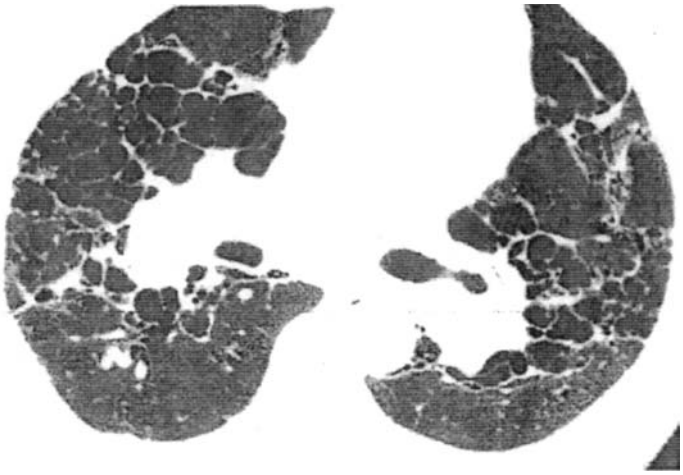

Figure 31 Cystic sarcoidosis. Extensive cysts in a perihilar (axial) distribution. The distribution of cysts along bronchovascular bundles is a clue to the diagnosis of sarcoidosis.

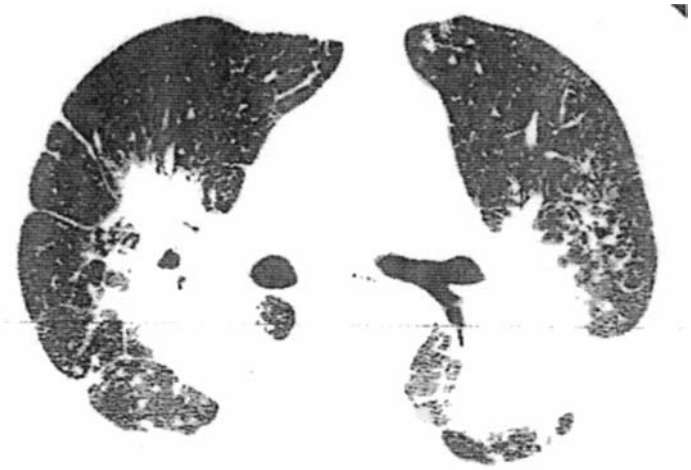

Figure 32 Sarcoidosis. HRCT at level of carina demonstrates confluent alveolar opacities, forming dense mass-like lesions, radiating out from the hilae. A few scattered nodules are also present.

lung zone predominance (Figs. 33–35), irregularly thickened bronchovascular bundles (88%), small nodules (< 3 mm in diameter) mainly in peribronchovascular and subpleural locations (50%) (Figs. 36–38), confluent nodular opacities with airbronchograms (31–44%) (Figs. 39–40), crowding and

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Figure 33 Sarcoidosis. HRCT scan shows large confluent masses radiating from the hilae; a mycetoma is evident in the left lung. Note the absence of honeycomb cysts in the peripheral subpleural regions.

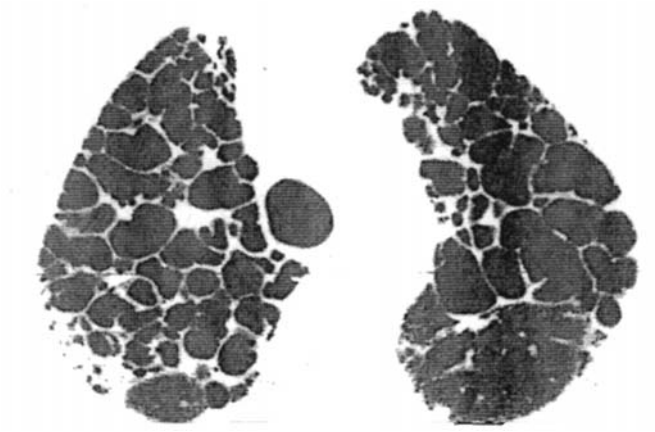

Figure 34 Advanced fibrocystic sarcoidosis. HRCT lobes (apices) demonstrate nearly complete replacement of lung parenchyma by cysts. The upper lobe predominance is typical of sarcoidosis.

retraction of bronchi and vessels near the hila; and traction bronchiectasis (133,134). The predilection of sarcoidosis for upper and mid lung zones, along bronchovascular bundles and lymphatics, is in sharp contrast to IPF/UIP, which has a predilection for the basilar and subpleural regions (Fig. 31). With

advanced disease, architectural distortion and cystic destruction may occur (133,134) (Fig. 34). Specific CT features have prognostic significance. Focal nodular, alveolar, or GGOs suggest an active inflammatory component, whereas distortion of lung parenchyma, linear bands, bronchiectasis, cystic radiolucencies, and bullae reflect irreversible fibrosis (17,135,136).

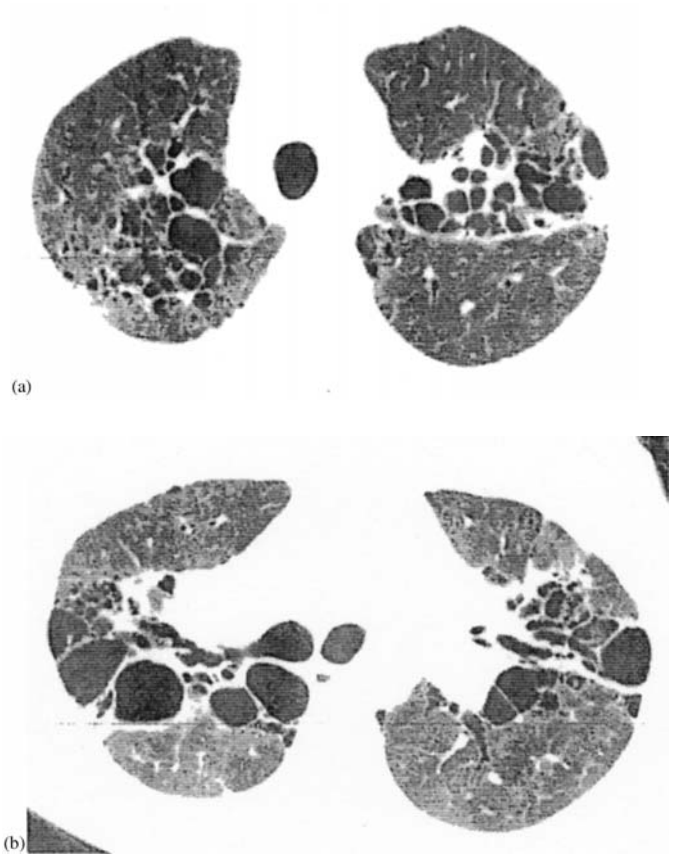

Figure 35 (a) Sarcoidosis. HRCT scans demonstrate multiple cysts in an axial distribution. The sub-pleural regions are relatively spared. The presence of cysts in the apices, in a bronchovascular location, is a clue to the diagnosis of sarcoidosis. (b) Sarcoidosis. HRCT from the same patient at the level of the carina. Multiple cysts are present; again, the perihilar distribution is classical for sarcoidosis. (c) Sarcoidosis. HRCT scan in the same patient in the lower lobes. Focal bronchiectatic changes and scattered ground glass opacities. (d) Sarcoidosis. HRCT form the same patient (basilar regions). A few scattered cysts are evident, but the lung architecture is less disrupted compared to cuts from the upper and mid lung zones. Bronchial dilatation, consistent with traction bronchiectasis, is also present.

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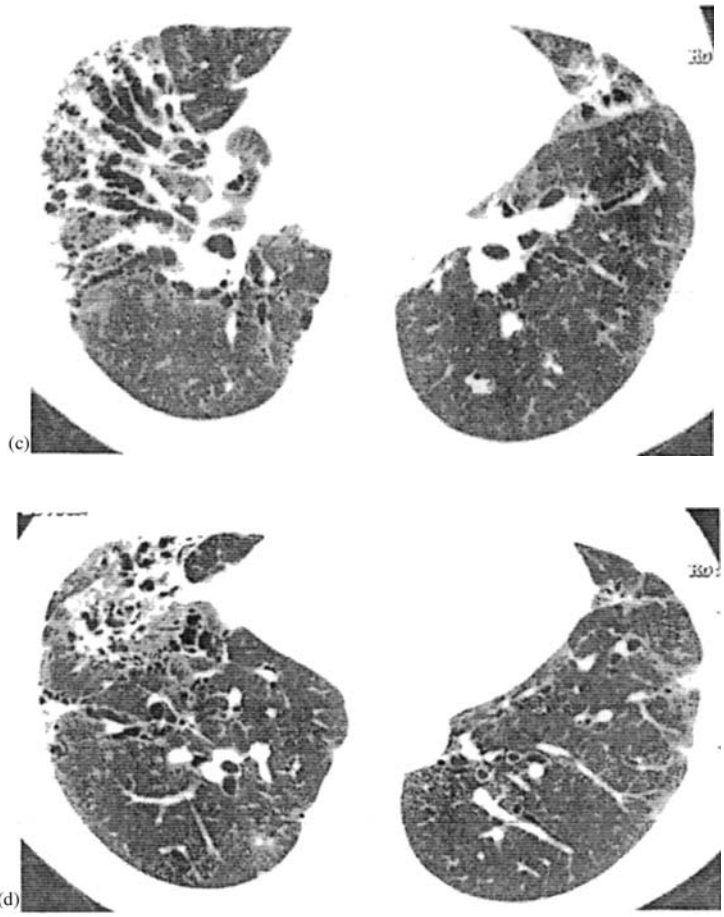

Figure 35 Continued.

Extensive cystic changes on CT scan may resemble UIP. However, the preferential distribution of the cystic lesions along bronchovascular bundles and lymphatics in an axial distribution distinguishes sarcoidosis from UIP (137) (Fig. 32). In addition, nodularity predominates in sarcoidosis, whereas UIP is predominantly reticular. Thickened bronchovascular bundles, thickened interlobular septa, and subpleural nodules are also found in lymphangitic carcinomatosis and lymphoma (105). In lymphangitic carcinomatosis, there is greater involvement of the interlobular septa and subpleural interstitium, with septal nodularity, beading, and polygon formation. Large nodules (11–30 mm), often containing air bronchograms, are common in lymphoma (105).

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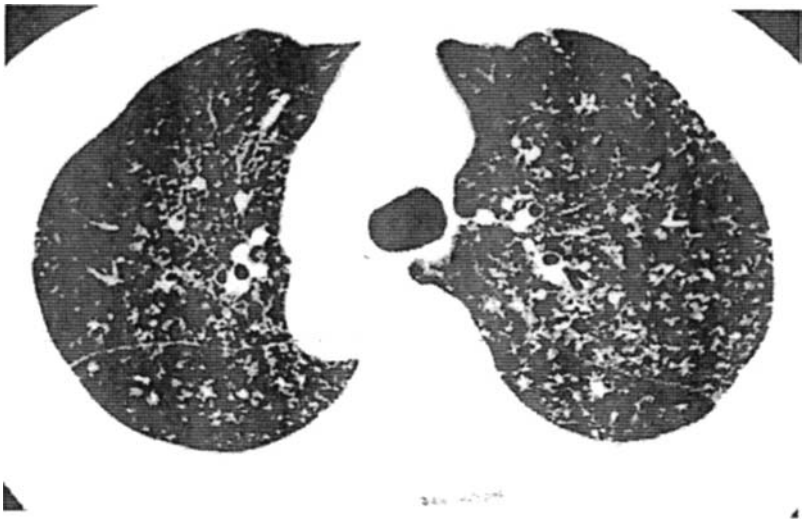

Figure 36 Sarcoidosis. HRCT scans (upper lobes) demonstrates innumerable, 1-3 mm "miliary" nodules. Transbronchial lung biopsies confirmed the diagnosis of sarcoidosis. The nodules resolved completely with corticosteroid therapy.

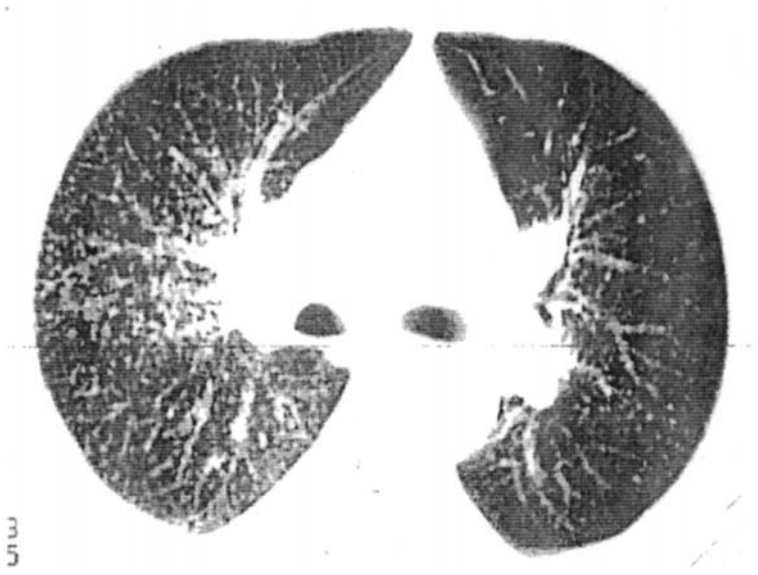

Figure 37 Pulmonary sarcoidosis. HRCT scan at the level of the carina demonstrates innumerable nodules; the distribution along bronchi gives some of the bronchi a "beaded" appearance.

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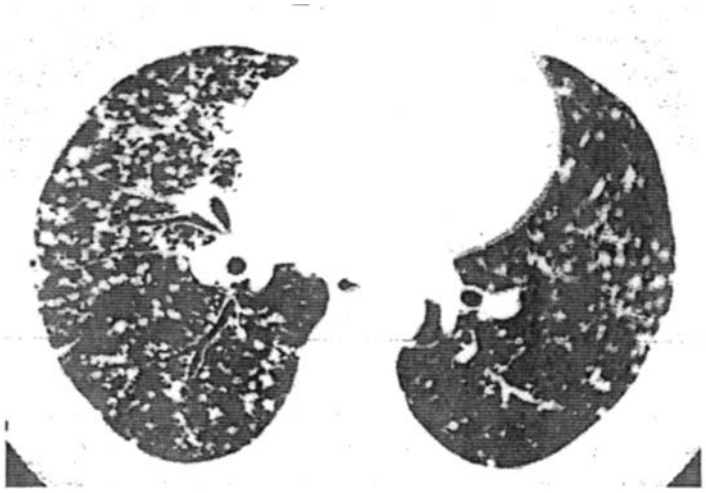

Figure 38 Sarcoidosis. Macroscopic nodules are interspersed throughout both lung fields. Note the absence of reticular or honeycomb change.

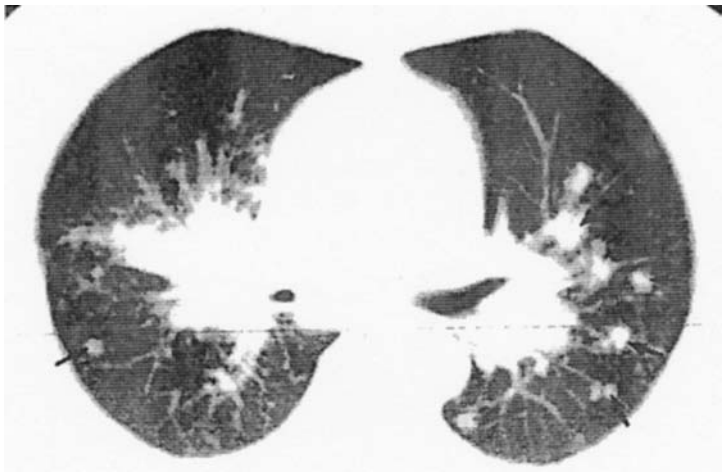

Figure 39 Sarcoidosis. HRCT scan demonstrates multiple macroscopic nodules (arrows), bilateral hilar lymphadenopathy, and extensive alveolar infiltrates radiating from the hilae along the bronchovascular bundles. These features are typical for sarcoidosis. (Reproduced with permission. From Ref. 163.)

Given the variable natural history and potential for spontaneous remissions, treatment of sarcoidosis is controversial. Indications for treatment should be focused and circumscribed. We recommend corticosteroids for patients with progressive or persistent sarcoidosis (pulmonary or

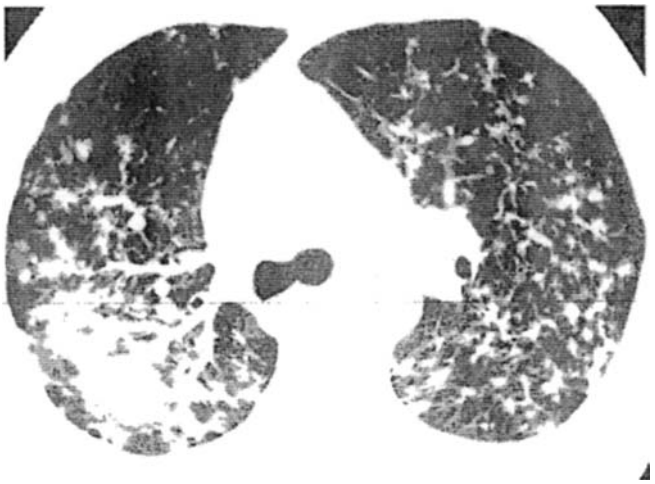

Figure 40 Sarcoidosis. CT image (5-mm collimation) of a 34-year old woman demonstrates multiple bilateral ill-defined pulmonary nodules and small irregular linear opacities, coalescing to form a conglomerate mass in the right upper lobe. (Reproduced with permission. From Ref. 13.)

extrapulmonary) (129,130). Immunosuppressive or cytotoxic agents or antimalarials (e.g., hydroxychloroquine) can be considered for patients who do not benefit from corticosteriods or experiencing adverse effects from (130).

J. Langerhans Cell Granulomatosis (LCG)

Pulmonary Langerhans cell granulomatosis (LCG), also termed Langerhans cell histiocytosis or pulmonary eosinophilic granuloma (EG), is a rare disease in smokers, presenting as chronic interstitial lung disease or pneumothoraces (54,138). Histologically, pulmonary LCG is characterized by inflammatory, cystic, nodular, and fibrotic lesions distributed in a bronchocentric fashion (53,54,138). Numerous cystic lesions are characteristic of LCG, but the CT appearance differs markedly from IPF/UIP. In LCG, CT demonstrate numerous thin-walled cysts with irregular or bizarre shapes, distributed predominantly in the middle or upper lung zones (53,54,139,140) (Figs. 41–43). The cysts are usually round and less than 10 mm in diameter, but may coalesce, occasionally exceeding 3 cm in size (139,140) (Figs. 43a–d). Irregular peribronchiolar nodules, ranging in size from 1 to 15 mm, are also a prominent feature, being observed in 67–78% of patients; the nodules represent granulomas (53,54,139,140), whereas the cysts represent destroyed and dilated bronchi and small airways (141) (Figs. 43a–d). The cysts or nodules are associated with areas of intervening normal lung tissue (53,139). There is no central or peripheral predominance; the costophrenic angles tend to be spared

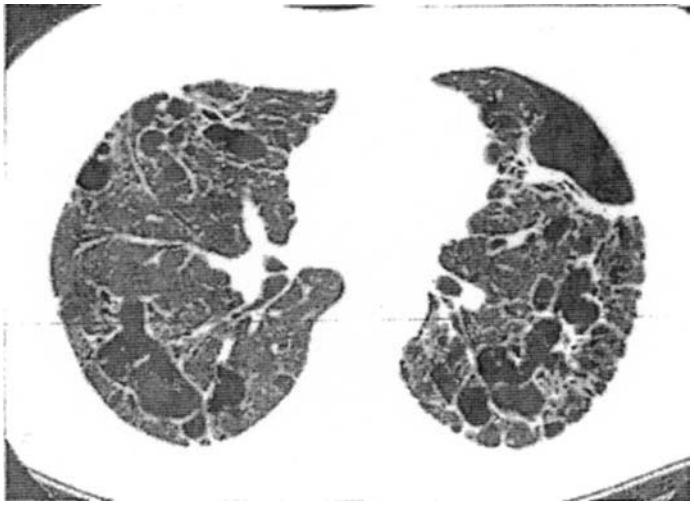

Figure 41 Langerhans cell granulomatosis (LCG). HRCT demonstrates extensive cysts, with some bizarre shapes.

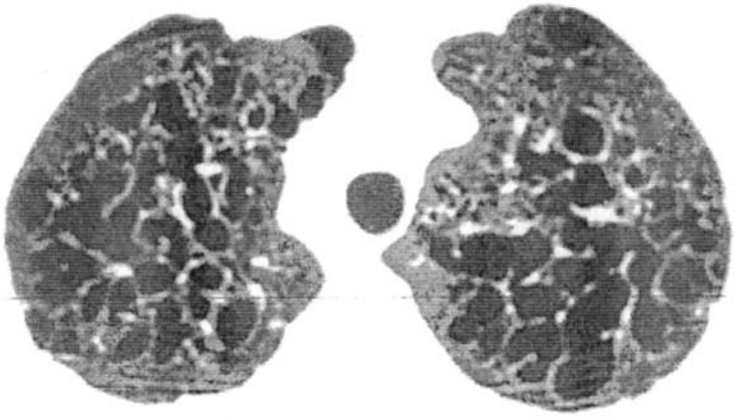

Figure 42 Langerhans cell granulomatosis. HRCT (apices) demonstrates extensive cystic destruction of upper lobes in a 38 year old male with LCG. In addition, scattered nodules are present. The combination of cysts, nodules, and upper lobe predominance strongly suggests LCG.

(53,54). Other CT abnormalities in LCG include cavitated nodules (17%), thick-walled cysts (39%), reticulation (22%), GGOs (22%), and irregular interfaces (22%) (139). Pleural effusions or hilar adenopathy are rare (54,138,139). CT features may progress from nodules to cavitated nodules, thick-walled

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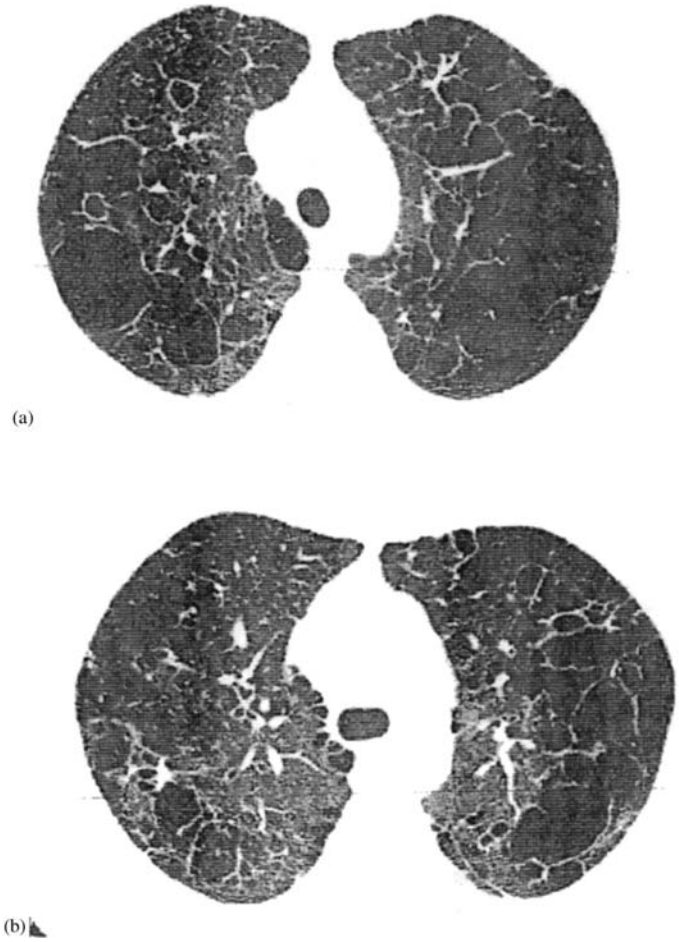

Figure 43 (a) Langerhans cell granulomatosis (LCG). HRCT scan (upper lobes) demonstrates marked destruction of lung parenchyma by cysts, some of which have coalesced and assumed bizarre shapes. A few faint nodules are visible. (Reproduced with permission. From Ref. 84.) (b) Langerhans cell granulomatosis. CT from the same patient demonstrates extensive cystic radiolucencies. Marked peribronchiolar thickening and scattered dense nodules are present, consistent with an active inflammatory component. (Reproduced with permission. From Ref. 84.) (c) Langerhans cell granulomatosis. HRCT from the same patient demonstrates extensive cysts, but also prominent bronchiolar thickening, nodules, and bronchial dilatation. These features are consistent with an active granulomatous process within the small airways. (d) Langerhans cell granulomatosis. HRCT from the same patient at the level of the carina shows prominent bronchial thickening, nodules, and patchy ground glass attenuation consistent with an active inflammatory process. Only a few cystic radiolucencies are apparent.

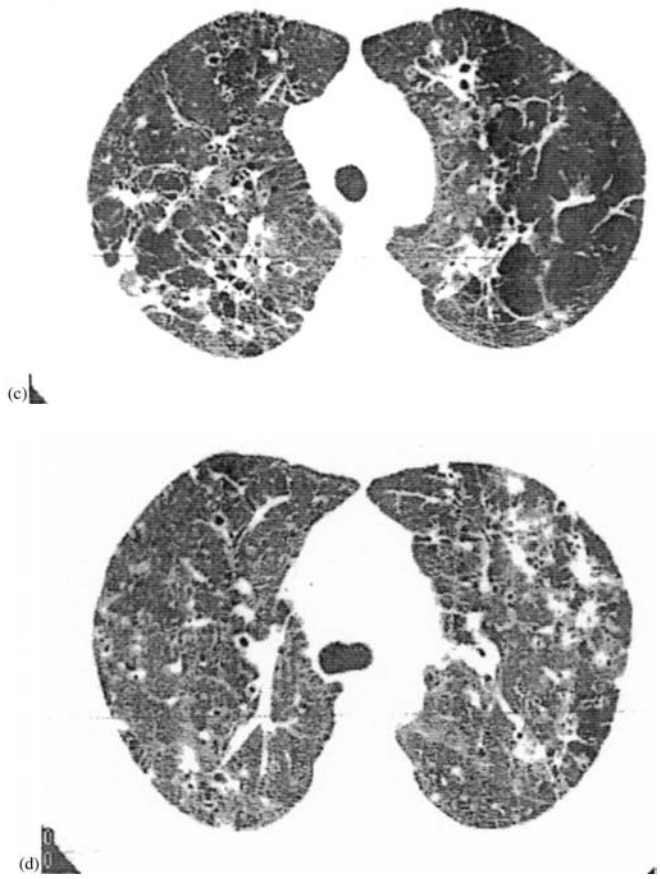

Figure 43 Continued.

cysts, thin-walled cysts, and confluent cysts (139,140). Nodular opacities, thick-walled cysts, and GGOs may regress, whereas thin-walled cysts, linear opacities, and emphysematous lesions do not regress (53,140).

Therapy for pulmonary LCG is controversial. Cessation of cigarette smoking is mandatory. Anecdotal responses have been claimed with corticosteroids, vinca alkaloids, and immunosuppressive or cytotoxic drugs, but data affirming efficacy are lacking (53,54,138). Lung transplantation is an option for patients with end-stage pulmonary LCG (54).

K. Lymphangioleiomyomatosis (LAM)

Lymphangioleiomyomatosis (LAM) is a rare disorder seen in women primarily of childbearing age. Cardinal clinical features include progressive airflow

obstruction, recurrent pneumothoraces, hemoptysis, or chylothoraces (56,142,143). Pathologically, LAM is characterized by an abnormal proliferation of atypical smooth muscle cells and cyst formation (56,142,143). HRCT may be virtually pathognomonic of LAM. Innumerable, small thin-walled cysts (ranging in size from a few millimeters to > 6 cm) are distributed diffusely throughout the lungs (55,144–146) (Figs. 44–46). The intervening lung parenchyma is normal. Cysts may assume bizarre shapes as multiple cysts coalesce (Fig. 44a). Nodules, reticular lines, or irregular lung-pleural interfaces are not found in LAM. Rarely, severe, diffuse cystic LCG can mimic LAM. However, in contrast to pulmonary LCG (138,139), LAM involves the lung

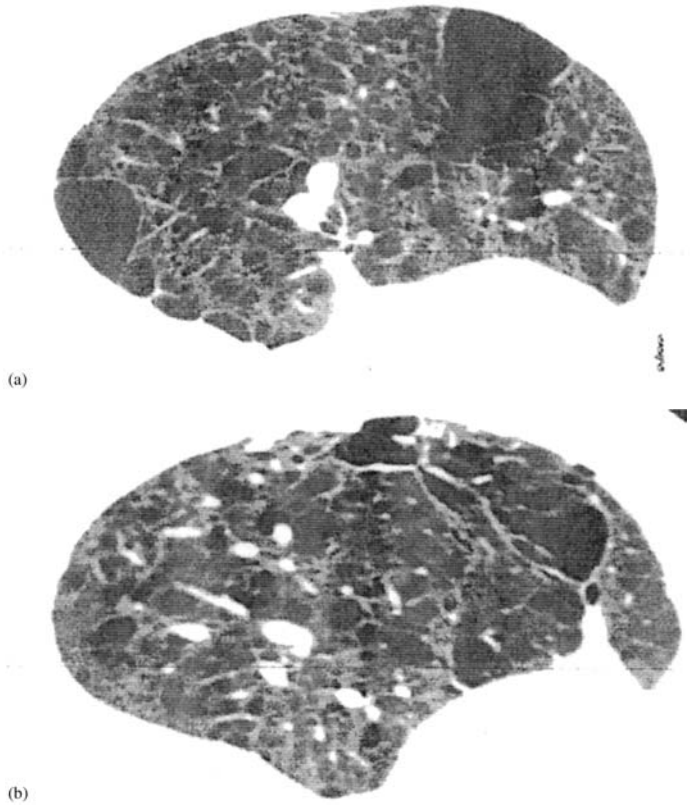

Figure 44 (a) Lymphangioleiomyomatosis. CT in a 44-year old woman with LAM demonstrates multiple, thin-walled cystic radiolucencies. Note the two large lesions, representing confluent cysts. (Reproduced with permission. From Ref. 85.) (b) Lymphangioleiomyomatosis. HRCT from the same patient showing innumerable well-defined cysts. The cysts are distributed diffusely, without a propensity for a particular region or zone of the lung.

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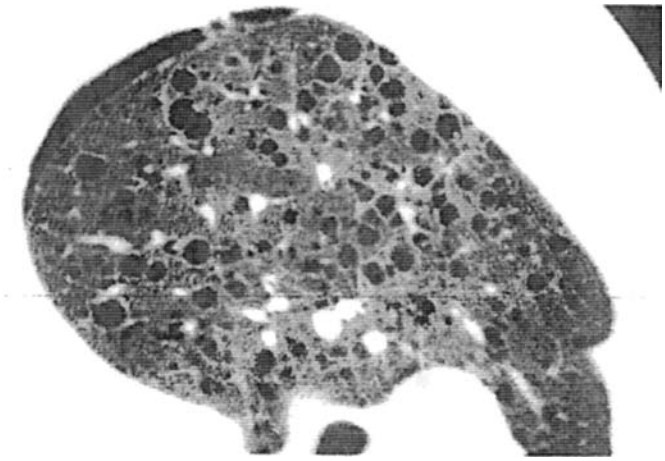

Figure 45 Lymphangioleiomyomatosis. CT demonstrates multiple, thin-walled cystic radiolucencies throughout lung parenchyma. Note small associated pneumothorax, a typical finding in LAM. (Reproduced with permission. From Ref. 85.)

diffusely, with no upper or mid lung predominance (147); further, a nodular component, which is a prominent feature of pulmonary LCG, is lacking in LAM (144,145) GGOs, not cited in early reports of HRCT in LAM (147,148), were noted in 12% (8 of 66) and 59% (22 of 37) patients in two more recent series (143, 149). Semiquantitative and quantitative analysis of the extent of disease by HRCT correlated well with physiological parameters (e.g., FEV_1, DLCO, and gas exchange at rest and exercise) (147) and exercise performance (55). Other radiological findings include pneumothoraces (29–81%), and pleural effusions (21–39%) (143,148,149). Mediastinal or intrathoracic lymphadenopathy is unusual (149), but abdominal CT scans reveal cysts or angiomyolipomas in kidney, spleen, or pelvic organs or retrocrural or para-aortic lymphadenopathy in 30–80% of patients with LAM (56,143,150,151). Cystic radiolucencies may also be observed in any chronic lung diseases, but the distribution and nature of these cystic lesions differs from pulmonary LAM. Honeycomb cysts, a prominent feature of IPF and collagen vascular disease–associated pulmonary fibrosis, are distributed in the peripheral (subpleural) regions of the lung and are nearly invariably accompanied by linear bands and distortion of lung parenchyma (13,152). In addition, those disorders are patchy and heterogeneous, whereas LAM is more homogeneous. Severe cystic lesions in LAM resemble emphysema, but the cysts in LAM are more regular and have well-formed walls (148,151).

The prognosis of LAM (with or without therapy) is poor, with inexorable progression over 5–15 years (56,142,143). Ten-year survival ranges from 23 to 78%; most deaths are due to progressive respiratory

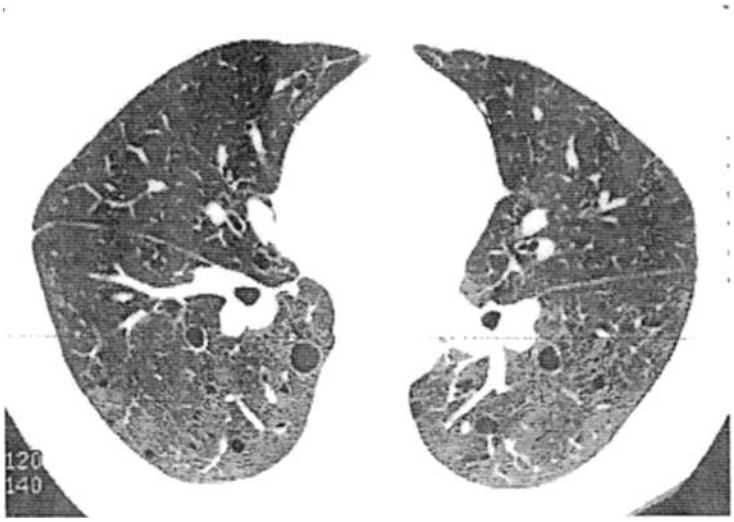

Figure 46 Lymphangioleiomyomatosis (LAM). HRCT scan from a 28-year old female with a history of recurring pneumothoraces. Multiple, well circumscribed cysts are present bilaterally, with large areas of intervening normal lung parenchyma. (Reproduced with permission. From Ref. 84.)

failure (56,142,143,148,149,151). Optimal therapy is controversial. Treatment strategies designed to ablate the effects of estrogens are used but are of unproven benefit. Patients with life-threatening, debilitating disease and progressive airflow obstruction may be candidates for lung transplantation.

In summary, HRCT is invaluable to narrow the differential diagnosis of interstitial lung disorders, and in some cases, the pattern may be pathognomonic (e.g., UIP, LAM). HRCT may enable clinicians confidently to identify UIP when HRCT features are classic. However, when CT features are atypical for UIP, surgical lung biopsy is often essential to establish a specific histological diagnosis.

VI. Relationship of HRCT Features and Pulmonary Function in UIP

The global extent of disease on HRCT correlates roughly with severity of functional impairment in IPF/UIP (6,10,153); the pattern of CT does not (6, 154). The extent of disease on HRCT correlates better with DLCO than lung volumes or spirometry (6,10,153). In one study of 39 patients with IPF, the global extent of disease on CT showed a moderate but significant correlation with forced vital capacity (FVC) and DLCO; the extent of GGOs

correlated with FVC (10). The extent of GGOs and global extent of disease on CT correlated with arterial Po_2 at peak exercise (10). In another study of pulmonary fibrosis complicating Hermansky-Pudlak syndrome, the extent of disease on HRCT correlated with FVC and DLCO (155). British investigators assessed HRCT scans in 68 patients with CFA; emphysema was present concomitantly in 14 patients (6). Both the extent of fibrosis on CT and emphysema were independent determinants of functional impairment. Among the 14 patients with emphysema, lung volumes (FVC and total lung capacity) were preserved and DLCO and Pao_2 were reduced. In patients *without* emphysema on CT, the extent of disease on CT correlated best with percentage of predicted DLCO ($r = -0.68$), oxygen desaturation with exercise ($r = -0.64$), and the physiologic component of the clinical-radiographic-physiological (CRP) score ($r = -0.62$); spirometry or lung volumes were less useful. However, these global correlations are of limited practical value in individual patients. Pulmonary physiological tests are essential to document the extent of impairment and monitor the course of the disease.

VII. Prognostic Value of HRCT Pattern in Patients with UIP

Specific HRCT patterns such as GGOs or HC correlate with specific histological features and are of prognostic value (8,9,28,156). Semiquantitative scoring systems have shown that the extent of GGOs on CT correlates with the degree of histological inflammation on SLBx (32,49), whereas CT fibrotic scores correlate with the pathological extent of fibrosis (7,32,49). GGOs may represent interstitial or alveolar inflammation (13,49), intra-alveolar granulation tissue (8), fibrosis of intralobular and alveolar septa (157), or a mixture of fibrosis and inflammation (13). Although pure GGOs may reflect alveolitis, GGOs in association with reticular lines and dilated bronchi or bronchioles (traction bronchiectasis or bronchiolectasis) invariably represents fibrosis (13). A reticular pattern (intersecting linear lines) reflects fibrosis within alveolar ducts, septae, or spaces, but an inflammatory component may coexist (8,13). Honeycomb change HC reflects irreversible destruction of alveolar walls and fibrosis (13,65,157).

The predominant pattern on HRCT (i.e., GGOs, reticular pattern, or HC) may be useful in assessing potential responsiveness to therapy (6–10,13, 28, 156, 158). Early studies in patients with IPF or CFA (not all of whom had SLBx) affirmed that a pattern of "predominant GGO" on HRCT predicted a higher likelihood of response to corticosteroid therapy (Fig. 47a and b) and improved survival compared to reticular patterns or HC (8–10,28,49,57,69,87,156,157,159). Further, on serial CT scans, GGOs improved with corticosteroid therapy in 33–44% of patients, whereas reticular

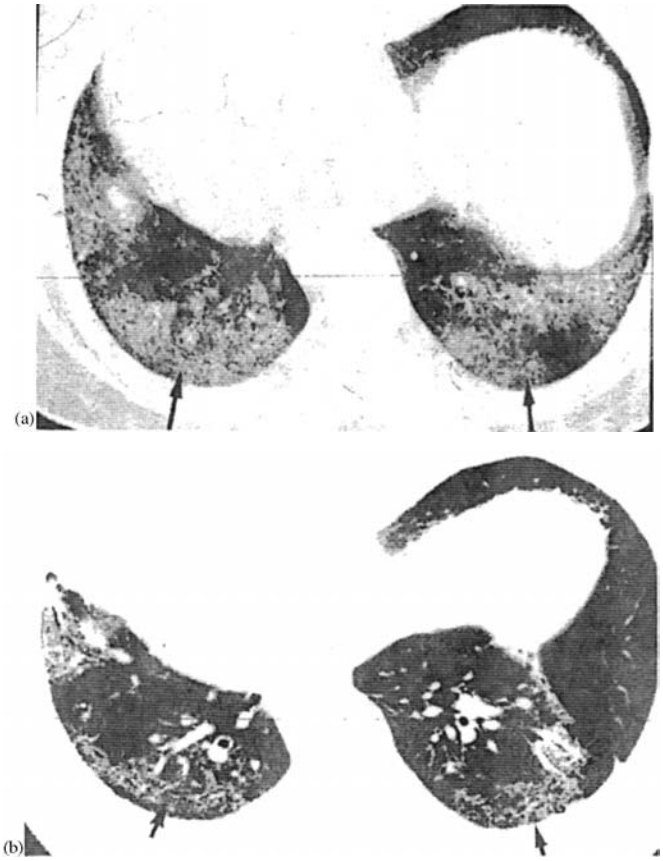

Figure 47 (a) Ground glass opacities. HRCT (lower lobes) from a 29-year old woman in whom open lung biopsies in 1991 demonstrates changes consistent with IPF. Focal alveolar (ground glass) opacities (see arrows) are seen at the posterior aspects of both lung bases. Note the patchy and sub-pleural distribution. (Reproduced with permission. From Ref. 164.) Although she was initially labelled as idiopathic pulmonary fibrosis, manifestations of systemic lupus erythematosus (SLE) developed over the next 2 years. (b) Resolving ground glass opacities. Following 3 months of high dose prednisone, marked regression of the GGO was noted. Some residual reticular lines and GGO persisted. Unfortunately, over the next 3 years, her disease progressed and she ultimately died of respiratory failure while awaiting lung transplantation.

patterns or HC *never* improved (6,10,13,87). However, these sentinel studies may be misleading. Extensive or ''predominant'' GGO is rarely found in UIP. Most patients with IPF display mixed patterns, with predominantly reticulation or HC admixed with scattered GGOs (10,13,28). In two series of patients with IPF, a predominant GGO pattern on CT was observed in only 4 of 39

(10%) (10) and 8 of 76 patients (10%) (28), respectively. Mixed patterns (i.e., both reticular and GGOs) were observed in 87.5% (10) and 64% (28), respectively. *Pure* reticular patterns were observed in only 2.5% (10) and 24% (28). Since surgical (open or thoracoscopic) lung biopsies were obtained in a minority of patients in these studies (10,28), it is likely that predominant GGOs in patients with presumed IPF/CFA in these and other series represented histological diagnoses other than *UIP* (e.g, DIP or cellular NSIP) (45,57,61).

Notwithstanding these limitations, we will briefly review these sentinel studies. In 1992, Lee et al. correlated initial CT patterns with response to corticosteroid therapy in a cohort of 19 patients with IPF (only 7 patients had surgical lung biopsies) (9). The extent of GGOs on CT correlated with improvements in DLCO, FVC, and FEV_1 following corticosteroid therapy. Patients with HC and minimal GGOs showed minimal or no improvement. In 1993, British investigators reported a series of 56 patients with pulmonary fibrosis in whom serial CT scans had been performed (156). Twenty-one patients had lone CFA (which is synonymous with IPF); 35 had fibrosing alveolitis (FA) associated with progressive systemic sclerosis (FASSc) (156). Initial CT scans were assessed for both extent and pattern of disease, and correlated with results of pulmonary function tests (PFTs). A reticular pattern was defined as the presence of intersecting lines in a fine network or honeycomb change. Initial scans were categorized as grade 1 (GGOs more extensive than reticular pattern) ($n = 10$); grade 2 (GGOs = reticular) ($n = 18$); grade 3 (reticular > GGO) ($n = 28$). Repeat CT scans were performed at a median interval of 16 months. On serial CT scans, the global extent of disease worsened in 15 patients decreased in 19 patients, and did not change in 22 patients. Importantly, reduction in the global extent of disease was due to regression of GGO in 18 of 19 patients; a reticular pattern *never* regressed. Overall, GGOs regressed in 21 patients and increased in 5 patients; in 4 patients, GGOs evolved to a reticular pattern. Among 28 patients with initial grade 3 CT, the reticular pattern increased (worsened) in 10 patients and did not change in the remaining 18 patients. In a subset of 40 patients treated between scans, the extent of disease decreased in 6 of 6 patients with grade 1 CT compared to 5 of 13 patients with grade 2 and 5 of 21 patients with grade 3 ($P < .001$). This relationship was independent of disease distribution, extent, or type of FA. Increases in the extent of disease (i.e., worsening) was more frequent in grade 2 (5 of 13 patients) and grade 3 (6 of 21 patients) compared to grade 1 CT (0 of 6 patients). Changes in FVC and CT were concordant in all but two cases. Changes in DLCO were also concordant with CT in all but three patients. Importantly, PFTs improved in 8 of 11 patients exhibiting regression of GGOs on serial CT scans. In another retrospective study by these investigators, the prognostic value of HRCT scans was evaluated in 76 patients with lone CFA and 66 with FASSc (28). Similar to the previous report, CT scans in patients with lone CFA were categorized as grade 1 ($n = 8$), grade 2

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(n = 18), or grade 3 (n = 50). Response to therapy and 4-year survival were best with grade 1 CT and worst with grade 3. Response to corticosteroid or immunosuppressive therapy was evaluated in 27 patients after 3–6 months. PFTs improved in all 4 patients with grade 1 CT but in only 4 of 12 patients with grade 2 and 0 of 11 patients with grade 3 CT. Although overall mortality was 50% (38 of 76 patients died), none of 8 patients with grade 1 CT died during a mean follow-up of 41 ± 11 months). By contrast, 45% of the patients with grade 2 and 80% of the patients with grade 3 CT died during that time frame. A subsequent study by these investigators analyzed CT scans in 111 patients with FA (54 patients had lone CFA; 57 patients had $FASS_C$ [154]). Serial CT scans were performed in 34 patients treated with corticosteriods (with or without cyclophosphamide). GGOs regressed in 15 patients (44%); overall extent of disease on CT diminished in all 15 of these ''responders.'' In striking contrast, reticular patterns worsened or persisted in *every* patient. Spanish investigators performed serial CT scans in 23 patients with IPF (10). The extent of GGOs increased in 7 patients, decreased in 7 patients, and did not change in nine patients; reticular patterns *never* regressed. Terriff et al. assessed serial CT scans in 26 patients with FA (19 patients had CFA/IPF; 7 patients had FA associated with collagen vascular disease) (159). With treatment, GGOs on CT often diminished, but later reappeared or progressed to irregular opacities or HC in the same areas. Japanese investigators retrospectively reviewed serial CT scans in 29 patients with IPF (157). Although GGO initially regressed with therapy, progression to HC eventually ensued in 26 of 29 patients (90%); areas of GGO preceded the development of HC in that location. Hartman et al. performed serial CT scans in 12 patients with UIP treated with corticosteriods; GGOs worsened or progressed to reticular lines or HC in 9 patients and improved in 3 patients (87). These various studies suggest that a ''predominant GGO pattern'' on HRCT predicts a better prognosis and greater responsiveness to therapy compared to reticular or mixed patterns. Unfortunately, grade 1 (i.e., predominantly GGOs) patterns are rarely observed in UIP. In addition, despite early regression in some patients (28,154), GGOs usually progress inexorably to reticular abnormality and HC (87,156,157,159). Given the potential for fibrosis to evolve over time, the value of CT in predicting long-term prognosis is modest.

VIII. Prognostic Value of Fibrotic Scores on CT

As has been discussed, the presence of a reticular or honeycomb pattern is associated with fibrosis and a lack of responsiveness to therapy (10,154,156). Quantifying the degree of fibrosis (by assessing reticular opacities or HC) may be prognostically useful in IPF/UIP. Complex quantitative scoring systems (using stereological methods) correlate well with histology on open lung biopsy

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(160), but are too cumbersome to be of practical value. The degree of CT fibrosis on semiquantitative scoring systems is an important independent predictor of mortality (7,32,161). Daniil et al. assessed the degree of fibrosis semiquantitatively in a cohort of 12 patients with UIP and 15 patients with NSIP (44). A fibrotic score was devised based upon the extent (scored 0 = absent to 3 = throughout the lung) and type of fibrotic change (e.g., HC, interstitial thickening, and traction bronchiectasis). The total fibrosis score (range 0–9) was derived as the sum of the individual scores for each component (44). Patients with UIP had a higher median fibrosis score (5 vs 3; $P = .011$) compared to patients with NSIP. We developed a semiquantitative CT scoring system (32) (Table 3) and prospectively applied this to a cohort of 38 patients with IPF (all were treated with high-dose corticosteroids) (7). Surgical lung biopsies and pretreatment CT scans were quantitatively scored. GGOs (CT-alv) and linear (reticular) opacities (CT-fib) were graded on a scale of 0 to 4. Highly significant correlations were found between CT fibrotic scores ($P = .009$) and pathology fibrosis scores (by SLBx) ($P = .03$). Correlations between CT-alv and pathology cellularity scores were statistically significant but less exact. Pretreatment CT alveolar (CT-alv) and fibrotic (CT-fib) scores predicted responsiveness to therapy and mortality. Responders to prednisone

Table 3 Semiquantitative HRCT Scoring for Ground-Glass and Interstitial Infiltrates

Ground-Glass Score	
0	No alveolar disease
1	GGO involving < 5% of the lobe (minimal but not normal)
2	GGO involving up to 25% of the lobe
3	GGO involving 25–49% of the lobe
4	GGO involving 50–75% of the lobe
5	GGO involving > 75% of the lobe
Interstitial Score	
0	No interstitial disease
1	Thick interlobular septal thickening; no discrete honeycombing
2	Honeycombing (± septal thickening) involving up to 25% of the lobe
3	Honeycombing (± septal thickening) involving 25–49% of the lobe
4	Honeycombing (± septal thickening) involving 50–75% of the lobe
5	Honeycombing (± septal thickening) involving > 75% of the lobe

GGO, ground-glass opacity.
Source: Ref. 32.

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therapy had higher CT-alv scores compared with nonresponders (NR) or stable patients ($P = .004$). Further, survivors had higher CT-alv scores and lower CT-fib scores compared with those who died during follow-up. Severe fibrosis on pretreatment CT (CT fibrosis score of ≥ 2 [representing interstitial infiltrate or HC in up to 25% of the lung] predicted subsequent mortality over a 3-year period with a sensitivity of 80% and a specificity of 85%. In that study, HRCT was a better predictor of survival than PFTs or CRP scores. Further, the addition of PFTs, CRP scores, or pathology scores did not improve the ability of HRCT to predict survival. British investigators also found that HRCT fibrotic scores predicted survival in a cohort of 115 patients with UIP awaiting lung transplantation (161). By univariate analysis, 12 variables correlated with 2-year survival on the transplant waiting list. However, by multivariate stepwise regression analysis, only DLCO percentage of predicted and HRCT-fibrotic scores were independent predictors of survival. The risk of death increased by 4% for every 1% decrease in DLCO percent predicted and increased by 106% for each unit increase in HRCT-fibrotic score. Receiving operating curve (ROC) analysis performed on the logistic regression model gave the best fit (predictive value) using a combination of DLCO and HRCT-fibrotic scores (area under the curve [AUC] of 0.907) compared with AUC of 0.802 for DLCO and 0.863 for HRCT-fibrotic score and 0.693 for FVC percentage predicted. The optimal points on the ROC curves for discriminating between survivors and nonsurvivors corresponded to 39% predicted DLCO and to an HRCT-fibrotic score of 2.25. The curve resulting from the model combining these two parameters yielded a sensitivity and specificity of 82% and 84%, respectively, for discriminating survivors from nonsurvivors. The area under the ROC curve for HRCT score in this study (161) was remarkably similar to our earlier study by Gay et al. (7). In a subsequent study, we assessed the accuracy of fibrotic scores on CT in assessing underlying histology and predicting survival in a cohort of 168 patients with IIPs (UIP = 106, NSIP = 33, RBILD/DIP = 22, other = 7) (46). An HRCT score ≥ 2 in any lobe was highly predictive of UIP; sensitivity for detecting UIP was 90%; specificity 86%; positive predictive value 92%; negative predictive value 69%. Cox regression analysis quantified the impact of a CT interstitial score ≥ 2 in any lobe on mortality while taking into account other features such as age, sex, smoking history, physiological status, time from onset of symptoms, and biopsy pattern. As shown in Table 4, in the absence of histological information, the presence of a CT interstitial score ≥ 2 in any lobe was associated with an increase in mortality (relative risk [RR] of 3.35, 95% CI 1.21, 9.25; $P = .02$). Mortality risk was even higher when a histological diagnosis of UIP was substantiated on SLBx (RR 28.46, 95% CI 5.47, 147.98; $P = .0001$). These studies suggest that the degree of fibrosis on CT is a surrogate marker for the histological pattern of UIP and a severe CT fibrotic score predicts a worse survival.

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Table 4 Risk Ratios from Cox Proportional Hazard Models Predicting Survival in Patients with IIP

Parameters in model	Risk ratio (95% CI)	*P* value
Model excluding histological subclassification		
Patient age	1.01 (0.97, 1.05)	0.73
Female gender	0.53 (0.25, 1.14)	0.11
Positive smoking history	0.43 (0.18, 1.01)	0.05
Physiological score of CRP	1.07 (1.02, 1.12)	0.009
Time of onset of symptoms	1.04 (0.96, 1.14)	0.32
CT-fib ≥ 2 in at least one lobe	3.35 (1.21, 9.25)	0.02
Model including histological *subclassification*		
UIP diagnosis	28.46 (5.47, 147.98)	0.0001
Patient age	0.99 (0.95, 1.03)	0.55
Female gender	0.31 (0.13, 0.72)	0.006
Positive smoking history	0.30 (0.13, 0.72)	0.009
Physiologic score of CRP	1.06 (1.01, 1.11)	0.02
Time of onset of symptoms	1.02 (0.93, 1.12)	0.73
CT-fib ≥ 2 in at least one lobe	0.77 (0.29, 2.04)	0.60

IIP = idiopathic interstitial pneumonia; CRP = clinical, radiographic, and physiologic score (162); CT-fib = interstitial component of the high resolution computed tomograph (see text for details).
Source: Ref. 46.

Further, we assessed the ability of CT to discriminate UIP from NSIP (27). A diagnostic algorithm was used to classify the HRCT pattern as definite UIP, probable UIP, indeterminate (equal probability of UIP or NSIP), probable NSIP, or definite NSIP. Criteria favoring UIP included basilar/subpleural distribution; more reticular infiltrates, and less GGOs. Conversely, criteria favoring a diagnosis of NSIP included more diffuse distribution, less reticular infiltrates, and more GGOs. When HRCT was interpreted as "definite" or "probable" UIP, mortality was higher compared to patients with "indeterminate" CT scans or scans more consistent with NSIP (Fig. 48a). Importantly, a combination of biopsy and HRCT findings provided the most discrete separation of patient survival. Patients meeting both CT and histological criteria for UIP had a higher mortality than histological UIP in the absence of HRCT criteria for UIP (HR = 2.03; $P = .04$). The hazards ratio (HR) for a patient with both histological and CT UIP compared to patients with histological NSIP was 11.4 ($P = .002$) (Fig. 48b). Median survival was worse in patients with histological UIP and CT scans consistent with "probable or definite" UIP (median survival of 2.08 years) compared to patients with histological UIP but an atypical CT for UIP (median survival of 5.76 years) (46). These data are similar to data from a smaller cohort of patients with either NSIP (n = 15) or UIP (n = 12), where

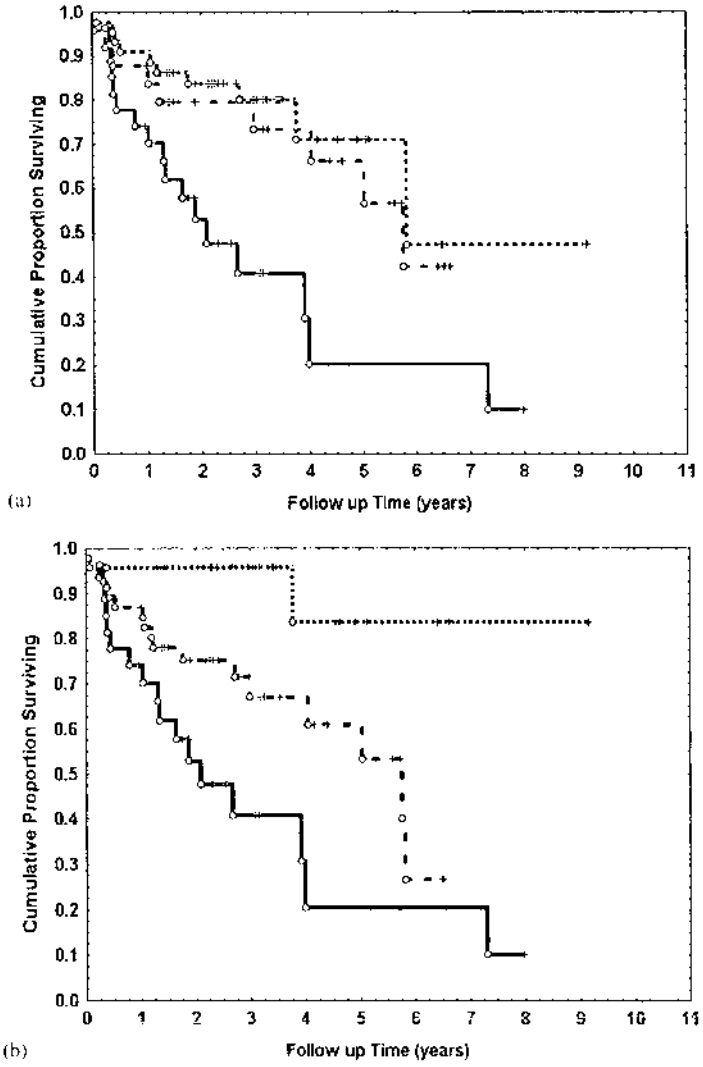

Figure 48 (a) Kaplan-Meier survival curves for patients with a HRCT diagnosis of NSIP (n = 44, dotted line, ...), indeterminate (n = 25, dashed line, ----), and UIP (n = 27, solid line, ——); last follow up visit = +, death = ○; p = 0.01. (Reproduced with permission. From Ref. 27). (b) Kaplan-Meier survival curves for patients grouped by combining HRCT and histopathologic features. Patient groups were: histopathologic pattern showed NSIP and HRCT was interpreted as indeterminate or NSIP (n = 23, dotted line, ...), histopathologic pattern showed UIP and HRCT was interpreted as indeterminate or NSIP (n = 46, dashed line, ----), and histopathologic pattern showed UIP and HRCT was interpreted as UIP (n = 27, solid line, ——); last follow up visit = +, death = ○; p = .001. (Reproduced with permission. From Ref. 27.)

survival was worse when HRCT scans were "typical of CFA" compared to "atypical for CFA" (44). These data suggest that HRCT "typical" of UIP represents more advanced disease than patients with histological UIP but CT scans that are atypical for UIP.

IX. Conclusions

In summary, HRCT is invaluable to characterize the morphology of IIPs and other ILDs. HRCT scans can narrow the differential diagnosis and, in some patients, enable a confident diagnosis of UIP or other ILDs (e.g., LCG or LAM). As has been discussed, diffuse, thin-walled cysts in a woman are highly specific for LAM. In addition, irregular cysts and nodules, with a mid and upper lung zone predominance, strongly suggests LCG. GGOs are observed with a variety of disorders; in this context, lung biopsy is required to substantiate a specific histological diagnosis. The predominant pattern on CT (e.g., GGOs, reticular patterns, or HC) also has prognostic significance. A pattern characterized as predominantly GGOs is associated with an improved prognosis and a higher rate of responsiveness to therapy compared to reticular or HC patterns. Such patients should be treated aggressively with corticosteroids and/or immunosuppressive agents. By contrast, patients with a predominantly reticular or HC pattern have a poor prognosis and low rate of response to therapy. Although serial CT scans may provide objective information about the course of the disease, the role of serial CT scans remains controversial.

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8

Other Imaging Techniques for Idiopathic Interstitial Pneumonias

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I. Introduction

Diffuse interstitial lung disease (ILD) is a generic term encompassing a broad range of largely unrelated conditions that share the propensity to cause breathlessness and/or cough associated with bilateral abnormal opacities of various types on conventional chest radiographs or computed tomographic (CT) scans. The idiopathic interstitial pneumonias are a subset of diffuse interstitial lung diseases characterized by expansion of the interstitial compartment (i.e., that portion of the lung parenchyma sandwiched between the epithelial and endothelial basement membranes) by an infiltrate of inflammatory cells. The inflammatory infiltrate is sometimes accompanied by fibrosis either in the form of abnormal collagen deposition or proliferation of fibroblasts capable of collagen synthesis. Recent international consensus has defined these entities more specifically, with idiopathic pulmonary fibrosis (IPF) being based upon the histological appearances of a usual interstitial pneumonia (UIP) pattern, thus distinguishing it from other interstitial fibrosing pneumonias of known causes and those with different histological patterns of unknown cause (1).

The diagnosis and monitoring of idiopathic interstitial pneumonias relies to an increasing extent on the use of high-resolution CT scanning (HRCT) of the chest (2–4). HRCT allows earlier diagnosis of IPF, helps to narrow the differential diagnosis based on the CT pattern, and allows the identification of the extent of associated emphysema (2,5,6). HRCT can also help to increase the level of diagnostic confidence for IPF when the clinical or radiological features are uncertain. This chapter aims to review the available literature on

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the use on non-CT imaging modalities in these conditions. In general, these other imaging techniques have been investigated for their potential roles in the diagnosis and evaluation of disease severity and monitoring of functional impairment in the idiopathic interstitial pneumonias, with the aim of identifying a modality enabling prognostic information.

II. Gallium 67 Citrate Scanning (Ga 67)

Gallium 67 is an amphoteric radionuclide produced by a cyclotron and has a half-life of 78 h (7). It is known to concentrate in inflammatory foci found in a variety of granulomatous and infectious disorders. Patients undergoing gallium scanning are injected intravenously with Ga 67 citrate based upon body weight. They return 48–72 h later for anterior and posterior scans of the whole body. Pulmonary uptake may then be visualized qualitatively or quantified. This usually involves the product of the regional percentage of total lung area, the regional signal intensity, and an index of the parenchymal texture (e.g., patchy or diffuse process). In general, pulmonary uptake is referenced as a percentage of the injected dose against systemic background uptake from a designated low-uptake tissue/organ.

With regard to idiopathic interstitial pneumonias, the early use of Ga 67 in the 1980s was aimed at providing a noninvasive estimate of the degree of parenchymal pulmonary inflammatory involvement in the hope of staging disease severity and to monitor therapeutic effectiveness against the perceivably reversible ''alveolitic component'' of IPF. In a detailed study of 30 patients with IPF and 19 control patients (not having interstitial or malignant disease), the pulmonary Ga 67 index was determined in conjunction with lung biopsy morphological analyses and bronchoalveolar lavage (BAL) fluid analysis (8). Over 70% of patients with IPF demonstrated pulmonary uptake of Ga 67 at 48 h with significantly higher Ga 67 indices than controls. Furthermore, there was a positive correlation between the pulmonary Ga 67 index and the interstitial and alveolar cellularity scores from open lung biopsies, as well as the neutrophil percentage count from BAL. This suggested that the accumulation of Ga 67 in the lungs corresponded to the degree of parenchymal inflammation, and led to recommendations that semiquantitative gallium scanning be considered for monitoring disease activity. By contrast, it was already known that the technique lacked specificity and any correlation with physiological parameters of severity to enable it to become a diagnostic tool in IPF.

More recently further studies have reproduced these early findings of detection using quantitative gallium indices in patients with connective tissue disease–associated interstitial pneumonia and hypersensitivity pneumonitis (9). Thus, in 113 patients, a lung/liver gallium index was significantly

higher in the groups with active interstitial lung disease (5.7) and non-infective bronchiolitis (4.1) compared with nonsmoking normal individuals (3.0; $P < .05$). However, specificity was poor and neither correlation with other physiological severity indicators nor prognostic information were forthcoming.

It has been suggested that the mechanism of pulmonary uptake of Ga 67 in smokers is a macrophage-mediated process, although being an immunologically mediated, and albumin-linked process in connective tissue disease (CTD)–associated interstitial pneumonia (9). Others have demonstrated that Ga 67 is taken up by activated lymphocytes (10), although the affinity for transferrin receptors on proliferating cells has also been proposed as the cellular basis for its uptake (11).

What is clear from these studies is that gallium scanning will provide a semiquantitative measure of the intensity of inflammation that correlates with other measures such as BAL (12). It will also identify a reduction in inflammatory response to corticosteroid therapy, which suppresses the gallium scan intensity. However, prior administration of corticosteroids reduces the correlation between such independent markers of inflammation and reduces the sensitivity of the scanning technique under these conditions (13).

Recently, the application of Ga 67–labeled transferrin as an intravascular pulmonary marker of capillary permeability was compared with the conventional Ga index in 20 patients with interstitial pneumonia (17 with IPF, 2 with collagen vascular disease and, 1 each with acute interstitial pneumonia or eosinophilic pneumonia) (14). An increase in pulmonary vascular leakiness was detectable in patients with active disease compared with inactive disease or control subjects. No such difference was noted with the Ga index. This suggests that gallium-labeled markers of pulmonary vascular leak may be more sensitive than conventional gallium scanning for assessing disease activity, and may open up a further field of research for this technique. In sarcoidosis, the value and limits of gallium scanning were set out in multicenter studies during the 1980s (15). In sarcoidosis, gallium scans can be helpful where other investigations are not diagnostic, particularly in the nonpulmonary organs (16), although early reports of prognostic value have not been borne out (17, 18).

In summary, the initial optimism from its potentially diagnostic role in nonpulmonary sarcoidosis and early studies of gallium scanning in the idiopathic interstitial pneumonias has been tempered by a number of factors. First, its lack of specificity and labor/time intensive set up. Second, its lack of correlation with clinical outcome or physiological measures of disease severity. Finally, it has been superceded by HRCT as the preferred imaging modality in diagnosis and monitoring of progression in interstitial pneumonias.

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III. Technetium 99m–Diethylenetriamine Penta-Acetate and Technetium 99m–Pertechnegas Scanning

Technetium 99m–penta-acetate (Tc 99m–DTPA) aerosol clearance is an index of alveolar-capillary membrane permeability and more particularly a measure of epithelial integrity (19,20). An aerosol of Tc 99m–DTPA is produced using pressurized air. A jet nebulizer creates diameters of less than 1 μm, which the patient inhales during normal tidal breathing through a mouthpiece with one-way valve and nose clip. A gamma camera detects counts over the lungs. DTPA inhalation continues until a sufficient count is picked up from the lung region. Then the gamma camera images from the back and records counts through a 60-min period from regions of interest over the lung fields and a suitable background reference point on the body (e.g., kidneys). Solute clearance is calculated from the rates of radionuclide disappearance from the lungs. Both mono- and biexponential clearance analyses may be obtained from the disappearance curve (Fig. 1).

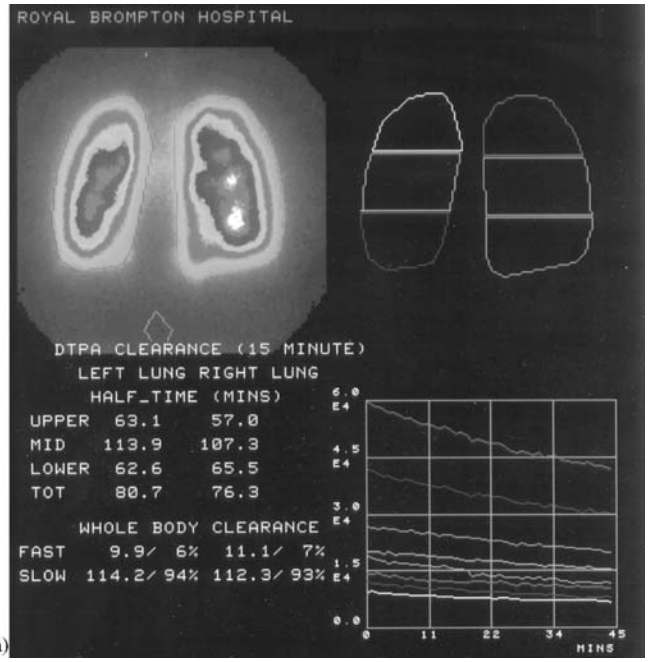

Figure 1 DTPA scans. (a) Normal scan demonstrating whole lung half-time clearance greater than 40 min, with fast and slow phase components. (b) Increased clearance in a patient with idiopathic pulmonary fibrosis. (Courtesy of Professor R. Underwood, Dept of Nuclear Medicine, Royal Brompton Hospital, London, UK.)

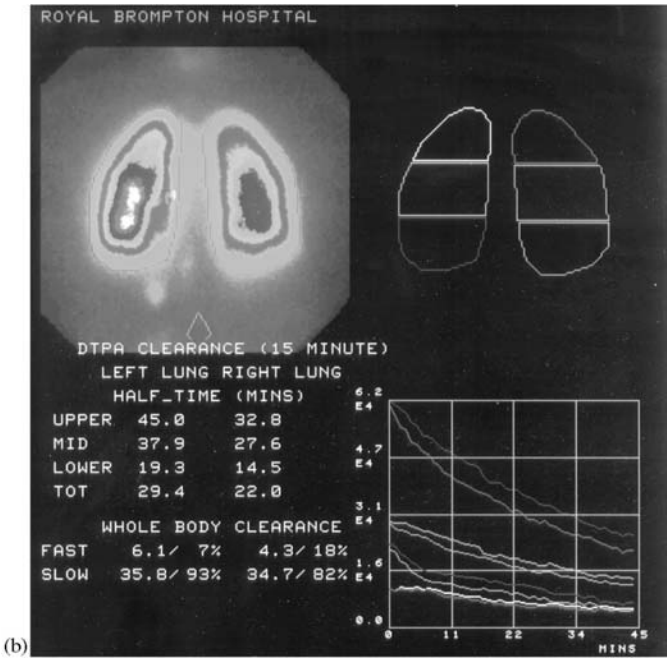

Figure 1 Continued.

In healthy nonsmokers, pulmonary clearance of DTPA follows a monoexponential (21). Beyond 40 min, the further decrease in DTPA activity is slow, and since interest lies in clearance values above normal, a cut off scintigraphy time of 40 min is utilized. In smokers without coexistent lung disease, the clearance can be separated into fast and slower component curves (21). Increased pulmonary clearance has been demonstrated in a number of lung conditions: acute respiratory distress syndrome (ARDS), human immunodeficiency syndrome (HIV) disease, and permeability edema following cocaine use (22). Increased permeability results from inflammation in the lung parenchyma in a variety of diffuse parenchymal lung diseases such as IPF, dermatomyositis, hypersensitivity pneumonitis, sarcoidosis, radiation pneumonitis, and pneumoconiosis (23–38) often when there is little other evidence of lung disease. An increased DTPA clearance is a sensitive marker of inflammation, and a normal clearance certifies absence of inflammation (39).

In 1979, a study of DTPA lung clearance in interstitial lung disease was reported on 10 patients with systemic sclerosis and 7 normal volunteers. Lung clearance half-times were significantly higher from patients, particularly from the lower lung zones. This established its place as an imaging study of potential value in the investigation of interstitial lung diseases (40). In addition to its potential role in the detection of diffuse parenchymal lung disease, DTPA

scanning has been used as a measure of prognosis in sarcoidosis, asbestosis, and fibrosing alveolitis (26,31,32). Persistently rapid clearance is associated with a higher risk of subsequent deterioration in lung function.

A study of DTPA in a heterogeneous group of patients with diffuse ILD demonstrated that persistently normal clearance predicts clinical and physiological disease stability based on lung function and selects patients with a favorable prognosis (32). In HIV disease, a biphasic pattern of clearance is seen in *Pneumocystis carnii* pneumonia (PCP) with early rapid clearance of DTPA from the lung (41). More recently, further attempts have been made at analyzing and refining the characteristic components of the DTPA clearance curve in patients with UIP IPF (42). Whereas previous studies have analyzed pulmonary clearance of aerosol as a monoexponential approximation to the clearance curve, and have therefore been strongly influenced by the fast component, these workers analyzed clearance using a biexponential model. This dual course is always abnormal (39). The half-time for the fast component (range 1.2–10.7 min) was an independent predictor of survival, unlike the half time of the monoexponential curve (range 9.4–66.4 min). Multivariate Cox regression in this cohort of 106 patients yielded a 25% increase in hazard of death, for every year of patient age, for a 1-min reduction in the fast component half time. Importantly, no studies have demonstrated a correlation between aerosol DTPA clearance and other pulmonary function parameters such as lung function tests or HRCT. Thus, although complementing other prognostic markers, it has been suggested that specific components of DTPA clearance may provide additional information regarding expected survival [42]. This awaits further validation. It has been suggested that a structural correlate of the fast component is damaged pulmonary epithelium and the physical basis of the slow component is normal lung. Although this is a simple, attractive hypothesis, it remains speculative.

Unfortunately, DTPA scanning lacks specificity and adds little to other diagnostic techniques. Moreover, it cannot be employed in cigarette smokers, as airway inflammation increases the clearance of the isotope (43–46).

Technetium 99m–pertechnegas (pertechnegas) is a newer radio-aerosol ventilation agent used to measure pulmonary clearance that has recently been investigated in the setting of diffuse parenchymal lung disease. Pertechnegas is produced by heating an ethyl alcohol rinsed saline solution of Tc 99m-pertechnetate to 2500 °C in a graphite crucible in a mixture of 4% oxygen in argon (47). Like DTPA, it traverses the alveolar epithelial membrane and is cleared into the systemic circulation where it is detectable. Clearance rates in nonsmoking control subjects have been estimated at $\sim 9.2\%\,\mathrm{min}^{-1}$, based on assumed alveolar permeability coefficients of $-0.23 \times 10^{-7}\,\mathrm{cm\,s}^{-1}$ and alveolar capillary layer thickness 0.25 μm from physiological measurements (48,49). These estimates for clearance are up to nine times greater than for DTPA ($0.59–1.56\%\,\mathrm{min}^{-1}$); this is likely due to the smaller mass median

aerodynamic diameter (MMAD $< 0.1\mu m$ for more than 80% of pertechnegas particles compared with $0.7\,\mu m$ for DTPA). The implication is that more pertechnegas particles are deposited in the alveoli (50,51). A further purported advantage of pertechnegas is that no aerosol device is needed. Rather the subject inhales directly from the gas generator for a shorter period of time. Thus, a reduced risk of surrounding contamination and consequent improved image quality, shorter inhalation time and faster data acquisition are provided.

Lung clearance by pertechnegas has been shown to be significantly shorter for a heterogeneous group of patients with interstitial pneumonias compared with controls (5.8 ± 2.2 vs 8.5 ± 2.4 min) (52). In a recent study of pulmonary membrane permeability changes in small cohorts of patients with interstitial lung diseases (8 patients with CTD, 10 with hypersensitivity pneumonitis, 9 with idiopathic interstitial pneumonia, and 10 with sarcoidosis), time to half clearance of pertechnegas from the lung was significantly reduced in all groups compared with age-matched nonsmoking controls except in the sarcoidosis group. The investigations speculate on the reasons for a lack of increased mean clearance in the sarcoidosis patients; either the alveolar capillary membrane is not affected in the same way as with the other diseases, or indeed because the sarcoidosis cohort had no clear functional impairment and therefore no active inflammation [53]. An ultrastructural study to identify deposition and binding regions of the radio-aerosol would indeed be a fascinating step forward in this area. Interestingly, and in contrast to several previous studies with DTPA, there was a positive correlation between the clearance half-time and functional severity, as assessed by forced vital capacity (FVC), total lung capacity (TLC), and D_{LCO} (carbon monoxide transfer factor) [53]. These data suggest another promising radionuclide scanning technique for measuring inflammatory loss of alveolar-capillary membrane integrity. However, as with DTPA scanning, it seems to lack specificity, and is subject to the same necessity for smoking cessation for some months prior to measurement.

Indium 113 labeling of DTPA can minimize the effect of smoking on pulmonary clearance when compared with Tc 99–DTPA. However, clearance occurs from the bronchial systems proximal to the alveoli, which may not be directly relevant to the disease under investigation (54). Nevertheless, further evaluation in conjunction with validated disease severity indices may provide the prospect of a new functional severity predictor that could be used alongside the established, but relatively crude, prognostic tools at our disposal to date. Thus, one important aim of further work would be to predict trends that justify treatment of patients before symptoms develop; as has been suggested with serial studies of DTPA in fibrosing alveolitis and radiation-induced fibrosis (32,55).

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IV. Ventilation-Perfusion Scanning

Conventional ventilation-perfusion (VQ) scanning has no place in the investigation of interstitial lung diseases, particularly in the context of excluding pulmonary embolism. In a study of 45 patients with end-stage pulmonary fibrosis awaiting lung transplantation, a wide range of "abnormal" scans potentially suggesting pulmonary embolic disease were identified; one-third had normal VQ matching, and one-third had severe mismatch, with the other third displaying intermediate mismatching [56]. In order to seek an explanation for the mismatched V/Q in patients with IPF, eight such patients were studied by inspiratory and expiratory CT scans. Regional ventilation and perfusion were compared using planar and tomographic (single-photon emission computed tomography, SPECT) scintigraphy; ventilation assessed by inhalation of krypton 81 m gas and perfusion by intravenous injection of Tc 99m–albumin macroaggregates. The results demonstrated that V/Q mismatch seen on scintigrams (that may have suggested pulmonary embolism) were very commonly due to the nonperfused large cystic airspaces (honeycombing with presumed concurrent vascular obliteration) that are often seen on CT scans in IPF and represented physiological dead space. Implicit in the conclusions were the inaccuracy of VQ scanning in IPF, and potentially erroneous interpretation without corresponding CT scans (57). Techniques other than VQ scanning should be used for identifying pulmonary emboli in interstitial pneumonias.

V. Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) of the pulmonary parenchyma represents a challenge in view of the limited signal caused by low proton density and physiological motion artefacts. However, research and developments in signal acquisition and processing continue to bring this multiplanar imaging modality closer to the investigative toolbox of the pulmonary physician interested in interstitial pneumonias. The current state of the literature is presented here. A small study comparing MRI with the pathological findings in patients with chronic infiltrative lung disease, which included 5 cases of IPF and 3 of extrinsic allergic alveolitis among the 22 patients studied, identified the predominant pattern of abnormality on MRI as parenchymal opacification. Other patterns included parenchymal opacification and reticulation, reticulation only, nodularity, and septal thickening. Parenchymal opacification on MRI was associated mainly with inflammatory infiltration (alveolitis), although a correlation with interstitial fibrosis was evident in two patients. By contrast, the small numbers with a reticulation pattern on MRI were associated with histological fibrosis. Nodules were identifiable as granulomas

due to sarcoidosis in all three such cases. Thus, MRI of the lung parenchyma has evolved to the stage of identifying the predominant pattern of interstitial lung involvement (58). Similarly, increased lung signal intensity (compared with the normal control lungs of healthy volunteers) from cardiac-gated spin-echo of the lungs of 14 patients with a heterogeneous mix of interstitial lung diseases (before and after single-lung transplantation) was notable in native lungs with interstitial lung disease and in transplanted lung during rejection episodes or pneumonia (59) (Fig. 2). Others have demonstrated the ability of standard MRI techniques to assess crude measures such as proportion of diseased to normal lung or airspace opacification corresponding to ground-glass opacities as accurately as HRCT in patients with interstitial diseases including the interstitial pneumonias [60,61]. These studies provide a basis for the future noninvasive assessment of interstitial pneumonias by MRI, thus avoiding ionizing radiation; although the current spatial resolution

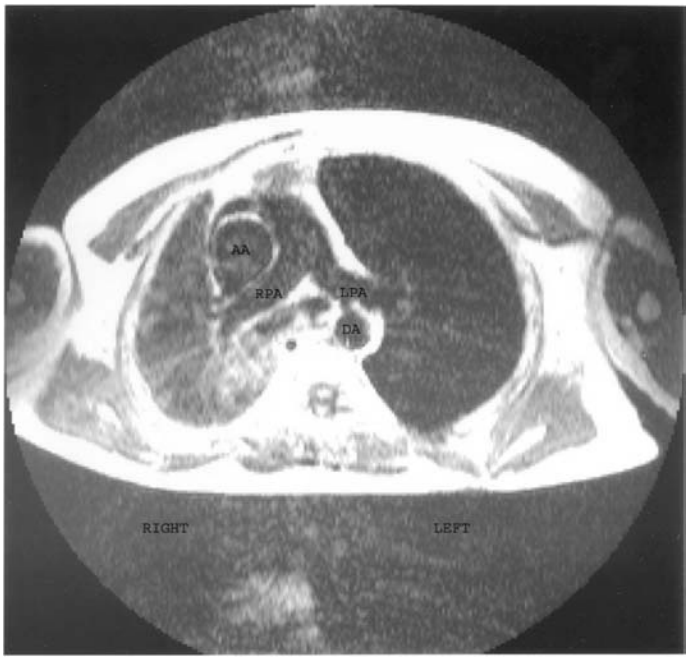

Figure 2 A spin-echo image (TE 40 ms) in a transverse plane at the level of the pulmonary bifurcation in a patient with pulmonary fibrosis and right lung transplantation. The cross-sectional area of the native lung is smaller than the transplanted lung and its signal intensity is higher. AA, ascending aorta; DA, descending aorta; RPA, right pulmonary artery; LPA, left pulmonary artery. (Courtesy of Dr R. Mohiaddin, MRI Unit, Royal Brompton Hospital, London, UK.)

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remains limited to gross abnormalities. It is acknowledged that significant further developments are required to improve lung signal intensity sufficiently to enable detection of less severe parenchymal changes, and indeed the combination of coexistent inflammatory and fibrogenic processes that characterize idiopathic interstitial pneumonias. Newer MRI techniques under investigation that may widen the potential of this modality in this context include the utilization of very short echo times, ultrafast turbo-spin-echo acquisitions, breathhold imaging, sodium imaging, contrast agents (aerosols or perfusion imaging), hyperpolarized noble gas imaging, and oxygen enhancement (62). Although MRI has been underutilized for imaging of the thorax compared with CT, and currently remains an inferior and indeed experimental modality in interstitial lung disease, it has been emphasized that its potential as a functional imaging technique based upon pulmonary function as opposed to morphology (CT) may yet be realized (63).

VI. Positron Emission Tomography

Positron emission tomography (PET) scanning remains a research tool in the study of interstitial lung diseases. It has been applied experimentally to the investigation of patients with sarcoidosis with demonstration of pulmonary uptake of fluorine 18–deoxyglucose (FDG 18) that may enable quantification as well as information regarding disease distribution (64,65).

PET scanning has been evaluated to measure pulmonary vascular permeability as a marker of disease severity in interstitial lung disease. The pulmonary transcapillary escape rate (PTCER) of transferrin labeled with gallium 68 was determined in 16 patients with hypersensitivity pneumonitis, sarcoidosis, or IPF. PTCER was significantly elevated in those deemed to have active disease (based upon a clinical-radiological-physiological score, CRP) compared with inactive disease or normal subjects. However, there was poor correlation between PTCER and radiological or CRP scores. Another difficulty that emerged was finding an appropriate method for quantifying the regional heterogeneity in PTCER that may have been masked by the crude averaging of signal intensities [66]. PET has not emerged as a practical method for diagnosis, monitoring disease severity, or prognosis in idiopathic interstitial pneumonia, although it continues to be utilized as a research technique in this field.

VII. Other Techniques

A number of other nuclear imaging modalities have been used in the investigation of interstitial lung disease for assessing disease activity.

A. Thallium 201 Scanning

Thallium (Tl 201) scanning is a well-established myocardial imaging technique for identifying stress-related ischemia. It has been proposed as a marker of lung activity in a range of lung diseases, particularly acquired immunodeficiency syndrome (AIDS) (67,68). It has the theoretical advantages over Ga 67 of immediate imaging results, lower levels of ionizing radiation, and imaging with portable cameras. However, it suffers from similar inadequacies to Ga 67 and other non-CT modalities of a lack of specificity for diagnostic and monitoring purposes in interstitial lung diseases, and has not been evaluated in the context of interstitial pneumonias.

B. J001X Tc 99 m Scintigraphy

J001 is an acylated peptidopolygalactoside isolated from the membrane proteoglycans of *Klebsiella*. It binds avidly to macrophages and is absorbed into the respiratory tract by inhalation when labeled with Tc 99 m. In scleroderma, ^{99mTc}J001X scintigraphy was positive in 12 of 17 patients [69]. However its sensitivity for active lung involvement in patients with rheumatoid arthritis was less than the reported values in patients with scleroderma (70).

C. Indium 111–Labeled Leukocyte Scintigraphy

This whole body technique has previously been used to diagnose pyogenic infections in pyrexias of unknown origin. However, indium 111 (In 111) labeled leukocyte scintigraphy has been shown to produce diffuse pulmonary uptake in patients with drug-induced pneumonitis, although poor specificity is once again a major limitation of this technique in diffuse parenchymal lung disease (71).

VIII. Conclusions

Gallium imaging is not of any proven value for evaluation of IPF. Aerosols containing Tc 99 m–DTPA, a hydrophilic agent, or other radionuclides are cleared more rapidly when there is increased capillary permeability, and may provide an index of lung inflammation. The clinical role of these agents remains unproven but under ongoing evaluation. Magnetic resonance imaging may in the future be useful as a tool for discriminating between lung inflammation and established fibrosis. Other imaging modalities such as PET and leukocyte-labeled scans remain experimental.

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9

Bronchoalveolar Lavage in Interstitial Lung Disease

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I. Introduction

Shortly after the introduction of the technique of bronchoalveolar lavage (BAL) through a flexible fiberoptic bronchoscope (1), it was apparent that the inflammatory cells retrieved by BAL were different in interstitial lung diseases versus normal volunteers (2–5). This led clinicians to begin using the results of BAL to manage patients with various interstitial lung diseases (5,6). This has been a controversial concept (7,8). In this chapter, we will discuss some of the background information that research using BAL has taught us about interstitial lung diseases, how that information has been used in the diagnosis and management of interstitial lung diseases, and the technical aspects of BAL which appear to be important to make the results understandable.

II. Background

Idiopathic pulmonary fibrosis (IPF) is the most common nongranulomatous lung disease studied by BAL. Initial studies of IPF patients demonstrated that increased neutrophils was the most common feature (5). There was an apparent subset of IPF patients with increased lymphocytes, who had a better prognosis (5,9). Increased lymphocytes in the BAL was associated with an increased cellularity on open lung biopsy (10). As our understanding of the idiopathic interstitial lung diseases changed, we now recognize the important difference between the usual interstitial pneumonitis seen with IPF and the changes of nonspecific interstitial pneumonitis, which is associated with a better prognosis (11,12). The improved prognosis for lymphocytic lavage may just be an

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indication of a case of nonspecific interstitial pneumonitis. Unfortunately, there have been few studies looking at the significance of BAL findings related to the new pathological definitions of idiopathic interstitial lung disease.

The eosinophil was another cell studied in IPF. It was noted that an increased number of eosinophils was associated with a worse prognosis in patients with IPF (9,13). The eosinophil is rarely seen in BAL fluid. In this case, patients with over 2% eosinophils had a worse prognosis. Although these observations were made using the old classification system, they probably are still valid.

BAL studies of various cytokines have been elevated in patients with IPF. This includes the cytokine interleukin-8 (IL-8) (14–16). This proinflammatory cytokine is a neutrophil chemoattractant. Elevated levels of IL-8 have been described in several other diseases, including acute respiratory distress syndrome (17). It has also been found to be increased in pulmonary infections such as pneumonia and cystic fibrosis (18,19). Increases in the tumour necrosis factor (TNF) levels have also been described in patients with IPF (20).

For the interstitial lung disease of known cause, collagen vascular diseases can have pathology similar to IPF. However, many of the cases of collagen vascular disease–associated pulmonary fibrosis reveals that the category of nonspecific interstitial pneumonitis based on computer tomographic (CT) scan or histology (21,22). The BAL findings are also somewhat different from those cases of 1PF (22,23). The general pattern is increased neutrophils, but increased lymphocytes are more commonly seen than in IPF. The finding of increased lymphocytes was associated with a better prognosis (24).

An area of interest in scleroderma has been the use of BAL to identify patients with early interstitial lung disease prior to symptoms. It has been found that patients with scleroderma have increased neutrophils and lymphocytes in their BAL fluid (23,25,26). Studies of patients with subclinical disease have been found to have increased neutrophils (27). White and colleagues studied scleroderma patients with increased neutrophils in their BAL but minimal clinical disease. Patients not treated with cytotoxic agents developed worsening disease with some deaths over the next 2 years. On the other hand, those patients who were treated with cyclophosphamide had a significantly slower progression of their disease and a lower mortality (28).

BAL has changed our whole view of the disease sarcoidosis. Historically, sarcoidosis was felt to be a disease of suppressed immunity, with anergy being a striking feature of the disease (29,30). This hypothesis was supported by the observation that a low number of peripheral blood lymphocytes was a common finding in sarcoidosis (31,32). There were some problems with this concept of suppressed immunity. The first was the hypergammaglobulinemia seen in some patients (33). Another issue was the marked effectiveness of corticosteroids in treating symptomatic disease (34).

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This apparent paradox was resolved as BAL gave the investigator the opportunity to sample the inflammatory response in the tissue. A striking increase in the number of lymphocytes was found in the BAL fluid of patients with active sarcoidosis (2). This led to a series of studies, mostly from Crystal's group at the National Institutes of Health in the United States. They demonstrated that major influx were helper T lymphocytes (CD4-positive lymphocytes) (3,35). Lavages from patients were found to have an increase in their CD4:CD8 lymphocyte ratio (3,36). Subsequent studies from this group demonstrated that these helper T cells were activated and releasing IL-2 (37). They also proved that the hypergammaglobulinemia seen in sarcoidosis was related to this T-cell activation. By coculture of peripheral blood lymphocytes with the cells of patients with sarcoidosis, they stimulatedgamma globulin release by the B cells (38). This probably was due to the stimulatory effect of IL-2. It was subsequently found by others that the level of peripheral blood gamma globulin was related to the number of CD4 cells found in the lung (39).

Several other groups also noted the increase in lymphocytes, especially CD4 lymphocytes in patients with sarcoidosis (4,40–43). In one study, it was found that untreated patients with a high CD4:CD8 ratio would have a significant worsening of lung function over the next 6 months (36). In patients with spontaneous resolution of their disease, the CD4:CD8 ratio would return toward normal, whereas the number of lymphocytes would be slower to fall toward normal (41). It was found that successful treatment with corticosteroids, methotrexate, or azathioprine would lead to a normalization of the CD4:CD8 ratio (44–46). This led to the hypothesis that during the resolution of sarcoidosis, suppressor lymphocytes would control granuloma formation, and the goal of therapy in sarcoidosis was to suppress helper T cell activation. However, this simplistic view has not proved to be a useful guide for treatment in all cases, since individual patients may have a more complex inflammatory reaction.

Sarcoidosis is an example of an interstitial lung disease which can have two phases: acute and chronic. During the initial, acute phase, lymphocytes may prove to be the important inflammatory mediator. However, during the chronic phase, other cells, including the alveolar macrophage and neutrophil, may be far more important (47). Less than half of patients with sarcoidosis go onto the chronic form of the disease (48,49). Macrophage activation may be crucial in the development of chronic disease. In most patients with sarcoidosis, interferon gamma production by the alveolar macrophage is increased (50). This is part of the Th1 response seen in sarcoidosis and other granulomatous diseases (51). IL-12, another important cytokine directing the Thl response, has been found to be increased in patients with sarcoidosis (52).

Other products of the macrophage that have been found to be elevated include oxygen free radicals (53). Macrophages from alveolar macrophages have also been found to release several proinflammatory cytokines. These

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include IL-1 (54) and IL-6 (55,57). The cytokine IL-8 has been associated with pulmonary fibrosis (58). Increased levels of IL-8 have been reported in some patients with sarcoidosis (57,58).

Another crucial cytokine found to be increased in sarcoidosis is TNF. Although TNF cannot usually be measured in the acellular component of the BAL fluid, increased levels of TNF are released by alveolar macrophages (59–61). The persistent release of TNF has been associated with chronic disease (61,62). Another granulomatous disease is hypersensitivity pneumonitis or extrinsic allergic alveolitis. Early BAL studies showed an increase in the percentage of lymphocyte in the BAL fluid of patients with hypersensitivity pneumonitis (63,64), and this observation has held up over time (65,66). One apparent difference between sarcoidosis and hypersensitivity pneumonitis was that the CD4:CD8 ratio was less than one in many cases of hypersensitivity pneumonitis (42,67). This observation has limited diagnostic value, because several hypersensitivity pneumonitis cases are characterized by a normal to increase in the CD4:CD8 ratio (68). This is particularly true in methotrexate pneumonitis (69) and chronic beryllium disease (70). Also, some cases of sarcoidosis may have an increased number of CD8-positive lymphocytes (71). An interesting feature of hypersensitivity pneumonitis has been that increased lymphocytes have been observed in patients exposed to antigens (e.g., farmers) who never develop disease (72,73).

The mast cell is also increased in patients with hypersensitivity pneumonitis (74–76). The mast cell can be somewhat difficult to find, since it is poorly stained with Wright-Geimsa (77). Mast cells should be sought if one suspects hypersensitivity pneumonitis (78). The finding of the mast cell is not specific, since it has been found to be increased in other diseases such as bronchiolitis obliterans with obstructing pneumonia, asthma, and radiation pneumonitis (79–81).

The macrophage also appears to play a role in hypersensitivity pneumonitis. Increased cytokines IL-6 and TNF have been released by alveolar macrophages of patients with chronic beryllium disease (82). Increased levels of the TNF receptor were found in the BAL fluid of patients with hypersensitivity pneumonitis (83). This would imply increased TNF activity. Exposing naive macrophages from asymptomatic farmers to *Micropolyspora faeni*, the cause of farmers's lung, led to marked increase in the release of TNF (84). These studies suggest that TNF may play a role in chronic hypersensitivity pneumonitis similar to that seen in sarcoidosis.

III. Use of BAL to Diagnose Interstitial Lung Disease

BAL may be useful to the clinician in evaluating the patient with an interstitial lung disease. Table 1 lists the various interstitial lung diseases and the general

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Table 1 Characteristics of BAL in Various Interstitial Lung Diseases

	Alveolar macrophages	Lymphocytes	Neutrophils	Eosinophils
Noninfectious Diseases				
Sarcoidosis	Foamy aspect	+	=	=/+
Hypersensitivity pneumonitis	Foamy aspect	++	+	=/+
Drug-induced pneumonitis	Foamy aspect	++	+	+
Idiopathic pulmonary fibrosis		+	+/++	=/+
Bronchiolitis obliterans with organizing pneumonia (BOOP)	Foamy aspect	+	+	+
Eosinophilic pneumonia		+	=	++
Alveolar proteinosis	Foamy aspect	+	=	=
Connective tissue disorders		+	=/+	=/+
Pneumoconiosis	Inclusion particles	= or +	+	=
Asbestosis	Feruginous bodies	=/+	+/++	=/+
Diffuse alveolar hemorrhage	Fe staining: +++	= or +	+	=
Carcinoma		= or +	=/+	=
Lymphoma		++	+	=
Infectious Diseases				
Bacterial	Intracellular bacteria Inclusion bodies	=	++	=
Viral		+	+	=/+
Tuberculosis		+	=/+	=

Source: Adapted from Ref. 124.

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BAL findings. It is immediately obvious that are very few specific patterns of neutrophils, lymphocytes, and eosinophils for any one disease. However, the information from lavage has been proposed to help characterize various common interstitial lung diseases (6).

One approach to the information of BAL is to consider the pattern of the differential cell counts of the BAL. Drent noted that patients with IPF, sarcoidosis, and hypersensitivity pneumonitis had relatively unique features (66,85). Patients with sarcoidosis had increased lymphocytes as their major feature, whereas those with hypersensitivity pneumonitis had increased lymphocytes and mast cells. IPF patients have increased neutrophils. Patients with sarcoidosis can have increased neutrophils, and IPF patients can have increased lymphocytes. However, by dealing with the general pattern of the cellular differential, Drent was able to discriminate the majority of patients using only the cellular cytology (66). This was developed into a computer program (86). In an updated version, the confounding effects of acute infection were also considered. The current program was able correctly to characterize more than 90% of patients with interstitial lung disease (87).

The use of the BAL differentials does require one to examine the clinical material gathered at the time of the BAL. As noted, increased lymphocytes have been one the persistent findings in sarcoidosis (6,85). Other granulomatous lung diseases can cause increased lymphocytes, such as hypersensitivity pneumonitis (63,67). Tuberculosis is an infectious granulomatous disease with increased lymphocytes (88,89). Table 2 lists many of the diseases associated with increased lymphocytes. It also lists some of the features used in distinguishing between different diseases. Although increased lymphocytes were not useful in diagnosing sarcoidosis, that information can be used with other studies to determine the cause of the patient's interstitial lung disease (90).

Some clinicians have used the CD4:CD8 ratio to enhance the specficity of BAL finding of increased lymphocytes to diagnose sarcoidosis (6,90). However, increased CD4:CD8 is not a universal finding in sarcoidosis. As the disease resolves, the ratio may go back to normal (41). Although some found the highest ratios in those with progressive disease (36), others found the highest ratios in patients with erythema nodosum and a good prognosis without therapy (91). Also, a group of patients with progressive sarcoidosis have an increased number of CD8 lymphocytes (71). The reported frequency of sarcoidosis patients with an elevated CD4:CD8 ratio varies from 40 to 80% (85,90,92,93). There are few other conditions which cause a CD4:CD8 ratio of greater than four, Thus, very high ratio is very supportive of the diagnosis of sarcoidosis.

There are a few situations where BAL findings are very supportive of the diagnosis of a specific interstitial lung disease. These are listed in Table 3, in which the clinical situation as well as the findings of the lavage are included. For example, the presence of increased neutrophils in a patient with known

Table 2 Common Diseases Associated with Increased Lymphocytes in BAL

Disease	Frequency of increased lymphocytes (%)	Other BAL features	Other Clinical Features
Sarcoidosis	> 70	Increased CD4:CD8 ratio in majority of cases	Chest radiograph showing hilar adenopathy; evidence of extrapulmonary disease
Hypersensitivity pneumonitis	> 90	Some cases have CD4 : CD8 > 1; Presence of mast cells very common	Clinical history; positive antibodies
Tuberculosis	> 70	Increased neutrophils	Positive smear and culture
Malignancy	> 20		Positive cytology; lymphoma may have positive lymphocyte markers
Idiopathic pulmonary fibrosis	> 20	Increased neutrophils	Features of pulmonary fibrosis; crackles on examination

scleroderma and who has increased interstitial marking on their chest CT scan. There is no need for an open lung biopsy to document the presence of a collagen vascular disease–associated pulmonary fibrosis. Another example is a patient who is status bone marrow transplant and develops acute infiltrates. With lavage, the initial fluid may be blood tinged and become bloodier with each aliquot of BAL fluid. This is characteristic of diffuse alveolar hemorrhagic syndrome (94). Increased eosinophils in the BAL fluid have been reported with a large number of conditions (95). However, when the eosinophil percentage is above 20%, the patient most likely has acute or chronic eosinophilic pneumonia. The difference is the duration and severity of symptoms, with acute eosinophilic pneumonia often causing acute respiratory failure (96).

Also listed in Table 3 are those conditions in which special examination of the BAL fluid allows for a specific diagnosis. This includes looking for an abnormal accumulation of proteinaceous material seen in alveolar proteinosis (97). The use of Papinicolau stains allows one to diagnose malignancy which may present as an interstitial lung disease. Examples here are lymphangitic spread of breast cancer or from bronchoalveolar cell carcinoma (98;99). Also

Table 3 Condition Which BAL Helps Make the Specific Diagnosis

Underlying condition	BAL Finding	Other supportive findings	Cause of interstitial lung disease
Collagen vascular disease	Increased neutrophils	Fibrosis of chest radiograph or CT scan	Collagen vascular disease–associated pulmonary fibrosis
Drug therapy*	Increased lymphocytes	Fibrosis and/or ground-glass opacity on HRCT	Drug-induced lung disease
Radiation therapy	Increased lymphocytes; increased neutrophils	Fibrosis and/or ground-glass opacity on HRCT in area of radiation	Radiation pneumonitis
None	> 20% eosinophils in BAL fluid	Diffuse infiltrates on chest radiograph	Eosinophilic pneumonia
None	Acid fast bacilli and/or positive culture	Diffuse infiltrate on chest radiograph	*Mycobacterium tuberculosis* pneumonia
Immunosuppression/HIV	Positive stain for *P. carinii* or cytomegalovirus		Pneumonia
Lupus Bone marrow transplant Goodpastue's syndrome	Blood in BAL; increase with each aliquot instilled	Diffuse infiltrates on chest radiograph; No culture evidence for infection	Diffuse alveolar hemorrhagic syndrome
None	Proteinaceous material in BAL fluid	Diffuse infiltrates	Pulmonary alveolar proteinosis

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Pneumocystis carinii and cytomegalovirus can be diagnosed by lavage (100). Likewise bacterial infection can be diagnosed if semiquantitative cultures of the BAL fluid are obtained (101).

IV. Use of BAL to Follow Disease

One of the hopes of lavage was to provide a measure of disease activity over time. However, serial lavage data provided little additional information in patients with IPF (102). In patients with pulmonary fibrosis, there seemed to little evidence of progression from one stage to the next of the disease. This may be because IPF patients are detected at a relatively late stage of their disease.

The use of BAL to monitor therapy has not been widely used in pulmonary fibrosis. Some have proposed BAL as a method to help direct therapy with drugs other than corticosteroids. Treatment with cyclophosphamide has been shown to reduce the neutrophil percentage in IPF patients undergoing serial BAL studies (103). Ziesche used information from BAL and transbronchial biopsy to help identify those patients with pulmonary fibrosis who were not making any interferon gamma. These patients were successfully treated with exogenous interferon gamma (104). As new strategies develop for the treatment of IPF, serial BAL may prove to be useful in monitoring therapy.

For scleroderma-associated fibrosis, BAL has already been shown to be useful for directing therapy. As noted above, White et al. used BAL findings to detect inflammatory changes in the lung in the asymptomatic patient. Based on the BAL findings, cytotoxic therapy was initiated and shown to improve survival (28). BAL findings may also help direct therapy in other high-risk populations.

The use of BAL in sarcoidosis has been more successful for monitoring disease and therapy. This may be because sarcoidosis patients can resolve, something which is not seen with IPF. The use of lavage to determine whether a patient still has disease is a clinically useful measure. Also, the presence of new inflammatory cells in the BAL can predict a worse prognosis. Neutrophils in the BAL fluid of a patient with sarcoidosis is associated with chronic disease, often fibrosis (105,106). The increase in neutrophils may be a reflection of increased IL-8 in the lung (107).

The use of serial BAL has also proved to be useful in monitoring therapy with sarcoidosis. Table 4 lists several studies which have used serial BAL studies to measure the effect of treatment on inflammatory cells. The studies look at the effect of systemic corticosteroids, inhaled corticosteroids, methotrexate, azathioprine, or cyclosporine on various inflammatory markers in the lung (45,46,108,109). In some of the cases cited, there was a change in the BAL fluid without a change in the disease status. For cyclosporine, the changes seen in the BAL fluid were not associated with improvement of

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Table 4 Effect of Therapy to Cells and Disease in Sarcoidosis: Results of Serial BAL Studies

Drug (Reference)	Effect on lymphocytes	Effect on alveolar macrophages	Effect on disease
Prednisone/ prednisolone (44,45)	Decrease in lymphocytes Decreased CD4:CD8 ratio Decreased IL-2 release	Decreased TNF release Decreased hydrogen peroxide release	Improvement
Inhaled budesonide (108)	Decreased in lymphocytes	Not studied	No change or improvement
Cyclosporine (109)	Decreased in lymphocytes Decreased CD4:CD8 ratio Decreased IL-2 release	Not studied	No change
Methotrexate (45)	Decrease in lymphocytes	Decreased TNF release Decreased hydrogen peroxide release	Improvement
Azathioprine (46)	Decrease in lymphocytes	Decreased TNF release	Improvement

disease (110). In a larger study of pulmonary patients treated for up to 18 months, a regimen of cyclosporine plus corticosteroids was no better than corticosteroids alone (111). For budesonide, there was no evidence of improvement at short-term follow-up despite improvement of BAL parameters (112). However, long term studies have shown persistent improvement in lung disease for those patients treated with budesonide versus placebo (113). As more specific medications become available, one will be able to test the action of these drugs on the whole inflammatory response. Infliximab, a monoclonal antibody to TNF, has been reported to treat sarcoidosis (114). It will prove interesting to see whether this specific agent affects the rest of the inflammatory response of the disease. In one patient, successful therapy with infliximab was accompanied by a significant drop in the number of lymphocytes in the BAL fluid (114).

V. Technical Aspects of BAL Handling

One of the most commonly encountered problems with interpreting BAL findings has been the variability between centers. Like any test, a standard approach may limit the variability of the results. Unfortunately, many clinicians do not follow standard protocols for obtaining the BAL fluid.

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Also, the interpretation of the cellular components of the BAL require some expertise. If this is lacking, then the results of the test may not be usable.

The volume of instilled fluid can lead to distinctly different cell population. Instilling 20 mL or less fluid leads to a sample of mostly bronchial airways, not alveoli (115). Studies looking at the results of aspirated fluid after 60-mL aliquots of instilled fluid have shown significant differences (116–118). For patients with interstitial lung diseases, these differences seem to level off after 120 mL of fluid has been instilled (116). Thus, the recommendation is to use at least 100 mL of instilled fluid during lavage (119).

Other examples of variables affecting the BAL fluid analysis are summarized in Table 5. The preparation of the slides for subsequent staining influences the differential count. The cytocentrifuge technique is commonly used, but the results are significantly different if a millipore filter method is used (120,121). The speed of the cytocentrifuge and the area examined have also been shown to change the results of the BAL cellular analysis (122,123). For an individual center, the specimens will likely be prepared in the same way. However, there may be significant differences between centers.

Occasionally, one is unable to acquire an adequate lavage sample. This should be recognized as reason why a sample may not truly reflect the alveolar sample. If the aspirated fluid represents less than 5% of the instilled fluid or there are more than 5% bronchial epithelial cells, the sample should be judged carefully (119). The sample probably contains a significant proportion of bronchial secretions, which may not reflect the inflammatory response of the

Table 5 Factors Which Affect BAL Results and Recommendations to Minimize Variation

Source of variability	Recommendation for minimizing variability (125)
Dwell time of aspirated fluid	Minimize, keep total to less than 60 s
Suction pressure during aspiration	The lower the better. Not to exceed 100 mm Hg
Volume instilled	At least 100 mL
Variability of lavage fluid returned	Report volume or percentage returned. When less than 5% returned, treat results cautiously
Handling of lavage fluid: Filtered versus nonfiltered Storage at room temperature or on ice	Keep standard
Cellular preparation: Cytocentrifuge versus membrane preparation	Keep standard

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alveoli. In some conditions, this may not be important. In cancer and tuberculosis, the clinician is looking for a specific cell or organism. In many cases of interstitial lung disease, it is the relative proportion of inflammatory cells in the BAL sample which are studied. For that interpretation, one needs an adequate alveolar sample.

VI. Conclusions

The use of BAL in the evaluation and management of interstitial lung disease has swung back and forth over the past 30 years. However, the samples obtained by lavage give unique information regarding the inflammatory response of the lung. In some diseases, the information is unique enough to make a specific diagnosis. In other situations, the BAL provides an assessment of the response of the lung, which has to be related to other information. The future of lavage will probably include the use of lavage to assess response to treatment.

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Pulmonary Fibrosis in Connective Tissue Disease

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I. Introduction

In this chapter, the features of interstitial lung disease of the more frequently encountered connective tissue diseases (CTDs) (rheumatoid arthritis [RA], systemic sclerosis [SSc], polymyositis/dermatomyositis [PM/DM], systemic lupus erythematosus [SLE], and Sjögren's syndrome [SS]) are reviewed. Pulmonary parenchymal disease occurs in all CTDs and makes a significant contribution to mortality, although pulmonary vascular disease in SSc (1) and bronchopneumonia in RA (2,3) and PM/DM (4,5) are also frequent pulmonary causes of death.

In the last two decades, interstitial lung disease in CTD has been widely studied. The prognostic significance of pulmonary function impairment in large unselected cohorts of patients has long been recognized, especially in SSc (6–9). However, a significant proportion of patients with CTD in unselected series have no evidence of interstitial lung disease, and in many others, minor functional impairment is not associated with chest radiographic abnormalities. Thus, although a range of parenchymal abnormalities was identified in small studies of histological findings in RA and PM/DM at open lung biopsy (10,11), definition of the full spectrum of disease was elusive. The advent of computed tomography (CT) disclosed a high prevalence of parenchymal abnormalities in all CTDs (12–17), and for the first time it was possible to compile large clinical series that included patients with limited disease, whereas excluding those without morphological evidence of pulmonary fibrosis. The use of CT stimulated a reappraisal of the diagnostic role of traditional modalities, including surgical lung biopsy and bronchoalveolar lavage. However, CT also

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created a new dilemma: the distinction between subclinical disease, manifesting only with subtle abnormalities on CT and pulmonary function testing, and clinically important pulmonary fibrosis warranting therapeutic intervention. This problem, which is probably the most frequent one facing chest physicians in the routine evaluation of CTD, has yet to be satisfactorily resolved.

II. Prevalence

Pulmonary involvement has now emerged as a major cause of death in CTD. However, the prevalence of interstitial lung disease is critically dependent upon the method of detection. Respiratory symptoms are not a reliable guide. Exertional dyspnea is a common complaint in CTD, especially in SSc (being reported by over 50% of patients in most series [18]). However, the presence of dyspnea does not consistently identify important lung involvement, but may merely represent the increased work of locomotion of arthritis or myositis; furthermore, patients severely limited by systemic disease may not exercise sufficiently to experience dyspnea despite significant pulmonary function abnormalities.

The definition of pulmonary fibrosis by chest radiography is also imprecise. In RA, for example, large cross-sectional studies have shown chest radiographic evidence of pulmonary fibrosis in less than 5% of patients (19,20), but interstitial disease is present on CT in 20–50% of patients (13–15). CT studies have revealed a similar prevalence of interstitial abnormalities in other CTDs (12,16,17). However, these figures do not necessarily equate to routine clinical practice. Selection bias is a major constraint. CT series have largely been performed at specialist respiratory centers; in only one series were consecutive unselected patients studied from a rheumatology clinic, and this disclosed a lower prevalence of lung involvement in RA (20%) (15) than in previous CT reports (13,14).

A further major difficulty is the distinction between clinically significant pulmonary fibrosis and limited subclinical CT abnormalities, which, in some cases, do not progress. Similarly bronchoalveolar lavage profiles in CTD often show a ''subclinical lymphocyte alveolitis'' (21), which does not seem to be a consistent precursor of overt pulmonary fibrosis. Exactly the same reservations apply to large cohort studies of pulmonary function indices; reductions in gas transfer, occurring in large subgroups of patients (6,15,22), are often minor, and not necessarily indicative of pulmonary fibrosis (rather than other pathophysiological processes, especially pulmonary vascular disease).

This problem applies also to biopsy and autopsy evaluation. For example, an early study was performed on RA patients volunteering for open lung biopsy (23). Pulmonary fibrosis was seen in over 50%, including many patients without overt clinical or radiological evidence of lung disease;

a figure which greatly exceeds estimates of the prevalence of clinically significant pulmonary fibrosis in RA. Difficulties in interpreting the significance of minor histological abnormalities apply equally to autopsy studies, with the further important proviso that, by definition, end-stage disease is evaluated. However, despite these difficulties, a reasonably accurate estimate of the prevalence of pulmonary involvement can now be made in CTD.

Interstitial lung disease is most prevalent in SSc, probably accounting for the relative paucity of studies of pulmonary fibrosis in other CTDs. The presence of pulmonary fibrosis is one of the American Rheumatism Association minor diagnostic criteria for SSc (24), and in autopsy studies, pulmonary fibrosis is found in over 70% of the cases (25,26). This figure overstates the frequency of pulmonary fibrosis in SSc in routine clinical evaluation; by definition, autopsy series describe SSc patients with major organ involvement. A large proportion of SSc patients screened for lung disease have subclinical involvement or lack clinical evidence of interstitial lung disease (27). However, the case for CT and pulmonary function screening for pulmonary involvement is strongest in SSc. Chest radiographic abnormalities are present in 25–65% patients (28), the highest prevalence being among those with CTDs, and CT identifies limited fibrosis in a further subgroup of patients with normal chest radiographic appearances (12,27). Pulmonary function abnormalities are present in a large majority in most series, although often confined to minor reductions in gas transfer.

Pulmonary fibrosis is also frequent in PM/DM, with at least 30% of patients having clinically overt disease (29) (although no single definitive evaluation has been performed of large numbers of patients). In RA, despite the low prevalence of lung disease on chest radiography (19,20), gas transfer is reduced in 20–40% of unselected patients (15,22). In SLE, biopsy and autopsy studies are confounded by selection bias and variations in histological diagnostic criteria, with a striking diversity in the prevalence of pulmonary fibrosis (4, 33, and 70%) (30–32). It is likely that, in many cases, trivial sequelae of infection or inflammatory complications were categorized as ''pulmonary fibrosis.'' The frequency of CT abnormalities has approximated 30% (17), but less than 5% of SLE patients have clinical or chest radiographic evidence of interstitial lung disease at the onset of systemic disease, and a progressive fibrotic lung disease develops during follow-up in a similar proportion (33).

The definition of the frequency of lung involvement in SS has been complicated by failure to distinguish between primary and secondary SS in early series (with lung abnormalities in secondary SS ascribable to the associated CTD), diagnostic variation, and failure to take smoking into account (34). Despite a high prevalence of cough (35), and the presence of lung function impairment in up to 25% (36), clinically overt interstitial lung disease approximates 10% in primary SS (37). Among the 10% of patients with

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dyspnea, most have histological abnormalities (fibrosis or lymphocytic infiltration) on transbronchial biopsy (38). Interstitial lung disease is evident in 10% on chest radiography (39) and in 35% of patients on CT (40). However, these figures cannot be equated with pulmonary fibrosis, as limited, potentially reversible lymphocytic infiltration is common in SS, with lymphocytosis being present in bronchoalveolar lavage fluid in 50% of patients with primary SS (41).

The frequency with which pulmonary disease precedes systemic manifestations complicates prevalence studies in CTD. Among patients developing pulmonary fibrosis, lung disease precedes typical systemic features in approximately 30% in PM/DM (42) and 20% in RA (43). In SSc, a small subgroup of patients have organ involvement (including parenchymal or pulmonary vascular disease) and autoantibody positivity, without overt cutaneous abnormalities (''systemic sclerosis sine scleroderma'') (44). Other patients have systemic features strongly suggestive of connective tissue disease but do not meet formal diagnostic criteria either at presentation or during follow-up. A further significant subgroup of patients has a mixture of features of SSc, RA, and PM/DM (''mixed connective tissue disease''), without satisfying diagnostic criteria for any single CTD.

Despite major discrepancies in published series, it can be concluded that a large minority of patients with SSc and PM/DM have clinically important pulmonary fibrosis. Interstitial disease is also obvious in up to 10% of patients with SS, and in less than 5% with RA and SLE, but subclinical involvement is present on lung function and CT evaluation in much larger subgroups.

III. Predisposing Factors

Although it is likely that major organ involvement tends to cluster in CTD, no consistent relationship has been documented between the severity of systemic and pulmonary disease. However, a number of predisposing factors have been identified. Genetic studies have shown that pulmonary fibrosis is associated with HLA-B8 and HLA-Dw3 positivity in RA (45), and with class II major histocompatibility complex (MHC) status (HLA DR3, 52a) in SSc (46). Serological markers are also important. Pulmonary fibrosis is linked to high levels of rheumatoid factor and prominent rheumatoid nodules in RA (19) and to the presence of antitopoisomerase antibodies and diffuse cutaneous involvement (Scl-70 positivity) in SSc (47,48). In PM/DM, the combination of diffuse lung disease with myositis and arthritis (the antisynthetase syndrome) is associated with antibodies to aminoacyl tRNA synthetases; anti-histidyl tRNA synthetase (Jo-1) is present in up to 30% of patients with inflammatory myopathy and in well over 50% of patients with inflammatory myopathy associated with diffuse lung disease, but in less than 5% of patients without

diffuse lung disease (49). Other less prevalent anti-tRNA synthetase antibodies (PL12, PL7, EJ, OJ) have also been linked to interstitial lung disease in PM/DM. Associations between autoantibody status and lung disease have not been documented in SLE and SS.

Environmental factors are also likely to be important. A wide variety of occupational exposures are known to cause SSc with pulmonary involvement, including d-penicillamine, tryptophan, bleomycin, pentazocine, vinyl chloride, benzene, toluene, and trichloroethylene (50), and the "toxic oil syndrome" in which scleroderma is associated with pulmonary involvement follows the ingestion of cooking oil containing rapeseed oil denatured with aniline (51). Smoking is believed by many to predispose to pulmonary involvement in CTD, although evidence of this is confined to RA (in which overt pulmonary fibrosis [19] and subclinical involvement [52] have both been linked to smoking); it is also possible that loss in pulmonary reserve due to smoking-related damage results in earlier detection of interstitial lung disease, thus leading to a spurious association.

IV. Pathogenesis

The pathogenesis of pulmonary fibrosis has been studied most intensively in SSc but it is likely that common pathogenetic mechanisms operate in all CTD. The most plausible model is autoimmune, with environmental factors triggering initial injury and a subsequent amplification of the immune response in genetically susceptible individuals (53,54). Factors promoting lung injury include tumor necrosis factor-α (TNF-α) (present early in disease and pivotal in animal models), transforming growth factor-β (TGF-β) (which colocalizes with collagen gene upregulation and facilitates connective tissue growth factor release) (55,56) and a helper T (Th1) to Th2 cytokine shift (57). Restricted T-cell responses to epitopes of deoxyribonucleic acid (DNA) topoisomerase I have been demonstrated both in SSc and in normal subjects; thus, the Scl70 antibody may induce a pathogenic immune response in individuals with responsive T-cell clones (58–60). CD45 RO ("memory") cells accumulate in the interstitium, especially in secondary lymphoid follicles with true germinal centers (61). A number of cytokines present in bronchoalveolar lavage fluid in SSc amplify injury including IL-8 (a neutrophil activator and chemoattractant), TNF-α, macrophage inflammatory protein (MIP)-1 (a macrophage and lymphocyte chemoattractant) and regulated on activation, normal T-cell expressed and secreted (RANTES) (which recruits and activates T cells) (62,63).

Following initial injury, amplified by proinflammatory cytokines, the other key pathogenetic process in SSc is the accumulation of connective tissue matrix cells and proteins (64–67). Among many identified growth factors,

connective tissue growth factor (which is upregulated by TGF-β and promotes collagen production) is likely to play a central role (68–70). TGF-β, endothelin (ET)-l, and coagulation cascade proteins are active in the lungs of patients with SSc (67,71,72); fibroblasts exhibit dysregulated type-1 collagen biosynthesis and impaired messenger ribonucleic acid (mRNA) downregulation. In lung tissue in SSc, ET-A receptors are decreased and ET-B receptors are increased (64,73).

V. Histological Subsets of Interstitial Lung Disease

Historically, interstitial lung disease was grouped generically in CTD, as pulmonary fibrosis or fibrosing alveolitis. In SSc, in particular, it was believed that the histological features were those of idiopathic pulmonary fibrosis (74) despite apparent differences in natural history (75), although in RA and PM/DM, organizing pneumonia (bronchiolitis obliterans organizing pneumonia) and lymphocytic interstitial pneumonitis (LIP) were also recognized (10,11). Following the reclassification of idiopathic interstitial pneumonias by the ATS/ERS International Consensus Committee (76), and increasing recognition of the entity of nonspecific interstitial pneumonia (NSIP), it has become apparent that lung disease in CTD can be subclassified in the same way. It is now clear that all forms of idiopathic interstitial pneumonia also occur in CTD, including usual interstitial pneumonia (UIP), NSIP, organizing pneumonia, desquamative interstitial pneumonia (DIP), respiratory bronchiolitis with associated interstitial lung disease (RB-ILD), LIP, and acute interstitial pneumonitis (AIP).

As in idiopathic disease, the histological features of UIP consist of patchy interstitial fibrosis, with an element of honeycombing, associated with a mild chronic inflammatory cell infiltrate and fibroblastic foci adjacent to established fibrosis. UIP in CTD is largely identical in appearance to idiopathic UIP, but there is an anecdotal impression that in some patients with RA, chronic inflammation is more intense and that the number of germinal centers is increased (Fig. 1). However, this observation might merely be indicative of coexisting follicular bronchiolitis rather than representing a true morphological feature of UIP in CTD. Early reports of a high prevalence of UIP in RA (10,77), PM/DM (11,78) and the description of UIP in other CTD predate recognition of NSIP as a discrete entity and overstate the true prevalence of UIP in these diseases.

NSIP was first recognised in CTD by Katzenstein and Fiorelli (1994); in their landmark description, 16% of their NSIP patients had CTD (79). It is now apparent that in SSc (80) and PM/DM (81), NSIP is more prevalent than UIP. The range of appearances in NSIP is similar in CTD and idiopathic disease, with the defining features being diffuse involvement of alveolar walls

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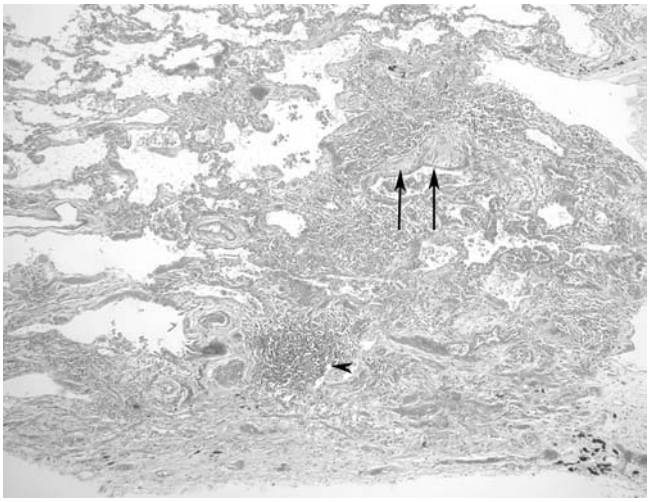

Figure 1 A case of usual interstitial pneumonia (UIP) from a patient with rheumatoid arthritis shows patchy subpleural fibrosis with occasional fibroblastic foci (*long arrows*), as well as a germinal center in the interstitium (*arrowhead*).

(categorized as cellular or fibrotic), temporally uniform disease, and a paucity or absence of fibroblastic foci (Fig. 2) (79). As in UIP, numbers of germinal centers may be profuse in CTD.

Other forms of interstitial pneumonia are morphologically similar in CTD and idiopathic disease. Although organizing pneumonia is sometimes associated with major interstitial fibrosis in CTD, especially in PM/DM (82), this also occurs occasionally in cryptogenic disease (83,84); moreover, this observation may represent coexistence of two separate histopathological processes in CTD in some cases. In other respects, organizing pneumonia does not differ histologically between idiopathic and secondary forms (Fig. 3). The same applies to occasional cases of DIP and RB-ILD. These smoking-related macrophage-mediated disorders, viewed by some as overlapping entities, are rare findings at biopsy in CTD (77,85–88), occurring exclusively in smokers, and are thus likely to represent a clinical or subclinical manifestation of smoking rather than a complication of systemic autoimmune disease.

Acute interstitial pneumonia, the histological counterpart of the clinical syndrome of the acute respiratory distress syndrome (ARDS) and the only acute interstitial pneumonia in the consensus classification system for idiopathic disease, has been reported in CTD, especially in SLE. The histological finding is one of diffuse alveolar damage (Fig. 4), and in SLE, the clinicopathological presentation of ARDS with diffuse alveolar damage is termed acute lupus pneumonitis (89–91). AIP has also been documented in

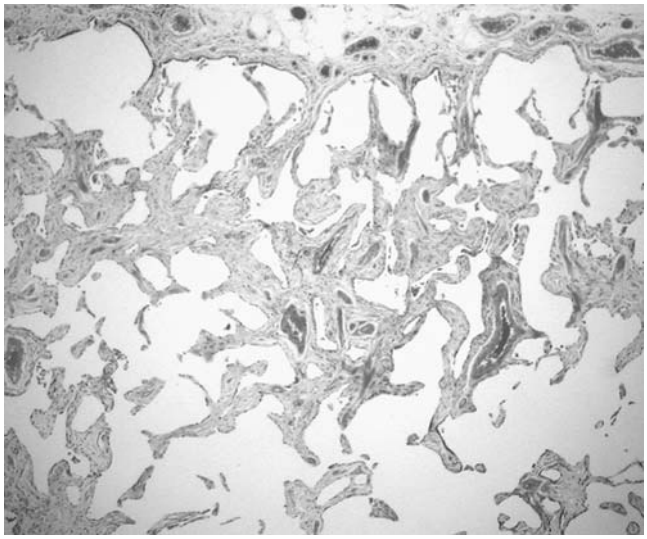

Figure 2 A case of fibrotic NSIP from a patient with scleroderma with diffuse involvement of the interstitium by chronic inflammation and fibrosis. The fibrotic component appears of a similar age throughout the biopsy with, in particular, no fibroblastic foci.

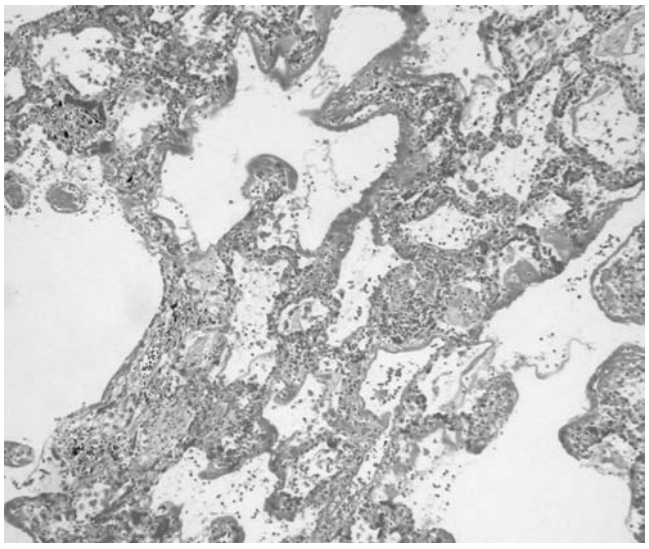

Figure 3 A case of organizing pneumonia (OP) with patchy filling of alveoli by buds of fibroblastic tissue together with a mild interstitial chronic inflammatory cell infiltrate.

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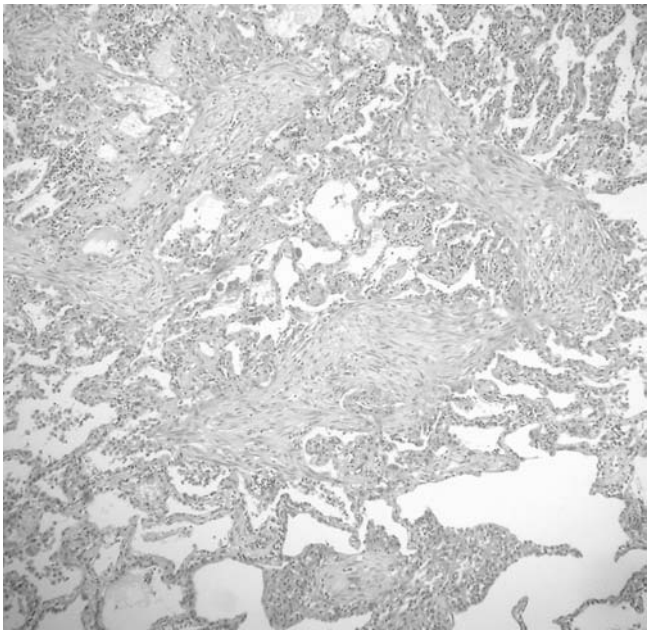

Figure 4 A case of diffuse alveolar damage (DAD) shows the interstitium to be expanded by plump fibroblasts and a mixed inflammatory cell infiltrate with the alveolar walls lined by hyaline membranes.

PM/DM (11,92), and in some of those surviving the acute episode, there are anecdotal reports of diffuse fibrotic disease as a long-term sequel, as is known to occur in idiopathic disease (93).

Follicular bronchiolitis and LIP are overlapping histological patterns of pulmonary lymphoid hyperplasia that may represent a continuum from peribronchiolar to interstitial lymphocytic infiltration. LIP is viewed by some as being a lymphoproliferative disorder (Fig. 5), but malignant transformation is rare, and it is now included within the idiopathic interstitial pneumonias (76). In fact, the LIP/follicular bronchiolitis continuum is seldom idiopathic, but most commonly occurs in immunosuppressed patients and in CTD, especially SS (39,94) and less frequently in RA (95,96), but it is also seen in other CTDs (97). Whether evolution to a predominantly fibrotic picture commonly occurs is not known, although increasing fibrosis has been reported in idiopathic disease (98).

With the subclassification of the interstitial pneumonias, certain characteristic groupings of histological subsets in individual CTD have become apparent. In SSc, it is now clear that the great majority of patients have NSIP or UIP. Organizing pneumonia has been reported in only a handful of patients (99), usually in association with penicillamine therapy, and LIP is

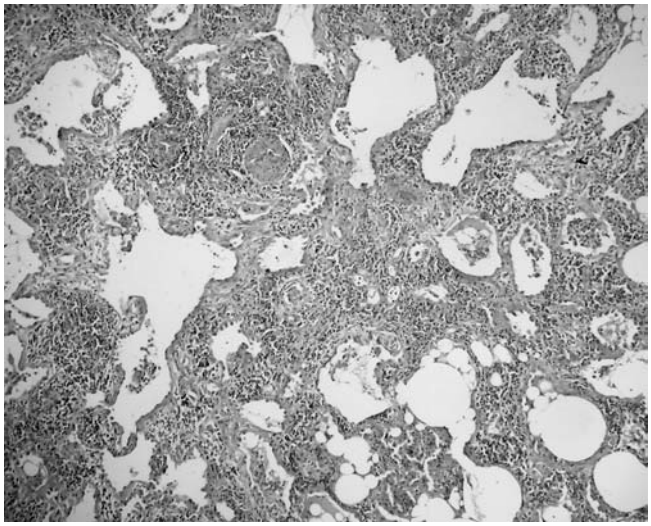

Figure 5 A case of lymphocytic interstitial pneumonia (LIP) with diffuse expansion of the interstitium by a dense infiltrate of small lymphocytes, plasma cells, and histiocytes.

seldom a feature of SSc. Although a small study disclosed an approximately equivalent prevalence of NSIP and UIP in SSc (100), it is likely that UIP was overrepresented owing to selection of patients with advanced disease. In a large series reported from a unit in which open or thoracoscopic lung biopsy was performed in patients with a wide range of disease severity, between 1984 and 1998, NSIP predominated (80). Of 80 biopsied patients, 62 had NSIP (78%), including 13 with cellular NSIP, and only 6 had UIP (8%); the remaining cases were largely made up of "end-stage lung" ($n = 6$, 8%) and RB-ILD in smokers ($n = 4$, 5%). The proportion of cellular NSIP in this series (20% of NSIP cases) is higher than reported in idiopathic disease. Importantly, the prevalence of NSIP did not differ before and after 1990 despite the fact that biopsy was performed by protocol before 1990 but only in selected cases subsequently. Thus, major distortion due to selection of patients for biopsy is unlikely, and it can now be concluded that NSIP is the predominant histopathological process in SSc.

In DM/PM, the pattern of lung involvement is more heterogeneous. A recent study of 22 biopsied patients disclosed NSIP in the majority (18, 82%), with the remainder having organizing diffuse alveolar damage (i.e., AIP) or organizing pneumonia (81). However, consolidation was occasionally evident on CT, and a significant prevalence of organizing pneumonia is recognized in PM/DM (11); the CT features of organizing pneumonia in PM/DM have been reported in a large series (101). This apparent discrepancy may reflect the fact that some patients with organizing pneumonia develop progressive

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pulmonary fibrosis: An overlap in histological and CT features between NSIP and organizing pneumonia is recognized in idiopathic disease (102). However, it is also possible that organizing pneumonia was overrepresented in previous reports, because a more acute and progressive course stimulated intensive investigation. A small subset of PM/DM patients with organizing pneumonia have a poor outcome (103), with rapid progression of disease despite treatment in some cases, and thus the prevalence of supervening UIP in rapidly progressive disease needs to be reappraised in further larger cohorts.

The prevalence of individual interstitial pneumonias has not been well characterized in other CTDs. Based upon a well-documented subgroup with rapidly progressive pulmonary fibrosis and a fatal outcome (104), it is suspected that a higher proportion of patients with RA might have UIP than in other CTD. However, interstitial lung disease in RA is truly heterogeneous; there is a significant prevalence of organizing pneumonia (10), and NSIP (79,105) and LIP (94,95) both occur. Obliterative bronchiolitis, a rare but often lethal complication of RA, is likely to be underrepresented in histological series, because clinical recognition is difficult except in advanced disease. The relative prevalence of all of these disorders is likely to be critically dependent upon selection factors, including restriction of biopsy to patients with advanced or progressive disease evaluated by subspeciality respiratory units. Rheumatoid lung has not been evaluated in sufficiently large series to allow secure histological generalizations.

The same constraints apply to SLE and SS. As discussed earlier, progressive pulmonary fibrosis is rare in SLE, and pathological descriptions predate the recognition of NSIP as a separate disorder. NSIP has been reported in SLE (79,89,106), but the relative prevalence of UIP and NSIP is not known. Organizing pneumonia (107) and LIP (97) are seldom encountered in SLE. In SS, LIP is much more frequent than other interstitial pneumonias (39), although there are anecdotal reports of NSIP at biopsy, including two cases known to the authors. Organizing pneumonia is rare in SS (108).

VI. Natural History and Prognosis

In most series, the natural history of pulmonary fibrosis in CTD has been compared to idiopathic pulmonary fibrosis (IPF), diagnosed clinically or at lung biopsy. However, the interpretation of the findings has been complicated by the inclusion of patients with minor impairment of pulmonary function and limited pulmonary fibrosis on chest radiography or CT. The prognosis of pulmonary fibrosis, in CTD and idiopathic disease alike, is linked to the severity of disease; severe lung function impairment and extensive disease on CT is associated with a higher mortality. It is difficult to quantify the

confounding effect of "lead-time bias" when contrasting the outcome of lung disease in CTD with IPF. This problem is compounded by the relatively low prevalence of clinically significant pulmonary fibrosis in RA, SLE, and SS compared to SSc and PM/DM. In SLE, no definitive statement is possible, and although interstitial involvement is often nonprogressive in SS (109–112), population analyses largely pertain to lymphocytic infiltration (LIP) rather than pulmonary fibrosis. In RA, despite a high mortality in a subset of patients with advanced disease (104), the heterogeneity of disease further complicates the definition of natural history, and no overall consensus has been reached on the prognosis of less extensive pulmonary fibrosis compared to other CTDs and idiopathic disease. In PM/DM, there are numerous reports of a fatal outcome in single cases or small patient groups, but until recently the same uncertainties prevailed as in RA. However, Douglas and colleagues have recently reported a cohort of 70 patients with overt diffuse interstitial lung disease in whom survival was consistent with idiopathic NSIP (and better than observed in historical control subjects with idiopathic UIP) (81). The same is likely to apply to subgroups of patients with SLE, SS, and RA based upon the frequent observation of stability of lung disease in these CTDs.

More is known about the natural history of pulmonary fibrosis in SSc. Early statements of outcome were confounded by the fact that studies of serial decline in pulmonary function indices were performed in unselected populations with individual connective tissue diseases rather than in the subset of patients with established interstitial lung disease (6–8). However, in a large cohort of over 60 SSc patients with overt clinical evidence of pulmonary fibrosis, survival was substantially better than in over 200 patients with IPF, and this difference persisted after matching for pulmonary function indices and the extent of disease on CT (75). Recently, serial trends in pulmonary function indices were evaluated in 62 SSc patients with biopsy-proven NSIP (80); over the 3 years after biopsy, pulmonary function indices changed little in the whole group, although occasional patients exhibited major progression of disease. Previous evaluations of serial pulmonary function trends had included patients without evidence of pulmonary fibrosis (6–9); lung function decline was greater than in normal populations, especially in the 2 years after the onset of systemic disease, but there was considerable individual variation, with a large subgroup of patients who remained stable during prolonged follow-up despite, in many cases, significant impairment in lung function. Taken together, these data indicate that pulmonary fibrosis in SSc has a better prognosis than IPF; a formal comparison with idiopathic NSIP has yet to be undertaken.

In SSc, total gas transfer (D_{LCO}) levels have consistently been linked to mortality (6,9,75,80,113); a reduction in D_{LCO} to less than 40% of predicted is associated with a 5-year survival of less than 10% (6). Other pulmonary function indices have generally been less predictive, although a severe restrictive defect is an adverse prognostic indicator (9). However, a major reduction in gas transfer

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is sometimes indicative of advanced pulmonary vascular disease rather than extensive interstitial fibrosis; the superior prognostic value of DLCO may reflect the fact that it captures both disease processes, unlike other functional measures.

VII. Clinical Features

Respiratory symptoms in CTD are modulated by limitations due to systemic disease, especially arthropathy and myopathy. Thus, the relationship between the severity of symptoms and the severity of lung disease (the degree of lung function impairment) are notoriously weak. This problem applies especially to exertional dyspnea, which is minimal in some cases despite moderate functional impairment, but it may be prominent in other patients in the absence overt interstitial lung or pulmonary vascular disease (resulting from inefficient locomotion in association with severe musculoskeletal disease). Exertional dyspnea is the most frequent initial respiratory symptom in SSc, RA, and PM/DM, although pleuritic pain is also a common presenting complaint in SLE (114). Cough due to tracheobronchial involvement is probably the most common presenting respiratory complaint in SS (115); when cough is prominent in other CTDs, secondary SS and reflux (especially in SSc) should both be considered.

The clinical findings in pulmonary fibrosis in CTD are similar to those in IPF with bilateral, predominantly basal crackles and, in advanced disease, tachypnea, cyanosis, and right heart failure. However, there is also a large subset of patients with limited pulmonary fibrosis, detected during screening investigations, in whom basal crackles are limited or absent. Digital clubbing is rare in CTD except in advanced rheumatoid lung.

VIII. Imaging Techniques

Plain chest radiography remains a mainstay of initial clinical evaluation, often serving to alert the clinician to a need for more intensive investigation. The most frequent appearance is a predominantly basal reticulonodular pattern resembling the radiographic appearance of IPF, although abnormalities are often limited in extent. This appearance, most often denoting NSIP or UIP, differs markedly from the classic radiographic features of organizing pneumonia (focal consolidation variably admixed with ground-glass attenuation). However, chest radiographic appearances are often difficult to interpret: the widespread use of CT has highlighted the insensitivity of the chest radiograph (12–17), and concurrent pleural involvement, a common feature of RA and SLE, is an important confounder. An apparently normal chest radiograph does not justify failure to investigate patients with respiratory symptoms more intensively.

The diagnostic value of CT in interstitial lung disease in general applies equally to CTD. CT is more sensitive than plain chest radiography in detecting pulmonary fibrosis in all CTDs (12–17) and is now part of routine evaluation in patients with suspected pulmonary fibrosis. However, the difficulty of interpreting the significance of minor CT abnormalities is highlighted by a recent prospective study of consecutive unselected patients with RA (15). Interstitial abnormalities were evident on CT in 20% of patients, and in most cases were identified clinically or on chest radiography. As the prevalence of clinically important pulmonary fibrosis in RA is considerably less than 20%, limited CT abnormalities should not be equated with the entity of rheumatoid lung in historic series.

The spectrum of CT appearances parallels the range of abnormalities reported in idiopathic disease. In UIP, a subpleural, predominantly basal reticular pattern is the rule, with microcystic disease or honeycombing, and little ground-glass attenuation in idiopathic disease (116), and this applied equally to SSc patients with UIP in a recent small study (117). The CT features of NSIP are more variable (118); in a study performed shortly after NSIP had been defined, it could seldom be distinguished from other idiopathic interstitial pneumonias (119). However, with increasing experience, it is now clear that NSIP patients presenting with the clinical features of IPF have more ground-glass attenuation (Fig. 6) and a finer reticular pattern, although the distribution differs little from UIP (116). In SSc, the spectrum of CT findings ranges from predominant reticular disease to prominent ground-glass attenuation, usually admixed with finer reticular abnormalities (120), but fibrosis is less

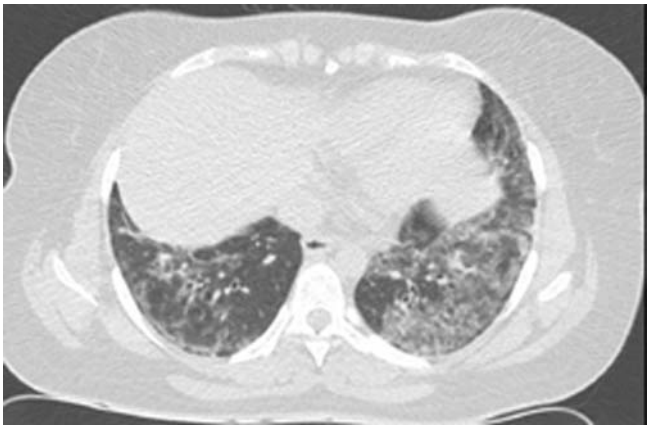

Figure 6 High resolution computed tomography image of a patient with Sci-70 positive systemic sclerosis and biopsy-proven fibrotic nonspecific interstitial pneumonia. The disease is predominantly basal and consists of a mixture of ground-glass attenuation and fine reticular abnormalities, in association with traction bronchiectasis.

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coarse than in IPF (121), which is in keeping with the high prevalence of NSIP in SSc (80).

In other CTDs, CT findings vary according to the frequency of individual histopathological processes. Abnormalities compatible with NSIP or UIP are most frequent, but consolidation, which is not seen in SSc, is often prominent in PM/DM (81,101,122) and may be admixed with ground-glass attenuation or with reticular abnormalities (122). This appearance generally corresponds to organizing pneumonia (Fig. 7), but it also occurs in a subset of patients with cellular NSIP (102). The distribution of organizing pneumonia is sometimes predominantly basal and subpleural, but is often bronchovascular, especially in PM/DM (101). A CT picture of widespread ground-glass attenuation, with or without irregular cystic abnormalities (Fig. 8), denoting LIP (123), is seen most often in SS, but it is also seen occasionally in other CTDs, especially RA and SLE. Mosaic attenuation is an occasional finding in RA and SS, which is in keeping with coexisting bronchiolitis (124,125), but it is not generally a feature of SLE, PM/DM, or SSc. In RA, CT evidence of bronchiectasis is also frequent (14).

The use of CT has undoubtedly reduced the need for thoracoscopic lung biopsy in CTD. Accumulated experience in the last decade has defined the expected pattern of interstitial involvement in individual CTDs, and CT findings often serve to confirm that disease is typical, obviating invasive diagnostic procedures. CT also provides a useful means of estimating the likelihood of

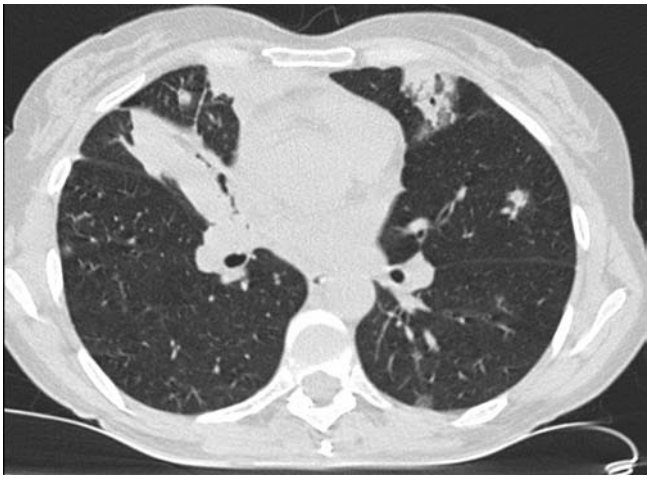

Figure 7 High resolution computed tomography image of a patient with sero-positive rheumatoid arthritis and biopsy-proven organizing pneumonia. There are areas of well-demarcated consolidation; note also the presence of bronchiectasis, a common CT feature of rheumatoid arthritis.

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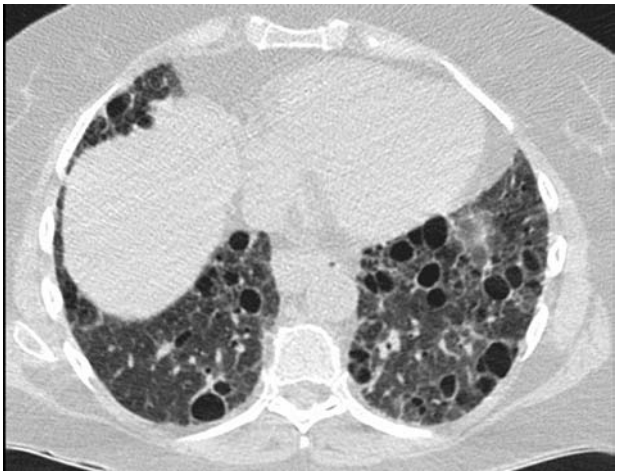

Figure 8 High resolution computed tomography appearances of a patient with Sjögren's syndrome and biopsy-proven lymphocytic interstitial pneumonia. Ground-glass attenuation has regressed and the predominant abnormality is irregular cystic air-space formation, a frequent late CT manifestation of LIP.

responsiveness to treatment in some patients, especially when disease is clearly fibrotic (prominent reticular abnormalities with little ground-glass attenuation) or predominantly inflammatory (with little or no reticulation) (126). However, the value of CT in this regard should not be overstated. In many SSc patients with an admixture of ground-glass attenuation and fine reticular disease on CT, the presence of significant inflammation cannot be excluded (127) even when the presence of traction bronchiectasis suggests predominant fine fibrosis.

Nuclear medicine techniques have a limited role in staging pulmonary involvement in CTD. Gallium 67 scanning is not a clinically useful modality; in SSc, gallium 67 uptake does not correlate with symptoms, chest radiographic appearances, or the degree of pulmonary function impairment (128). Interest in the quantification of pulmonary clearance of inhaled technetium-labeled diethylenetriamine penta-acetate (Tc 99m–DTPA clearance) has recently been stimulated by the observation that rapid Tc 99m–DTPA clearance is linked to a worse outcome in IPF (129). This observation is of particular interest in view of the recently promulgated "epithelial fibrotic" hypothesis in IPF (130); rapid Tc 99m–DTPA clearance is indicative of loss of epithelial cell integrity. In SSc, Tc 99m–DTPA clearance has been used to detect subclinical lung involvement (27), and in one study, persistently rapid clearance was associated with a high risk of subsequent functional deterioration, whereas normal clearance was a consistent predictor of functional stability (131). However, the technique is not readily available in many centers, and further studies are required to justify its widespread introduction.

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IX. Pulmonary Function Tests

As in most other forms of interstitial lung disease, plethysmographic and spirometric volumes are typically reduced in a restrictive pattern, with usually a greater reduction in DLCO. Arterial gases are often relatively well preserved, compared to IPF, even when interstitial disease is moderately extensive (132), and severe hypoxia denotes a poor outcome.

The accuracy of functional indices in staging the histological severity of disease (at lung biopsy) has not been attempted in CTD. However, in SSc patients with fibrosing alveolitis and no overt clinical evidence of pulmonary hypertension, DLCO levels reflect the extent of fibrosis on CT much better than other resting functional variables (133). In part, this is likely to reflect the high sensitivity of DLCO. Minor reductions in lung function indices below a highly variable normal range (80–120% of population-derived values) are difficult to interpret in the absence of established pulmonary fibrosis (15). For example, an apparently small reduction in the forced vital capacity to 75% of predicted normal represents, in reality, a reduction of between 5 and 35% from an unknown baseline. By contrast, the greater sensitivity of DLCO results in much greater reductions in patients with moderately extensive fibrosing alveolitis. The confounding effect of variation in baseline values is proportionately reduced, and the correlation of DLCO with the morphological severity of disease is proportionately stronger.

However, the greater sensitivity of DLCO creates its own problems in patients without clinical or radiological evidence of pulmonary fibrosis undergoing routine pulmonary function tests. A small isolated reduction in DLCO is common in CTD (15) and does not necessarily denote clinically significant pulmonary fibrosis or a higher likelihood of future progression. In some cases, fluctuations in gas transfer may represent vascular events, probably accounting for the observation of a population reduction in gas transfer in a large cohort of SSc patients in the winter (134). Thus, minor pulmonary function impairment in CTD disease can only be interpreted in conjunction with clinical and radiological data, especially CT evaluation. This problem is compounded by coexistent pathologies, including pulmonary vascular disease in SSc and SLE, pleural disease in RA and SLE, respiratory muscle weakness in PM/DM and SLE, and obstructive airways disease in RA and SS. Thus, the functional consequences of concurrent disease sometimes confound the interpretation of pulmonary function tests in established pulmonary fibrosis.

Maximal exercise testing in connective tissue disease is often impracticable owing to arthropathy or myopathy and has not been widely evaluated. In SSc, oxygen desaturation on maximal exercise correlates better with the extent of disease on CT than lung volumes (133) but is inferior to DLCO in this regard. The greatest utility of maximal exercise testing may lie in demonstrating lack

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of exercise desaturation in selected cases, reducing the likelihood that minor CT or resting functional abnormalities are clinically significant.

X. Bronchoalveolar Lavage

The performance of bronchoalveolar lavage (BAL) has become less frequent in CTD over the last decade. Early studies showed a high prevalence of a BAL lymphocytosis in CTD without, in many cases, overt clinical or radiographic evidence of pulmonary fibrosis, leading to the concept of ''subclinical alveolitis'' (21,41). However, despite initial expectations, there is no evidence that an isolated BAL lymphocytosis is a precursor of clinically significant interstitial lung disease in CTD, and in advanced pulmonary fibrosis, a BAL neutrophilia and/or eosinophilia is usual.

More recently, the prognostic role of BAL in CTD has been evaluated almost exclusively in SSc. A BAL neutrophilia has been associated with subsequent deterioration in pulmonary function indices in several studies (135–138), and is regarded by some as an important indication for immunosuppressive treatment. The prognostic value of BAL needs to be clarified. Extensive pulmonary fibrosis, as judged by CT, is strongly linked with a high BAL neutrophil content in SSc (139,140); it is likely that extensive disease is itself an important risk factor for subsequent deterioration. Thus, it is unclear whether BAL adds usefully to careful noninvasive (CT) staging of disease severity. The prognostic value of a BAL eosinophilia is similarly uncertain. In a large cohort of SSc patients with biopsy-proven NSIP, mortality was higher in patients with a BAL eosinophilia at presentation, after adjustment for the severity of pulmonary fibrosis (80), and an eosinophil influx into BAL fluid occurs in SSc when disease is minimal on CT (139); however, until these findings are reproduced, their significance will remain uncertain.

XI. Treatment of Pulmonary Fibrosis

Although clinicians have debated the efficacy of treatment in pulmonary fibrosis in CTD for much of the last two decades, there is no overall consensus on the indications for therapy and few studies address this crucial question. The central problem is to reconcile a potential therapeutic benefit with the significant likelihood of iatrogenic disease. It is widely accepted that reversible inflammatory disease should usually be treated in the hope of preempting progression to fibrosis. However, the place of intervention in overtly fibrotic disease is less straightforward, as the efficacy of current treatments in preventing progression of fibrosis remains uncertain. The problem is compounded by observation of limited stable disease in a significant subgroup of

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patients; it can be argued that, in many of these cases, treatment is of little benefit and may harm the patient.

However, in the absence of definitive data, there is broad agreement on a number of factors that should alter the threshold for intervention. As discussed earlier, the presence of prominent ground-glass attenuation or consolidation on CT implies a higher likelihood of reversible disease. Severe disease, as judged by symptoms, pulmonary function tests, and CT, usually merits therapy. It can be argued that the presence of extensive disease implies a previous ''track record'' of major progression, and thus a higher likelihood of progression in the future. Evidence of recent progression of disease, based upon symptoms, serial imaging, or pulmonary function deterioration, is an equally important justification of treatment. However, in extensive fibrotic disease, the clinician does not have the luxury of observation in order to define longitudinal behavior, as further declines in pulmonary function indices are likely to be irreversible and disabling. In SSc, the DLCO level can be viewed as a more accurate guide to intervention than other pulmonary function tests, as it reflects the morphological extent of pulmonary fibrosis more consistently (133), and has had prognostic value in many studies (6,9,75,80,113). Thus, treatment is usually warranted when a DLCO level of less than 50% of predicted is associated with moderately extensive disease on CT. However, the presence of ''outliers'' with disproportionate hypoxia or lung restriction, in SSc and other CTD alike, complicates this judgment, and highlights the danger of basing decisions upon any single measure of disease severity. Furthermore, progression of fibrosis is probably most likely early in the course of systemic disease, although this is documented only in SSc (7,9,113).

Thus, the decision to treat pulmonary fibrosis should be influenced by evidence of inflammation, disease extent, evidence of recent progression, and a recent onset of systemic disease. In many centers, the presence of increased BAL cellularity (and, less widely, rapid clearance of inhaled Tc 99m–DTPA clearance) are viewed as important ancillary grounds for intervention. However, many patients with pulmonary fibrosis are asymptomatic and have mild impairment of pulmonary function indices with limited disease on CT. In the absence of evidence of recent progression, immediate treatment may not be warranted. However, in that case, meticulous observation is essential. Most clinicians recommend serial pulmonary function tests and chest radiographs at 3- to 6-month intervals until stability has been documented for at least 18 months. Even in that case, regular monitoring investigations at annual intervals are warranted in the longer term.

Treatment regimens for pulmonary fibrosis in CTD are anecdotal, based on uncontrolled retrospective studies, or extrapolated from regimens used in idiopathic interstitial lung disease. Thus, it has been historical practice to treat advanced pulmonary fibrosis with corticosteroid therapy with or without an immunosuppressive agent.

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Retrospective therapeutic analyses have largely been confined to SSc. Early studies focused on penicillamine, which was widely prescribed for the systemic manifestations of disease, but no convincing evidence emerged from retrospective studies (141–144), and, more recently, a comparison between high- and low-dose penicillamine disclosed no pulmonary benefit; moreover, a significant subgroup of patients developed radiological evidence of pulmonary fibrosis de novo while receiving treatment (145). High-dose corticosteroid therapy has not been formally evaluated and is now regarded as being relatively contraindicated in SSc following publication of a case control study showing an increased prevalence of renal crisis in patients receiving prednisone doses in excess of 20 mg daily (146).

The most suggestive evidence comes from reports of the use of cyclophosphamide being associated with improvements in pulmonary function indices in small groups of SSc patients (147–49). In two larger populations of patients with evidence of alveolitis, as judged by BAL, cyclophosphamide was introduced de novo or following failure of corticosteroid therapy (136,137). Those treated with cyclophosphamide were shown to have a much better outcome on serial pulmonary function testing than other patients, including a subgroup with no evidence of BAL alveolitis. The study of White (137), in particular, has had a substantial impact in rheumatological circles, and it is now argued by some (although not in print) that a placebo-controlled study is difficult to justify. This unsubstantiated view has not prevented the continuation of ongoing placebo-controlled evaluations of oral cyclophosphamide in the United States and intravenous cyclophosphamide in the United Kingdom. The absence of controlled data should be stressed; given the significant morbidity associated with long-term cyclophosphamide usage, including a striking increase in the eventual prevalence of bladder cancer, it is essential that any therapeutic gain be shown in a controlled evaluation to outweigh the major drawbacks of this therapeutic approach. Intermittent pulses of intravenous cyclophosphamide may have a more acceptable side effect profile, judging from experience in Wegener's granulomatosus (150), but its relative efficacy compared to oral cyclophosphamide is uncertain. Published experience to date in CTD patients with pulmonary fibrosis has consisted of pilot data in a handful of patients (151,152).

Some physicians advocate induction therapy with oral or intravenous cyclophosphamide followed by a switch to another less toxic immunosuppressive agent (such as azathioprine) once improvement or stability of disease has been demonstrated with serial pulmonary function tests. However, although this approach appears to be logical, there is no published experience of the use of other immunosuppressive drugs in SSc.

There are no convincing therapeutic data relating to fibrotic lung disease (UIP or fibrotic NSIP) in other CTDs. Many physicians continue to prescribe high-dose oral or intravenous corticosteroids initially in patients with

rapidly progressive disease, but in other contexts, a combination of low-dose prednisolone and an immunosuppressive agent is more usual. This approach remains justifiable, given the lack of viable alternatives, but there is a pressing need to evaluate new antifibrotic agents in patients with CTDs, which would be ideally evaluated with sequential CT imaging as well as serial pulmonary function indices.

The paucity of therapeutic data applies equally to other pulmonary processes in CTD. Organizing pneumonia often responds as well to corticosteroid therapy as cryptogenic organizing pneumonia, and it is reasonable to follow a similar treatment protocol in the two diseases, with an initial high-dose approach and attempted withdrawal of treatment over the next 1–2 years. However, the recent vogue for rapid cessation of corticosteroids in cryptogenic disease (153) should be viewed with circumspection in CTD in view of the poor outcome in a significant minority of patients (103). If corticosteroids are to be withdrawn rapidly, a high index of suspicion for supervening pulmonary fibrosis is essential, with an early change to prolonged treatment if this occurs.

Aggressive empirical immunosuppression is warranted in AIP (93). In idiopathic disease, it is usual to institute pulsed intravenous treatment, generally with methyl Prednisolone initially (e.g. 750 mg daily), and to follow this, in nonresponders, with intravenous cyclophosphamide (e.g. 600 mg/m^2 BMI as a single dose, repeated if necessary seven to 10 days later). However, although unsubstantiated, the simultaneous introduction of both agents is justifiable in overwhelmingly severe disease, in view of the expected poor outcome and the need for a rapid therapeutic effect.

Corticosteroids are usually prescribed in LIP. Despite the paucity of published experience, it is clear that some patients respond well, and in others, stability is achieved. However, based solely upon anecdotal experience, the outcome appears to be highly variable, and, in view of the inflammatory, potentially reversible nature of this disorder, the early addition of an immunosuppressive drug appears logical in progressive disease.

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11

Cytokine Phenotypes and the Progression of Chronic Pulmonary Fibrosis

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I. Introduction

Fibrosis of the pulmonary interstitium is observed in a wide variety of disorders, including infectious diseases, autoimmune disorders of connective tissue, and disorders where the etiology is unknown, such as idiopathic pulmonary fibrosis (1,2). The clinical manifestations of interstitial pulmonary fibrosis are likely the consequences of an initial immune/inflammatory response to a persistent antigen, leading to continued tissue injury and progressive fibrosis. The underlying mechanisms which promote the development of a fibrotic pathological response, as compared to a normal reparative response, remains one of the many different enigmas of pulmonary fibrosis. The clinical management of these disorders is frequently difficult, requiring the use of potent and often cytotoxic and immunosuppressive therapies (3,4); however, these therapeutic approaches often fail and organ transplant is the last resort.

This treatment strategy reflects, in part, the limited understanding of the mechanisms which are involved in the initiation, maintenance, and resolution of normal interstitial lung responses. Both steroid-resistant sarcoidosis and idiopathic pulmonary fibrosis are examples of fibrotic interstitial lung diseases that follow the above scenario, as these disorders are characterized by the accumulation of leukocytes within the lung, followed by progressive fibrosis and subsequent loss of airspace. Interestingly, both of these disorders often progress independent of pharmacological strategies aimed at intervention. Thus, the clinical frustration of treating progressive interstitial pulmonary

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fibrosis lies both in the inability to identify the etiology and complete pathogenesis of the disease but also in the lack of truly efficacious agents to remit the disease progression.

Although the sequence of events in the pathology of many interstitial fibrotic lung diseases is not well characterized, numerous factors that regulate immune and fibrotic processes have been implicated in the development of chronic pulmonary disorders. These processes include potential viral infections (5,6), genetic variations (7,8), immune complexes (9), environmental factors (10), and effector cell activation (11). This last category has generated recent interest, as the classification of effector cells can no longer be limited to peripheral blood leukocytes, but must include resident stromal (fibroblasts and smooth muscle cells) and parenchymal cells that comprise the lung. Epithelial cells (12), endothelial cells (13), and fibroblasts (14) have all been identified as being effector cells via their ability to generate significant levels of regulatory cytokines/chemokines that participate in cytokine networks within the lung (Fig. 1). The contribution of nonimmune cells in the lung to the evolution of interstitial disease is more diverse than simply serving as a passive target, leading to lung injury, as these cells may actively contribute to the pathogenesis of fibrotic disease.

For example, pulmonary fibroblasts are critical to the evolution of interstitial fibrotic lung disease, as these cells can synthesize both cytokines and extracellular matrix. Although the processes which lead to an increase in extracellular matrix deposition are not totally clear, it is apparent that

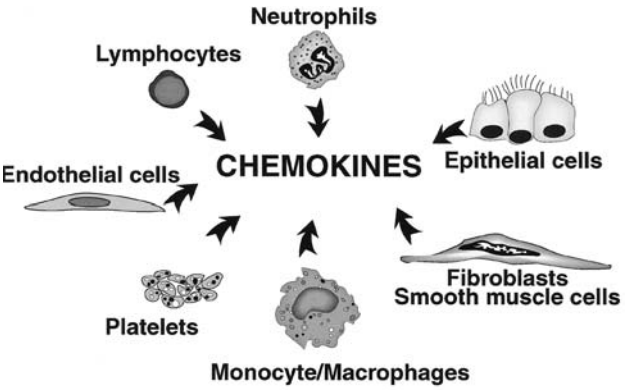

Figure 1 The expression of a variety of cytokines, especially chemokines, is not restricted to just leukocytes and other professional immune/inflammatory cells, as many resident cells have the capability to generate high levels of these mediators. The ability to express these mediators allows the resident cells to be effectors of the immune response.

inflammatory/immune cells, cytokines, and activated stromal cells themselves are contributing factors to end-stage disease (15). Moreover, it is likely that cytokine networks are ultimately responsible for cell-to-cell communication, which dictates the progression of pulmonary fibrosis. The development of chronic interstitial lung disease in humans is an enigmatic process, which is often characterized by fibroproliferation, matrix deposition, and chronic inflammatory cells. Although the mechanisms that dictate the progression of these pulmonary disease are not well known, certain of these disorders share common characteristics, including an unknown etiology, ill defined mechanisms of initiation and maintenance, and end-stage fibrosis.

Progressive pulmonary inflammation, as can occur in diseases such as idiopathic pulmonary fibrosis and end-stage sarcoidosis, is associated with substantial morbidity and mortality and efficacious therapeutic options are limited for the treatment of these diseases. However, mechanistically it is known that the progression of these disorders is likely controlled by cytokine networks that dictate their progression. Recent studies show that various cytokines affect fibroblast activation, proliferation, and collagen deposition during the evolution of chronic fibrotic lung disease. In particular, interferon gamma suppresses such fibroblast activities as proliferation and collagen production, whereas interleukin-4 (IL-4) and interleukin-13 (IL-13) augment fibroblast growth and collagen production. Interestingly, these two mediators are the prototypic cytokines that functionally define either type 1 or a type 2 immune responses. Thus, experimental models, which are characterized by either a Th1 or a Th2 response, will be useful in delineating the mechanisms which maintain and resolve chronic interstitial lung inflammation. These experimental systems will prove to be especially important, as the degree of inflammation and fibroblast activation/proliferation during the pathogenesis of chronic pulmonary inflammation may be dependent upon a balance of Th1- and Th2-like cytokines which are expressed during the evolution of the disease.

II. Cytokines and Pulmonary Fibrosis

A variety of cytokines have been found associated with chronic pulmonary inflammation, including IL-1 (16), IL-4, IL-6 (17), IL-8 (18), IL-9, IL-13, macrophage inflammatory protein-1 α (MIP-1α) (19,20), monocyte chemoattractant protein-1 (MCP-1) (21,22), tumor necrosis factor (TNF), (23) transforming growth factors (24–25), granulocyte-macrophage colony-stimulating factor (GM-CSF) (26), macrophage-CSF (M-CSF) (26), and platelet-derived growth factor (PDGF) (27). Although this list is not all inclusive, it does contain representative cytokines which possess early activation and chemotactic, growth and differentiation, and remodeling activities. For example, TGF-β possesses a number of activities that would suggest a

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profibrotic role in lung disease. TGF-β directly increases the gene expression of extracellular matrix molecules by stromal cells, inhibits collagenase production, and influences fibroblast proliferation via the induction of fibroblast growth factors (24,25).

The identification of different cytokines from either patients or animal models that mimic human pulmonary fibrosis have provided clues that specific immune mediators are involved in the evolution of interstitial disease. However, a causal role of these cytokines in the initiation and maintenance of lung lesions has not been clearly established. Thus, the biomedical community is still far from understanding the mechanisms which dictate either the restoration of normal lung tissue or the progression to irreversible fibrotic derangements of the pulmonary interstitium following chronic lung disorders.

However, there is a growing body of scientific evidence suggesting that the cytokine profile of the natural immune/inflammatory response likely determines the disease phenotype responsible for either resolution or progression to end-stage fibrosis. Much of the supporting evidence is derived from studies demonstrating that interferons, especially interferon gamma, have profound suppressive effects on the production of such extracellular matrix proteins as collagen and fibronectin (28–31). Investigations have demonstrated that interferons can inhibit both fibroblast and chondrocyte collagen production in vitro, as well as decrease the expression of steady-state type I and III procollagen mRNA levels in these cells (32). In addition, the administration of interferon gamma in vivo can cause a reduction of extracellular matrix in animal models of fibrosis (30–31). This information supports the concept that interferon gamma, one, of the major Th1-type cytokines, possesses profound regulatory activity for collagen deposition during chronic inflammation. Interestingly, IL-4 and IL-13, major Th2-type cytokines, are potent stimuli for the production of fibroblast-derived extracellular matrix (33–35). These studies have demonstrated that IL-4 treatment of fibroblasts can increase steady-state levels of extracellular matrix mRNA and subsequent production of extracellular matrix protein. In addition, they have been identified as chemotactic factors for directed movement of fibroblasts (36). These studies lend support to the theory that the disease phenotype characterized by either Th1-or Th2- like cytokines may be paramount in determining the course of chronic pulmonary inflammation, leading to interstitial fibrosis.

III. Fibrosis as a Dysregulated Consequence of a Natural Process

The involvement of various cytokines in the initiation and maintenance of chronic lung disease with eventual end-stage fibrotic pathology may represent

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the results of a natural sequence of host responses, that have gone awry. Under a normal host defense paradigm, it is likely that the initial cell-mediated reaction involves the expression of interferon gamma and mediators that would classified as type 1 response (37). This sequence of events plays out over a number of days and, in the hypothetical case discussed here, does not include the involvement of the innate immune response. However, it is likely that innate immunity, or the inability of the innate immune response to function normally, may be intimately linked to the progression of chronic inflammation. The immune process involving a high interferon gamma response is extremely efficient in activating the phagocytotic and killing activities of neutrophils, monocytes, and macrophages, as well as inducing major histocompatibility complex (MHC) class II expression on antigen-presenting cells (APCs). This expression of the type 1 cytokine phenotype is an effective procedure for both the clearance of an antigen or pathogen that escapes the initial innate immune response and an important event for establishing communication channels between APCs and T cells. This latter activity facilitates the development of a cell-mediated immune response and brings to bear a more complex and sophisticated attack on the inciting, foreign agent. Therefore, the initial type 1 (interferon gamma) cytokine response serves a number of functions, including the simultaneous activation of phagocytic cells for antigen clearance and killing of pathogen and the establishment of communication links between APCs and lymphocytes.

Although the elevation in interferon gamma is indeed important in activating mononuclear cells (monocytes, macrophages, and lymphocytes), it also serves a key role in the regulation of fibroblast activation. The ability of interferon gamma to suppress fibroblast proliferation and collagen deposition has long been recognized as an important role of this cytokine in the negative regulation of wound healing. Interestingly, clinical trials designed to capitalize on this biological phenomenon of interferon gamma and potentially alter the progression of pulmonary fibrosis have been conducted with questionable success. The inability of interferon gamma to serve as an effective biological therapy is likely due to the adverse effects of this cytokine on a variety of in vivo systems.

One of the interesting conundrums regarding the continued maintenance of a long-term type 1 process is that the sustained expression of cytokines such as interferon gamma may come with a price to the host, as this response is associated with collateral tissue injury due, in part, to the nonspecific damage caused by phagocytic cells in a highly activated state. Significant pathology may occur to surrounding tissue in an area of inflammation, as these activated cells attempt to clear and destroy the inciting agent with its armament of proteases, oxygen radicals, and other mediator systems. Thus, if the initiating antigen or pathogen is not cleared by the first cell-mediated immune response, the host enters a transition phase, which is characterized by the appearance of

a different cytokine phenotype. The subsequent host response is represented by the expression of cytokines which would be classified as a type 2 immune process with accompanying levels of the prototypic cytokines IL-4, IL-5, and IL-13. The significance of this new mix of mediators to host defense lies in the fact that a different type of immune process is now available to aid in clearing the antigen or killing the pathogen, which has escaped a type 1 cytokine-directed immune attack. Produced in this different immune process is the production of an additional type of antibody, IgE, the mobilization of another granulocyte population, the eosinophils, and the production of type 2 cytokines. The switch to a more sophisticated immune response may be directed by the persisting antigen or pathogen that is still serving as a stimulus to the system. Independent of the mechanism, which remains an enigma, that results in the cytokine phenotypic switch, the host now has the ability to mount a continued response with renewed vigor of the host response.

The ability to mount a different type of a reaction is an important component of the global host response to get rid of a persisting foreign agent, as it brings to bear additional aspects of the immune/inflammatory armament. However, the switch phenomenon also serves as a key process to ensure the host is ultimately protected from an invading foreign agent even if it may have escape this second wave of cytokine-directed defenses. It is now recognized that specific cytokines, which control the type 2 lymphocyte response, are also the same cytokines that ultimately activate resident fibroblasts and cause these cells to proliferate and deposit collagen. Thus, if the antigen continues to persist and escape the different cytokine-directed responses, the final cytokine phenotype targets and activates the resident fibroblasts to proliferate, ''lay down bricks and mortar,'' and wall off the inciting agent away from the host. Cytokines such as IL-4 and IL-13, two key type 2 mediators, are important signals for fibroblast activation, as these cytokines bind to specific receptors on the fibroblast and result in collagen expression. With the above scenario in mind, the end-stage pathology observed in pulmonary fibrosis may be due to dysregulation of this final walling-off process. As the levels of type 2 cytokines like IL-4 and IL-13 rise, there is a concomitant increase in fibroblast activation, which normally would return to a quiescent level if the causative agent is cleared. Again, the persistence of the agent, coupled with continued expression of type 2 cytokines, finally drives fibroblasts to a highly active state (Fig. 2). If this process is linked to a genetic predisposition for chronic disease or undefined environmental factors, the underpins are formed for end-stage disease.

IV. Type 1/Type 2 Paradigm for Progressive Fibrosis

Some of the most common worldwide diseases which are dominated by Th2 cytokines and eventual end-stage fibrosis of target tissue are helminthic

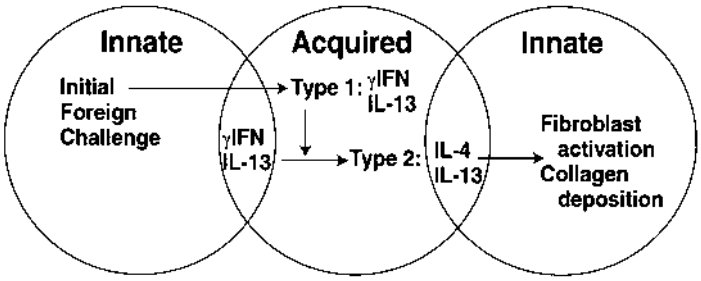

Figure 2 A schematic of the evolving immune/inflammatory response from an initial innate response to an acquired response and the eventual return to a primordial innate, fibroblast-directed response. The predominate cytokine phenotypes dictate each stage of these reactions, with type 2 cytokines predominating in the case of persisting antigen-driven responses. This finally leads to fibroblast activation and collagen deposition.

parasitic infections. For example, schistosomiasis is one of the world's most prevalent forms of chronic cell-mediated inflammation and possesses a cytokine phenotype characterized by high levels of IL-4, IL-5, IL-10, and IL-13, with corresponding low levels of interference gamma (INF-γ). In addition, the fibrotic response of the host during this disease greatly contributes to the morbidity associated with the parasitic infection. The vigorous fibrotic response to the schistosomal egg granuloma is the consequence of a parasite-induced, host-derived cytokine profile which likely allows the parasite to survive while effectively fibrosing or "walling off" the deposited parasitic egg. Interestingly, the treatment of murine schistosomiasis with exogenous IFN-γ significantly decreases collagen deposition associated with granuloma formation (38). Furthermore, IL-12–based vaccination was shown to suppress the fibrotic response normally induced by schistosomal infection (39). These studies lend support to the potential disparate role of Th1 and Th2 cytokines during the evolution of chronic disease with associated fibrotic processes. The opposing effects of Th1 and Th2 cytokines in fibrosis are further supported by a number of recent investigations demonstrating that IL-4 and IL-13 are important mediators of fibroblast activation (35,40).

Although there is little doubt that Th2 cytokines may predominate in specific parasitic infections, interesting data have recently accumulated suggesting that certain cell-mediated responses have characteristic Th2 cytokine profiles (41–44). Murine models of chronic graft-vs-host disease, as a result of experimental bone marrow transplant, have been characterized by a hypergammaglobulinemia, high levels of IgE, immune complex deposition in tissues, and elevated concentrations of IL-4 (41). When mice

with bone marrow transplant-graft-vs-host disease were treated with neutralizing IL-4 antibodies, IgE levels dropped, immune complex-induced lesions resolved, and splenomegaly was prevented. Interestingly, cyclosporine A, an agent known to suppress Th1 cytokine responses, caused an exacerbation of bone marrow transplant graft-vs-host disease in these models (45). Although clinical studies assessing longitudinal alterations in cytokine levels and corresponding changes in lung pathology are difficult to perform in human bone marrow transplants with subsequent graft-vs-host disease, it is known that fibrosis and associated cell proliferation associated with bronchiolitis obliterans may be a consequence of the transplant.

One of the more compelling pieces of information which may link the expression of Th2 cytokines to the evolution of chronic fibrosis-based inflammation is the association of fibroblast activation and the presence of eosinophils (46). A number of studies have demonstrated that asthma and parasitic infections are associated with both Th2 cytokine expression (IL-4 and IL-5) and a profound eosinophilia, as IL-5 is both an eosinophilopoietic and chemotactic factor for eosinophils. Although the mechanistic role of eosinophils and Th2 cytokines has been demonstrated in asthma and parasitic infections, the role of these cells and Th2 cytokines in other disease states is not as clear. However, recent data have demonstrated an eosinophilia in chronic inflammatory disorders in which fibrosis occurs. In addition, in vitro experiments have shown that eosinophils are capable of a time-dependent release of factors which stimulate human lung fibroblasts to undergo replication and synthesize extracellular matrix. The interactions between fibroblasts and eosinophils appear to be rather complex, as fibroblast-conditioned media has also been shown to prolong the survival of eosinophils. Nonetheless, studies have identified an increase in eosinophils in association with fibrotic changes in idiopathic pulmonary fibrosis. Thus, a potential fibrotic network, leading to end-stage pathology, may be established between the triumvirate of Th2–type cytokines, eosinophils, and fibroblasts.

V. IL-13 and TGF-β as Prototypic, Profibrotic Cytokines

The role of IL-13 and TGF-β as key mediators in the fibrotic process stems from recent studies using either a transgenic expression system for the overexpression of IL-13 or an adenovector-mediated gene transfer system for the overexpression of TGF-β1 (47–49). In the former experimental setting, IL-13 transgene expression under the control of the Clara cell 10-kD protein promoter in the lungs of mice demonstrated a unique model system to assess the in vivo biology of this cytokine. The overexpression of IL-13 resulted in an

eosinophilic inflammation, mucus hypersecretion, globlet cell hyperplasia, and subepithelial airway fibrosis.

Since IL-4 and IL-13 are related cytokines, they are both located on chromosome 5 and may have resulted from gene duplication during evolution, it is important to define specific differences between these two type 2 cytokines. Although both can share a common receptor, they differ in their ability to induce eosinophilic chemotaxis and survival, regulate T-lymphocyte proliferation, induce prostaglandin and INF-γ production, and cause epithelial cells to secrete electrolytes (50,51). One of the more striking differences between IL-4 and IL-13 is the significant profibrotic activity of IL-13. This cytokine appears to be able to increase the fibrotic process directly by stimulating fibroblasts to increase collagen expression and via a cytokine network that involves the expression of TGF-β1 (47). Data to support this latter aspect of an IL-13–directed cascade stem from the fact that IL-13 transgenic animals possess dramatically elevated levels of TGF-β1 associated with collagen deposition. Interestingly, a specific inhibitor of TGF-beta1, a TGF-β1 soluble receptor, negated the in vivo fibrosis caused by the IL-13 overexpressing mice (47). Thus, the effects of IL-13 in this process may be indirect. Further evidence to support the cytokine cascade effect of IL-13 has been provided by investigations demonstrating the ability of IL-13 to induce the expression of the chemokine MCP-1/CCL2, which may activate fibroblasts to express collagen. In one study IL-13 transgene overexpressed in a CCR2 knockout mouse, the receptor for the ligand MCP-1/CCL2, demonstrated reduced pulmonary fibrosis (52). Furthermore, the direct challenge of primary cultures of wild-type fibroblasts was found to increase collagen expression via a pathway that included the downstream expression of TGF-β (Fig. 3). Thus, there appears to be an IL-13–MCP-1/CCL2-TFG-beta circuit operative in the activation of fibroblasts and deposition of collagen. Table 1 contains a partial listing of cytokines and their role in the fibrotic process.

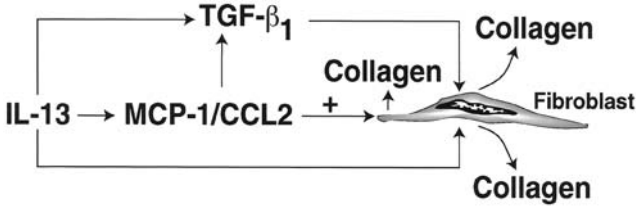

Figure 3 Cytokine cascades have been demonstrated to provide a mechanism for the activation of various immune/inflammatory pathways. This network appears to be operative in driving fibroblasts to become activated via a IL-13–MCP-1/CCL2–TGF-β1 connection.

Table 1 Certain Cytokines and Their Role in the Fibrotic Process

Cytokine	Effect on fibrotic process
IL-4	Minimal effect
IL-10	Suppresses
IL-12	Suppresses
IFN-γ	Suppresses
IL-13	Augments
TGF-β1	Augments
MCP-1/CCL2	Augments

VI. Exploiting an Antifibrotic Therapy by Targeting IL-13

The central role of IL-13 in the fibrotic process makes this cytokine an ideal target to develop new therapeutic approaches in regulating the progressive pathological response of end-stage disease. Recent studies have provided exciting evidence that reducing the biological activity of IL-13 can indeed reduce organ-based fibrosis. One of the first published studies to clearly show a therapeutic effect of targeting the biological activity of IL-13 utilized a parasite model induced by *Schistosoma mansoni* (50). This model causes the expression of a dominant CD4+ Th2 response with a subsequent polarized cytokine phenotype. Investigations into the role of IL-4 (a prototypic type 2 cytokine) in this model, using either IL-4 depletion or IL-4 −/− mice did not reveal a critical role for this type 2 cytokine in the developing pathology, including fibrosis, associated with schistosomiasis.

Interestingly, the developing granulomatous inflammation and accompanying fibrosis, which is a hallmark of the host response in this model, was significantly reduced in Stat6 −/− mice (53). Since IL-4 and IL-13 are the major activators of Stat6, the significance of IL-13 in the fibrosis associated with this model was explored. This was accomplished by using the soluble IL-13 receptor alpha 2 complexed with the Fc portion of an antibody (sIL-13Ralph2-Fc) (50). This therapeutic construct was shown to reduce collagen expression in vivo using histological, biochemical, and molecular analyses. The antifibrotic effects of sIL-13Ralpha2-Fc was not due to skewing the cytokine profile, as blocking the biological activity of IL-13 did not alter the production of INF-γ, IL-4, IL-5, IL-10, or IL-13. However, fibroblast activation appeared to be profoundly altered by IL-13, as in vitro studies demonstrate that fibroblasts express IL-13 receptors and, when occupied by IL-13, induced the expression of type 1 collagen.

Another exciting therapeutic approach for the treatment of fibrosis is the use of an IL-13 fusion cytotoxin, which is IL-13 tagged with a derivative of

Pseudomonas exotoxin (54). Once bound to the cell expressing an appropriate receptor, the IL-13 fusion cytotoxin destroyed that targeted cell. The strategy to use this construct is an outgrowth of investigations that have used the fusion protein selectively to target and eradicate solid tumor cells which express IL-13 receptors (55). Importantly, experimental animals did not demonstrate any negative effects from the prolonged systemic in vivo administration of the IL-13 fusion cytotoxin. In a recent in vivo study, IL-13–responsive cells were targeted via an intranasal administration of the IL-13 fusion cytotoxin in *Aspergillus fumigatus*–sensitized mice challenged by the airways with fungal spores (54). The experimental animals received 50, 100, or 200 ng of the construct per animal on days 14 through 28 after challenge. The experimental group exhibited a significant decrease in collagen deposition at day 28 postchallenge with a concomitant decrease in lymphocytes in the bronchoalveolar lavage of the animals (Fig. 4). These studies further support a role for targeting IL-13 as an efficacious means to block the progression of pulmonary fibrosis.

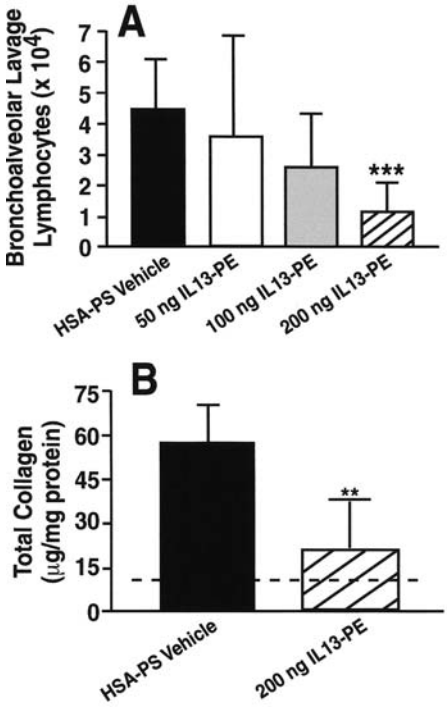

Figure 4 Efficacy of an IL-13–toxin construct as it alters the pathological benchmarks of inflammation and fibrosis in an animal model system.

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VII. MCP-1/CCL2 as a Profibrotic Chemokine

Even though the chemokine MCP-1/CCL2 was originally described as a specific chemotactic agent for the elicitation of mononuclear cells, it has now gained significant attention as a mediator involved in the maintenance of fibroblast activation and collagen deposition associated with pulmonary fibrosis (56). Whereas a variety of cells have the capability to synthesize MCP-1/CCL2, it is the pulmonary fibroblasts isolated from patients with idiopathic pulmonary fibrosis that appear abundantly to produce this chemokine (57). Furthermore, experimental models of fibrosis of the lungs and kidney are characterized by an increase in levels of MCP-1/CCL2, and interventional therapies targeting MCP-1/CCL2 have begun to identify a role for this CC chemokine in chronic interstitial lung disease (58,59). For example, immunoneutralization of MCP-1/CCL2 in an experimental model of bleomycin-induced lung fibrosis reduced the elicitation of mononuclear cells by over 30%, whereas depletion of MCP-1/CCL2 reduced the fibrotic response associated with crescentic nephritis (58).

In an additional investigation, MCP-1/CCL2 was defined as playing a significant role leading to collagen deposition (56). In this study, primary cultures of lung fibroblasts treated with escalating doses of MCP-1/CCL2 demonstrated a concentration-dependent increase in the amount of radioactive hydroxyproline incorporated into fibroblast-derived collagen. This experiment was paired with an additional set of studies showing that MCP-1/CCL2 induced a concentration-dependent increase in TGF-β by the challenged fibroblasts. The indirect regulation of collagen synthesis by MCP-1/CCL2 via TGF-β appeared to be a likely mechanism, as MCP-1/CCL2 stimulated fibroblasts in the presence of TGF-β antisense oligionucleotide did not express type 1 collagen. However, MCP-1/CCL2–treated fibroblasts in the presence of TGF-β sense oligionucleotide did not cause a reduction in type 1 collagen (56). These studies are important, as they demonstrated that chemokines are important to the fibrotic process and that the type 2 cytokines are likely to be operative in this scenario, as IL-13 is a major cytokine known to induce the expression of MCP-1/CCL2.

VII. Fibroblasts as Effector Cells of Chronic Inflammation

There is little doubt that the development of novel therapeutic strategies for pulmonary fibrosis should target the lung fibroblast as a key component of this progressive process. One of the main reasons is that data now underscore the effector role of the fibroblast as a cell that is intimately involved in the recruitment and activation of leukocytes into the interstitial space of the lung. The fibroblast can no longer be viewed as a passive bystander cell to this

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process, but a true player in the evolving response in the lung. Furthermore, the balance between normal tissue repair and excessive collagen deposition appears to be dictated by the nature of the interaction between immune cells and lung fibroblasts during inflammatory conditions, as key communication loops have been identified between fibroblasts and macrophages, lymphocytes, eosinophils, and mast cells (59).

In a further attempt to delineate the contribution of various fibroblast populations to interstitial lung inflammation, investigations were preformed to determine the phenotype of fibroblasts isolated from either control lungs and inflammatory lung lesions typified by either type 1 or type 2 cytokines (60). The latter fibroblast populations were recovered from either developing lung lesions initiated by the purified protein derivative (PPD) from *Mycobacterium* (an INF-γ driven type 1 response) or initiated by *Schistosoma* egg antigen (SEA) from *Schistosoma mansoni* (an IL-4–, IL-5–, IL-13–driven type 2 response). Alterations in MCP-1/CCL2, CCR2, procollagens I and III, and TGF-β were assessed in the different fibroblast populations. Data generated from the type1 or type 2 fibroblasts point to an important role of a type 2 phenotype in the progression of fibroblast activation and collagen deposition, as the fibroblasts isolated from the type 2 inflammatory lesion generated two-fold more MCP-1/CCL2 than similar number of type 1 fibroblasts (60). In addition, Western blot analysis demonstrated that total CCR2, the receptor for MCP-1/CCL2, was markedly increased in untreated type 2 fibroblasts, as compared to the other populations. Although all three fibroblast populations exhibited MCP-1/CCL2–dependent TGF–β synthesis, only the normal the type 2 fibroblasts demonstrated an MCP-1/CCL2 requirement for procollagen mRNA expression. Collectively, these investigations suggest that specific lung fibroblasts are able to establish a cytokine network that involves MCP-1/CCL2, CCR2, and TGF-β, leading to collagen deposition.

VIII. Conclusions

Growing clinical evidence suggests that an aggressive tissue repair response by the host is responsible, in part, for a variety of destructive fibroproliferative diseases found in the lung. Historically, the lung fibroblast was considered to be a passive bystander cell during the development of chronic lung disease, and it only participated in the late stages of the disease via collagen expression. Emerging evidence now demonstrate that fibroblasts are active participants to this process, at multiple steps, and mechanistically contributes to the overall development of chronic lung disease. Fibroblasts in the lung are now seen as a heterogeneous group of cells that can take on specialized roles during the different stages in the evolving inflammatory lung response. The cytokine environment expressed at the lung lesion dictates the different phenotypes of

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these fibroblasts. This phenomenon is especially important as developing inflammatory lesions take on either a type 1 and type 2 cytokine profile, which profoundly influences the fibroblast's role in the chronic lung response. The type 2 phenotype leads to fibroblast proliferation and matrix deposition, whereas the type 1 response is more quiescent. These observations may hold a key for the development of future treatment strategies for fibrotic lung disease, as the effector activity of the fibroblast may become a useful target to develop novel therapies.

Acknowledgments

This work was supported by National Institutes of Health grants p50HL60289, HL35276, and PO1 HL31963.

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12

CXC Chemokines in Angiogenesis Related to Pulmonary Fibrosis

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I. Introduction

Angiogenesis, defined as the growth of new capillaries from preexisting vessels, is a pervasive biological phenomenon that is at the core of many physiological and pathological processes. Examples of physiological processes which depend upon angiogenesis include embryogenesis, wound repair, and the ovarian/menstrual cycle. In contrast, chronic inflammation associated with chronic fibroproliferative disorders as well as growth and metastasis of solid tumors are associated with aberrant angiogenesis. Angiogenesis is similar to, but distinct from, vasculogenesis, which describes the de novo formation of the vascular system from precursor blood islands during embryogenesis. Development of the heart and great vessels occurs via vasculogenesis, whereas organs which require invasion of blood vessels for development (brain, lung, kidney) are supplied by angiogenesis (1). *Neovascularization* is a term which can be used interchangeably with angiogenesis, but may be more appropriately reserved for describing aberrant angiogenesis which accompanies pathological, processes such as tumorigenesis or chronic inflammation.

Inflammation and angiogenesis, although distinct and separable, are closely related processes (2). The histological appearance of chronic inflammation includes the presence of "granulationlike" tissue, a prominent feature of which is neovascularization. The marked increase in the metabolic demands of tissue which proliferates, repairs, or hypertrophies must be accompanied by a proportional increase in capillary blood supply. This form of aberrant angiogenesis is analogous to angiogenesis associated with tumorigenesis or

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disorders associated with chronic fibroproliferation. This absolute dependence on neovascularization suggests several important characteristics of angiogenesis. First, the vascular system must be able to respond rapidly to increased tissue metabolic needs with increased microvasculature. Second, because of the high metabolic cost of angiogenesis, under basal conditions, the process must be tightly controlled, occurring only when necessary. Indeed, angiogenesis occurs rarely in the adult organism. Finally, in the absence of such strict control, pathophysiology, or disease is likely to result. although a variety of factors may be involved in the regulation of angiogenesis, this chapter will focus on the CXC chemokine family of cytokines.

II. CXC Chemokines, CXC Chemokine Receptors, and Angiogenesis

A variety of factors have been described that promote angiogenesis (3–8). In parallel, a growing list of angiogenesis inhibitors [i.e., angiostatin, endostatin, vasostatin, endothelial monocyte–activating polypeptide II (EMAP-II), metalloprotease and thrombospondin domains (METH-1 and METH-2)], have been identified (19–26). Although these factors are important in the regulation of angiogenesis, these molecules may not fully account for all of the modulation of the neovascular response in pathological conditions, such as tumorigenesis or chronic fibroproliferative disorders. CXC chemokines are characteristically heparin-binding proteins. On a structural level, they have four highly conserved cysteine amino acid residues, with the first two cysteines being separated by one nonconserved amino acid residue; hence the name CXC (27–40). Although the CXC motif distinguishes this family from other chemokine families, a second structural domain within this family dictates their angiogenic potential. The NH_2-terminus of the majority of the CXC chemokines contains three amino acid residues (Glu-Leu-Arg: the ELR motif) which precedes the first cysteine amino acid residue of the primary structure of these cytokines (27–40). The family members that contain the ELR motif (ELR+) are potent promoters of angiogenesis in physiological concentrations of 1 nM to 100 nM (35). In contrast, members of the family that lack the ELR motif (ELR−) and, in general interferon inducible, are potent inhibitors of angiogenesis in physiological concentrations of 500 pM to 100 nM (35,41–43). On structural/functional level, this suggests that the CXC chemokine family is a unique family of cytokines because of their ability to behave in a desparate manner in the promotion and inhibition of angiogenesis (Table 1).

A. Angiogenic (ELR+) CXC Chemokines

The angiogenic members of the CXC chemokine family include interleukin-8 (IL-8/CXCL8), epithelial neutrophil-activating protein-78 (ENA-78/CXCL5),

Table 1 The ELR+ and ELR− CXC Chemokines Are Aniogenic and Angiostatic Factors, Respectively

Angiogenic CXC Chemokines Containing the ELR Motif (ELR+)
Interleukin-8 (IL-8, CXCL8)
Epithelial neutrophil-activating protein-78 (ENA-78, CXCL5)
Growth-related gene alpha (GRO-α, CXCL1)
Growth-related gene beta (GRO-β, CXCL2)
Growth-related gene gamma (GRO-γ, CXCL3)
Granulocyte chemotactic protein-2 (GCP-2, CXCL6)
Platelet basic protein (PBP)
Connective tissue–activating protein-III (CTAP-III)
Beta-thromboglobulin (β-TG)
Neutrophil-activating protein-2 (NAP-2, CXCL7)
Angiostatic Interferon-Inducible CXC Chemokines that Lack the ELR Motif (ELR−)
Interferon-γ–inducible protein (IP-10, CXCL10)
Monokine induced by Interferon-γ (MIG, CXCL9)
Interferon-inducible T-cell α-chemoattractant (I-TACT, CXCL11)

CXCL nomenclature is derived from a new classification system (40).

growth-related genes (GRO-α, GRO-β, and GRO-γ; CXCL1, CXCL2, and CXCL3, respectively), granulocyte chemotactic protein-2 (GCP-2/CXCL6), and NH_2-terminal truncated forms of platelet basic protein (PBP), which include connective tissue-activating protein-III (CTAP-III), beta-thromboglobulin (β-TG), and neutrophil-activating protein-2 (NAP-2) CXCL76 (35,44–4). ELR+ CXC chemokines have been shown to mediate both in vitro endothelial cell chemotactic and proliferative activity, as well as in vivo angiogenesis in a direct manner using bioassays of angiogenesis (35,44–48). These experiments prove that ELR + CXC chemokines have a direct effect on the endothelial cell, and that this angiogenic activity is distinct from their ability to induce inflammation. Furthermore, ELR+ CXC chemokines have been found to induce the expression of the metalloproteinases, MMP-2 and MMP-9, by tumor cells during tumorigenesis (49–51). The production of MMP-2 and MMP-9 are not only important for promoting endothelial cell migration in extracellular matrix (ECM) during angiogenesis, but are also involved in enhancing tumor cell migration in ECM leading to metastasis. Therefore, ELR+ chemokines not only have a direct effect on endothelial cell chemotaxis and proliferation, but also have an indirect effect in mediating their migration through ECM via the local production of MMPs.

Although stromal cell–derived factor (SDF-1/CXCL12) is a non-ELR CXC chemokine, it remains unclear whether this ELR-CXC chemokine inhibits or promotes angiogenesis. SDF-1/CXCL12 has been found to induce in vitro migration of human umbilical vein endothelial cells (52,53). Mice with

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targeted disruption of the SDF-1 gene perinatally die (54). This appears to be muti-factorial and includes defects in B-cell and myeloid progenitors, suggesting that SDF-1/CXCL12 is involved in lymphopoiesis and myelopoiesis. In addition, these mice demonstrate cardiac ventricular septal defects (54). Recently, targeted disruption of the receptor for SDF-1/CXCL12, CXCR4, has demonstrated that this CXC chemokine receptor is essential for vascularization of the gastrointestinal tract, hematopoiesis, and cerebellar development in these mice (5,56). In contrast to these findings, SDF-1 can attenuate the angiogenic activity of either ELR+ CXC chemokines, basic fibroblast growth factor (bFGF) or VEGF using the in vivo cornea micropocket assay of angiogenesis (57). Thus, the role of SDF-1/CXCL12 in modulating angiogenesis in the context of tumorigenesis or chronic fibroproliferative disorders awaits further study as to whether SDF-I/CXCL12 directly contributes to the pathogenesis of angiogenesis-dependent disorders.

B. CXCR2 Is the Putative Receptor for Angiogenic (ELR+) CXC Chemokine–Mediated Angiogenesis

The fact that all ELR+ CXC chemokines mediate angiogenesis highlights the importance of identifying a common receptor. This would provide the unique opportunity to target a putative receptor for ELR+ CXC chemokine–induced angiogenesis in the context of tumorigenesis and chronic fibroproliferative disorders. The candidate CXC chemokine receptors are CXCR1 and/or CXCR2. Only IL-8/CXCL8 and GCP-2/CXCL6 specifically bind to CXCR1, whereas all ELR+ CXC chemokines bind to CXCR (27–39). The ability of all ELR+ CXC chemokine ligands to bind to CXCR2 supports the notion that this receptor mediates the angiogenic activity of ELR+ CXC chemokines.

Evidence to support this contention is (1) ELR+ CXC chemokines bind endothelial cells (58). (2) CXCR2 is the receptor used by all ELR+ CXC chemokines (27,28,37,59,60), and all ELR+ CXC chemokines are potent angiogenic factors (35). (3) The expression of CXCR2 in human burn wound granulation tissue, melanoma, breast cancer, and head and neck squamous cell carcinoma is uniquely found in association with microvascular endothelial cells in areas of neovascularization and not on normal adjacent endothelium (61–64). (4) CXCR2 is a member of the G-protein–coupled seven-transmembrane receptor (GPCR) family (65). GPCR activation leads to dissociation of the heterotrimeric protein complex ($G_{\alpha\beta\gamma}$) to α and $\beta\gamma$ subunits that mediate downstream regulation of several intracellular signaling pathways (i.e., cAMP/protein kinase A [PKA], protein kinase C [PKC], phospholipase C [PLC], phosphoinositide 3-kinase [PI3-K], Ras, Raf, and mitogen-activated protein kinases [MAPK] (66–68). Some of these signaling pathways are identical to

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signal transduction by receptor protein tyrosine kinases that are important for cellular proliferation, migration, and regulation of apoptosis (66–68) (5). The human Kaposi's sarcoma herpes virus encoded GPCR (KSHV-GPCR) is homologous to CXCR2, is constitutively active and further augmented with IL-8/CXCL8 and/or GRO-α/CXCL1 binding, and linked to angiogenesis of Kaposi's sarcoma (69–72). In fact, transgenic mice expressing KSHV-GPCR within hematopoietic cells develop angioproliferative lesions in multiple organs that morphologically resemble KS lesions (73). These findings suggest that the expression of only one viral chemokine receptor–like gene can lead to the histopathological recapitulation of KS. This supports the notion that a CXCR2-like receptor facilitates angiogenesis and tumorigenesis of this angiosarcoma. Moreover, the function of the KSHV-GPCR for inducing KS lesions requires ELR+ CXC chemokine ligand signal coupling, as mutation of the binding domain of this receptor followed by transgenic expression under the same promoter leads to *no* lesion formation in mice (S. Lira personal communication). (6) A point mutation of CXCR2, not CXCR1, results in constitutive signaling and cellular transformation similar to KSHV-GPCR (74). In fact, persistent activation of CXCR2 by an angiogenic ELR + CXC chemokine leads to similar cellular transformation as seen with either the point mutation of CXCR2 or KSHV-GPCR (74). Thus, the potential expression of CXCR2 on endothelial cells in the presence of persistent juxtacrine (i.e., pericyte, smooth muscle cell, fibroblast, or tumor cell) and paracrine (i.e., smooth muscle cell, fibroblast, epithelial cell, or tumor cell) stimulation with ELR+ CXC chemokines has important implications in promoting an angiogenic environment of the endothelium.

Although studies have suggested that both CXCR1 and CXCR2 may be expressed on human endothelial cells (75,76), other studies have suggested that CXCR2 is the putative receptor for ELR+ CXC chemokin–mediated angiogenic activity (77,78). Addison and colleagues have demonstrated that CXCR2 is detected in human microvascular endothelial cells at both the mRNA and protein levels. In addition, the expression of CXCR2, not CXCR1, was found to be functional in mediating endothelial cell chemotaxis. Moreover, this response was sensitive to pertussis toxin, suggesting a role for G-protein–linked receptor mechanisms in mediating endothelial cell chemotaxis (77). Furthermore, the importance of CXCR2 in mediating ELR+ CXC chemokine–induced angiogenesis was demonstrated in vivo using the cornea micropocket assay of angiogenesis in CXCR2 +/+ and −/− animals. ELR+ CXC chemokine–mediated angiogenesis was inhibited in the corneas of CXCR2 −/− mice and in the presence of neutralizing antibodies to CXCR2 in the rat corneal micropocket assay. These studies have been further substantiated using CXCR2 −/− mice in a wound repair model system (78). Devalaraja and associates have examined the significance of CXC chemokines in wound healing (78). In this study, full excisional wounds were created

on CXCR2 wild-type (+/+), heterozygous (+/−), or knockout (−/−) mice. Significant delays in wound healing parameters were found in CXCR −/− mice, including decreased neovascularization. These in vitro and in vivo studies establish that CXCR2 is the receptor that mediates ELR+ CXC chemokine–dependent angiogenic activity.

C. Interferon-Inducible (ELR−) CXC Chemokines Are Inhibitors of Angiogenesis

The angiostatic members of the CXC chemokine family include PF4/CXCL4, monokine induced by interferon-gamma (INF-γ) (MIG/CXCL9), and INF-γ–inducible protein (IP-10/CXCL10) (79–84). IP-10/CXCL10 can be induced by all three interferons (lFN-α, IFN-β, and IFN-γ) (27,79–84). MIG/CXCL9 is unique in that it is only induced by IFN-γ (27,79–84). Recently a new ELR- member of the CXC chemokine family, IFN-inducible T-cell alpha chemoattractant (I-TAC/CXCL11), has been cloned and its expression appears to be induced primarily by IFN-γ (85). I-TAC/CXCL11, similar to IP-10/CXCL10 and MIG/CXCL9, inhibits neovascularization in the corneal micropocket (CMP) assay in response to either ELR+ CXC chemokines or VEGF. These findings suggest that all IFN-inducible ELR− CXC chemokines are potent inhibitors of angiogenesis. Moreover, this interrelationship of IFN and IFN-inducible CXC chemokines and their biological function are directly relevant to the function of IL-18 and IL-12 or other molecules that stimulate the expression of IFN. The capability of IL-18 and IL-12 to induce IFN-γ and subsequently IFN-inducible CXC chemokines may explain their ability to inhibit angiogenesis. Therefore, IL-12 and IL-18, via the induction of IFN-γ, will have a profound effect on the production of IP-10/CXCL10, MIG/CXCL9, and I-TAC/CXCL11. The subsequent expression of IFN-inducible CXC chemokines may represent the final common pathway and explain the mechanism for the attenuation of angiogenesis related to INFs.

All three IFN-inducible ELR− CXC chemokines specifically bind to the CXC chemokine receptor, CXCR3 (85,86) and the expression of CXCR3 mRNA has been associated with endothelial cells (87). Salcedo and colleagues have recently demonstrated that CXCR3 is expressed and functional on microvascular endothelial cells (76). Recently, Romagnani and colleagues have found that CXCR3 is expressed on endothelial cells in a cell cycle–dependent manner, and this expression mediates the angiostatic activity of IP-10/CXC10, MIG/CXCL9, and I-TAC/CXCL11 (88). In addition, we have confirmed these findings in vivo using specific neutralizing antibodies to CXCR3 (unpublished data). These findings provide definitive evidence of CXCR3-mediated angiostatic activity by angiostatic INF-inducible ELR− CXC chemokines.

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III. Role of Angiogenic (ELR+) and Angiostatic INF-Inducible (ELR−) CXC Chemokines in the Regulation of Angiogenesis Associated with Chronic Fibroproliferative Disorders

Angiogenesis is increasingly being recognized for its role in promoting the pathogenesis of chronic inflammatory/fibroproliferative disorders. For example, rheumatoid arthritis is associated with the unrestrained proliferation of fibroblasts and capillary blood vessels that leads to the formation of the pannus and destruction of joint spaces. Macrophages isolated from rheumatoid synovium produce proangiogenic factors (89). Recently, Koch and associates have found that IL-8/CXCL8 and ENA-78/CXCL5 represent major angiogenic factors in the synovium in rheumatoid arthritis (90). Psoriasis is a well known angiogenesis-dependent skin disorder that is characterized by marked dermal neovascularization. Keratinocytes isolated from psoriatic plaques demonstrate a greater production of angiogenic activity. Interestingly, this angiogenic phenotype is due, in part, to a combined defect in the overexpression of the angiogenic cytokine IL-8/CXCL8 and a deficiency in the production of the angiogenesis inhibitor, thrombospondin-1, resulting in a proangiogenic environment (91).

Coronary artery atherosclerosis continues to be the leading cause of morbidity and mortality in the United States (92). The pathogenesis of coronary atherosclerotic plaque formation is a complex process that demonstrates features of exaggerated injury and repair including recruitment of mononuclear cells, fibroproliferation, deposition of extracellular matrix, and angiogenesis, which lead to progressive fibrosis, calcification, and eventual luminal occlusion (93–95). Aberrant angiogenesis has also been demonstrated in atherosclerosis (89,96–98). Simonini and colleagues have demonstrated that IL-8/CXCL8 is a significant angiogenic factor in coronary atherectomy specimens (99). These investigators found IL-8 levels to be markedly greater in coronary atherectomy specimens as compared to control samples from the internal mammary arteries (99). IL-8/CXCL8 expression by immunohistochemistry was highly correlated with localization of factor VIII–related antigen expression on endothelial cells in the coronary atherectomy specimens. The contribution of IL-8/CXCL8 to net angiogenic activity from coronary atherectomy specimens was assessed using the rat cornea micropocket assay, and was found to represent the majority of the angiogenic activity. These findings suggest that, in human coronary atherosclerosis, IL-8/CXCL8 is an important mediator of angiogenesis and may be contributory to plaque formation via its angiogenic properties.

Evidence exists for angiogenesis in the lung. The angiogenic response of the bronchial circulation/systemic circulation in the lung is a fundamental response related to alterations in the pulmonary vascular resistance (100–104).

Compensatory neovascularization of up to 30% of the original pulmonary blood flow can occur in the bronchial circulation in all mammals in response to marked increases in pulmonary vascular resistance (104). In fact, Mitzner and colleagues (100) have recently demonstrated in the mouse that neovascularization from the systemic circulation can supply up to 15% of the normal pulmonary flow within 5–6 days postligation, and this angiogenic response can occur in the absence of any hypoxic stimulus. This response is an attempt to maintain blood flow to the metabolic pulmonary tissue, especially in reaction to injury and repair.

Idiopathic pulmonary fibrosis (IPF) is a chronic and often fatal pulmonary fibroproliferative disorder. The pathogenesis of IPF that ultimately leads to end-stage fibrosis demonstrates features of dysregulated/abnormal repair with exaggerated neovascularization/vascular remodeling, fibroproliferation, and deposition of extracellular matrix, leading to progressive fibrosis and loss of lung function. Although numerous elegant studies have examined the biology of fibroblast proliferation and deposition of ECM in interstitial lung disease, few studies have examined the role of angiogenesis/vascular remodeling that promotes fibrogenesis in these disorders.

The existence of neovascularization in IPF was originally identified by Turner-Warwick, who examined the lungs of patients with IPF and demonstrated neovascularization leading to anastomoses between the systemic and pulmonary microvasculature (105). Further evidence of neovascularization during the pathogenesis of pulmonary fibrosis has been demonstrated in bleomycin-induced pulmonary fibrosis following the perfusion of the vascular tree of rat lungs with methacrylate resin at a time of maximal bleomycin-induced pulmonary fibrosis (106). Using scanning electron microscopy, these investigators demonstrated major vascular modifications that included neovascularization of an elaborate network of microvasculature located in the peribronchial regions of the lungs and distortion of the architecture of the alveolar capillaries. The location of neovascularization was closely associated with regions of pulmonary fibrosis, similar to the findings for human lungs (105), and this neovascularization appeared to lead to the formation of systemic-pulmonary anastomoses (106). Although these studies supported the presence of angiogenesis, there have been limited investigations to delineate factors that may be involved in the regulation of this angiogenic activity during pulmonary fibrosis.

Our laboratory has demonstrated that in IPF lung tissue there is an imbalance in the presence of CXC chemokines that behave as either promoters of angiogenesis (IL-8/CXCL8) or inhibitors of angiogenesis (IP-10/CXCL10) (107). This imbalance favors augmented net angiogenic activity (107). Lung tissue from IPF patients have elevated levels of IL-8/CXCL8, as compared to control lung tissue, and demonstrate in vivo angiogenic activity that can be significantly attributed to IL-8/CXCL8 (107). Immunolocalization of

IL-8/CXCL8 demonstrated that the pulmonary fibroblast was the predominant interstitial cellular source of this chemokine, and areas of IL-8/CXCL8 expression were essentially devoid of neutrophil infiltration (107). This would seem to be discordant with the previous observations of augmented bronchoalveolar lavage fluid (BALF) IL-8/CXCL8 in IPF in association with BALF neutrophilia (108). However, this disparity, may be explained by the different compartments analyzed in these studies; that is, BALF versus lung interstitium. Moreover BALF neutrophilia may simply be a marker of disease without their involvement in the pathogenesis of IPF. In further support of IL-8/CXCL8's role as an angiogenic factor is its association with the regulation of angiogenic activity in psoriasis, rheumatoid arthritis, and non–small cell lung cancer (91,109–111). This supports an alternative biological role for IL-8/CXCL8 or other ELR+ CXC chemokines in the interstitium of IPF lung tissue.

In contrast to the increased angiogenic activity attributable to IL-8/CXCL8, we found a deficiency of the production of the angiostatic factor, IP-10/CXCL10, in IPF as compared to controls (107). Interestingly, IFN-γ, a major inducer of IP-10/CXCL10 from a number of cells, is a known inhibitor of wound repair, which is in part due to its angiostatic properties, and has been shown to attenuate fibrosis in bleomycin-induced pulmonary fibrosis (112). This supports the notion that the distal mediator of the affect of IFN-γ is IP-10/CXCL10, and an imbalance in the expression of this angiostatic CXC chemokine is found in IPF. These results suggest that attenuation of the angiogenic (IL-8/CXCL8) or augmentation of the angiostatic (IP-10/CXCL10) CXC chemokines may represent a viable therapeutic option for the treatment of IPF.

The pulmonary fibroblast is the predominant cellular source of IL-8/CXCL8 in the interstitium of IPF, supporting the notion that the pulmonary fibroblast has a pivotal role in mediating the angiogenic activity during the pathogenesis of IPF (107). Indeed, the pulmonary fibroblast has received increasing attention as a pivotal cell in the pathogenesis of IPF (113). Relative levels of IL-8/CXCL8 and IP-10/CXCL10 from IPF pulmonary fibroblast–conditioned media demonstrated a significant imbalance favoring IL-8/CXCL8–induced angiogenic activity. In contrast, normal pulmonary fibroblasts had greater levels of bioactive IP-I0/CXCL10 that favored a net inhibition of angiogenesis (107). The difference in expression of IL-8/CXCL8 and IP-10/CXCL10 between IPF and control pulmonary fibroblasts lends further support to the notion of a phenotypic difference between IPF and normal pulmonary fibroblasts which has been well described (114).

We have recently shown that ENA-78/CXCL5 is an additional important regulator of angiogenic activity in IPF (115). We found that lung tissue from patients with IPF expressed greater levels of ENA-78/CXCL5 as compared to normal control lung tissue. These higher levels of ENA-78/CXCL5 were

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associated with increased angiogenic activity as assessed by the corneal micropocket assay that was significantly attributable to ENA-78/CXCL5. The predominant cellular sources of ENA-78/CXCL5 were hyperplastic type II cells and macrophages. These hyperplastic type II cells are associated with areas of active inflammation and are often found in proximity to fibroblastic foci. This is in contrast to our previous findings that pulmonary fibroblasts were the predominant cellular source of IL-8/CXCL8, and suggests that the expression of chemokines with similar biological functions does not necessarily indicate redundancy (107). Furthermore, it is further support for the role of nonimmune cells in the pathogenesis of pulmonary fibrosis, and may explain the failure of conventional immunosuppressive agents in this disease.

The finding that both IL-8/CXCL8 and ENA-78/CXCL5 have important roles in the pathogenesis of IPF raises the question of the relative roles of IL-8/CXCL8 and ENA-78/CXCL5 in promoting angiogenesis in IPF. In our corneal micropocket model, we have previously shown that neutralizing antibodies to IL-8/CXCL8 significantly inhibit the angiogenic activity of IPF samples, and we have now also shown that anti–ENA-78/CXCL5 antibodies significantly inhibit the angiogenic activity of IPF samples. This is similar to previous findings in rheumatoid arthritis (90). As IL-8/CXCL8 and ENA-78/CXCL5 share the same receptor (CXCR2), one possible explanation is heterologous desensitization of the receptor, whereby neutralization of ENA-78/CXCL5 may overexpose the receptor to IL-8/CXCL8 (and vice versa), thereby resulting in desensitization of the receptor as is seen in chemotaxis assays at high concentrations of ligand (116). Our results do not show that either ENA-78/CXCL5 or IL-8/CXCL8 is more important but merely that they both play an important role in angiogenic activity in IPF. Furthermore, we cannot exclude that other angiogenic factors might be involved. Our laboratory has recently described CXCR-2 as the receptor that mediates the angiogenic activity of the ELR+ve CXC chemokines (77) As both IL-8/CXCL8 and ENA-78/CXCL5 bind to CXCR2, this may represent an attractive therapeutic target with respect to the inhibition of angiogenesis, thereby inhibiting or retarding the progression of IPF.

To determine whether the imbalance in the expression of these CXC chemokines is relevant to the pathogenesis of pulmonary fibrosis, the expression and biological activity of murine macrophage inflammatory protein-2 (MIP-2/CXCL2; an angiogenic ELR+ CXC chemokine homologous to human GRO-$\beta\gamma$/CXCL2/3) and the angiostatic CXC chemokine, IP-10/CXCL10, were correlated with the extent of fibrosis during bleomycin-induced pulmonary fibrosis in a murine model system (117,118). MIP-2/CXCL2 and IP-10/CXCL10 were temporally measured during bleomycin-induced pulmonary fibrosis from whole lung tissues, and were found to be directly and inversely correlated, respectively, with total lung hydroxyproline levels,

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a measure of lung collagen deposition (117,118). Moreover, if either endogenous MIP-2/CXCL2 was depleted by passive immunization with neutralizing antibodies or exogenous IP-10/CXCL10 was administered to the animals during bleomycin exposure, both treatment strategies resulted in marked attenuation of pulmonary fibrosis that was entirely attributable to a reduction in angiogenesis in the lung (117,118). These findings support the notion that angiogenesis is a critical biological event that supports fibroplasia and deposition of ECM in the lung during pulmonary fibrosis, and that angiogenic and angiostatic factors, such as CXC chemokines, play an important role in the pathogenesis of this process.

We have recently shown that IL-12 attenuates bleomycin-induced pulmonary fibrosis via induction IFN-γ (119). Moreover, the beneficial effects of IL-12 can be inhibited by simultaneous administration of anti–IFN-γ antibodies (119). These findings provide further support for IFN-γ, and thereby the INF-inducible chemokines, IP-10/CXCL10 and MIG/CCL9, as inhibitors of fibrosis. In contrast, an antibody to IL-12 was found to attenuate bleomycin-induced pulmonary fibrosis (120). Although these findings appear to the contradictory, they can be explained by the fact that during bleomycin-induced fibrosis, the IL-12 p40 subunit is preferentially expressed over IL-12 p70 (120). This is relevant because IL-12 p40 antagonizes the effect of IL-12 p70, thereby suppressing Th1-mediated responses (120). Furthermore, there was no change in levels of IFN-γ protein in this study (120). Interestingly, bleomycin-induced pulmonary fibrosis was shown to be attenuated in IFN-γ−/− mice (121). This would appear to contradict previous studies that have shown that IFN-γ inhibits wound repair (122) and attenuates fibrosis in bleomycin-induced pulmonary fibrosis (112,123). The difference may be related to timing, and IFN-γ may be necessary to initiate the inflammatory response, which was diminished in the IFN-γ−/− mice, and only exerts antifibrotic effects once the fibrotic process has been initiated. With the recent demonstration of the efficacy of IFN-γ treatment of IPF patients (124), the above studies substantiate that IFN-γ treatment of IPF may mediate its effect, in part, by shifting the imbalance of the expression of ELR+ and ELR− CXC chemokines to favor an angiostatic environment, leading to inhibition of dysregulated neovascularization/vascular remodeling, fibroproliferation, and deposition of extracellular matrix in IPF patients.

IV. Conclusions

Angiogenesis is regulated by an opposing balance of angiogenic and angiostatic factors. CXC chemokines are an unique cytokine family that contains members that exhibit on a structural/functional basis either angiogenic or angiostatic biological activity. The above studies have

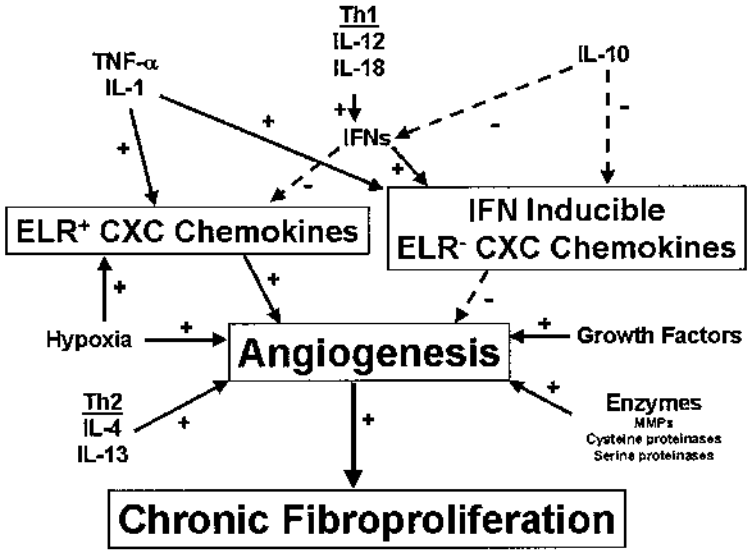

Figure 1 Interrelationship of angiogenic and angiostatic CXC chemokines with other factors in the regulation of angiogenesis/vascular remodeling that ultimately are important to chronic fibroproliferative disorders.

demonstrated that as a family, the CXC chemokines appear to be important in the regulation of angiogenesis associated with the pathogenesis of chronic inflammatory/fibroproliferative disorders (Fig. 1). These findings support the notion that therapy directed at either inhibition of angiogenic or augmentation of angiostatic CXC chemokines may be a novel approach in the treatment of chronic fibroproliferative disorders.

Acknowledgments

This work was supported, in part, by National Institutes of Health grants HL66027, CA87879, P01 HL67665, and P50 CA90388 (R.M.S.); HL04493 and the American Lung Association (J.A.B); and HL03906, P01 HL67665, and the Dalsemer Award from the American Lung Association (M.P.K.).

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13

Role of Polymorphonuclear Leukocytes in the Pathogenesis of Idiopathic Pulmonary Fibrosis

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I. Introduction

Idiopathic pulmonary fibrosis (IPF), also known as cryptogenic fibrosing alveolitis (CFA), is a progressive disorder characterized by the persistent deposition of collagen and extracellular matrix in the lungs that ultimately results in respiratory failure and death (1). The onset is insidious, and patients often present with complaints of progressive dyspnea and a dry, nonproductive cough. On physical examination, patients may demonstrate basilar inspiratory crackles, digital clubbing, and, in late stages, evidence of right heart failure such as an S_3 gallop, jugular venous distention, hepatomegaly, and pedal edema (2). Pulmonary function studies often reveal a restrictive lung defect with decreased vital capacity and total lung capacity, as well as decreased gas transfer (DL_{CO})(2). The diagnosis of IPF is made by demonstration of a histological pattern of usual interstitial pneumonia (UIP) on open lung biopsy specimens, and should be distinguished from other idiopathic interstitial pneumonias, such as acute interstitial pneumonia (AIP), desquamative interstitial pneumonia (DIP), and non-specific interstitial pneumonia (NSIP) (3).

Therapy for IPF is largely ineffective; neither corticosteroids (the mainstay of therapy) nor other immunomodulatory therapies (such as cyclophosphamide, azathioprine, or colchicine) have been shown to be effective (4–7). Currently, lung transplantation is the only option for cure of this disease, but is usually complicated by significant morbidity and mortality (8,9). As the median survival from time of diagnosis in patients with UIP is between 2.8 and 3.2 years (10), it is clear that new insights into the pathogenesis of this disease are necessary to develop more effective and specific therapies.

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II. Pathophysiology of IPF

Previous hypotheses regarding the pathophysiology of IPF postulated that a subclinical lung injury resulted in asymptomatic, acute inflammatory foci throughout the lungs (11,12). These inflammatory foci would become areas of chronic inflammation, which result in the deposition of collagen scar and progression to pulmonary fibrosis. However, recent evidence suggests that UIP need not be preceded by inflammation, but rather by the dysregulated migration and local proliferation of pulmonary fibroblasts that form the presumed pathological lesion, the fibroblastic focus (1,13,14). It should be noted though that patients with IPF often come to medical attention late in the course of disease when inflammation may have already subsided. Therefore, it remains possible that acute inflammation may indeed contribute early to the pathogenesis of IPF. Thus, this chapter will focus on evidence supporting a role for a key inflammatory cell, the polymorphonuclear leukocyte (PMN, neutrophil), in the pathogenesis of IPF. Neutrophils are of particular interest given their role in initiating lung injury, and the presence of these cells in experimental models of lung fibrosis and in the lungs of patients with various stages of IPF.

III. Mechanisms of Neutrophil-Mediated Lung Injury

Neutrophils circulate in blood as quiescent cells expressing low numbers of surface proteins or glycoproteins that serve as adhesion molecules or receptors for specific chemotaxins, opsonins, or inflammatory cytokines (15,16). Once recruited at sites of inflammation, neutrophils undergo a number of functional changes that mediate cellular adhesion, transmigration, and the release of toxic products. Neutrophils contain a large number of hydrolytic enzymes and other toxic molecules in their granules (15). In addition, these cells can generate various oxidant species, including superoxide anion, hydrogen peroxide, hypochlorous acid, and chloramines (17,18). The elaboration of various toxic molecules with overlapping biological effects is believed to have evolved to ensure adequate killing of invading microbial pathogens. Generally, the release of proteolytic enzymes and reactive oxygen species is compartmentalized to the phagolysosome during intracellular microbial killing and at the leading edge during the transmigration of neutrophils through endothelial and alveolar epithelial basement membrane (19,20). However, the extracellular release of these toxic substances can result in lung parenchymal and stromal cell injury, as well as degradation of key extracellular matrix components of lung tissue.

Human neutrophils contain azurophilic (or primary) granules, specific (or secondary) granules, gelatinase granules, and secretory vesicles (15). Enzymes contained within azurophilic granules include the serine proteases neutrophil elastase, cathepsin G, and proteinase-3, as well as the nonproteolytic

enzymes lysozyme and myeloperoxidase. The major components of specific granules are lysozyme, lactoferrin, and several members of the matrix metalloproteinase (MMP) family, which includes collagenase-2 (also referred to as MMP-8), gelatinase A (MMP-2), and gelatinase B (MMP-9). Of the enzymes found in neutrophilic granules, elastase, cathepsin G, proteinase 3, and MMP-9 have been most closely linked with degradation of extracellular matrix components in vitro (20,21) and neutrophil-dependent tissue injury in the setting of pulmonary inflammation in vivo (22–25). In particular, elastase can be elaborated in large quantities by neutrophils and has broad substrate specificity for a number of extracellular matrix components. In addition to elastin, neutrophil elastase can degrade collagen types III and IV, laminin, fibronectin, and core proteins of proteoglycans (26,27). Furthermore, elastase can cleave proenzyme forms of matrix metalloproteinases into the fully active form of these enzymes (21). Finally, elastase can modify the amplitude of neutrophilic inflammation by inducing the expression of chemokines from lung parenchymal cells (28), and by altering the biological activity of these chemotactic cytokines once released (29). Like neutrophil elastase and other serine proteases, neutrophil-derived MMPs can collectively degrade a broad range of extracellular matrix components, particularly type IV collagen (20,21,30).

The extracellular release of elastase and other serine proteases is counterbalanced by the action of the primary antiprotease, alpha$_1$-antitrypsin (31). Largely produced in the liver, alpha$_1$-antitrypsin is also contained within the azurophilic granules of neutrophils. This protein competes with elastin to bind free elastase, and once bound, inhibits the proteolytic activity of this enzyme. Even if bound to elastin or other matrix components, elastase can still be neutralized by secretory leukoprotease inhibitor (SLPI) (32). In contrast, matrix metalloproteinases, including MMP-9, are inhibited by a specific family of inhibitors referred to as tissue inhibitors of metalloproteinases (TIMPs) (33). An imbalance between protease and antiprotease expression favoring enhanced proteolytic activity has been speculated to mediate tissue injury in a number of destructive inflammatory diseases of the lung, including emphysema, bronchiectasis, and acute lung injury (27,34). Similarly, exuberant expression of neutrophil-derived serine proteinases and MMPs has also been observed in the airspace and lung interstitium of patients with IPF (33,35,36). These proteases may propagate abnormal tissue remodeling in IPF by directly injuring lung parenchymal cells, altering the magnitude of neutrophilic inflammation, and by destruction of lung alveolar architecture. Alternatively, enhanced protease expression has been shown to be overly compensated by the even greater expression of TIMPs and other antiproteases in the lung interstitium of IPF patients (36,37), which would have the net effect of diminished proteolytic activity and progressive extracellular matrix accumulation.

In addition to proteolytic enzymes, the neutrophil is a rich source of reactive oxygen species (18,38). Nicotinamide adenine dinucleotide phosphate

(NADPH) oxidase is the predominant enzyme involved in oxidant production. Upon neutrophilic activation, NADPH oxidase is assembled at the phagolysosome or at the region of cell membrane in contact with target cells or matrix. This enzyme mediates the generation of superoxide anion (O_2^-), which is subsequently dismutated to hydrogen peroxide (H_2O_2). Hydrogen peroxide is a relatively weak oxidizing agent. However, neutrophil-derived myeloperoxidase catalyzes the conversion of H_2O_2 into the potent oxidant hypochlorous acid (HOCl) (39). Oxidants, particularly HOCl, are directly injurious to stromal and parenchymal cells, including endothelial and alveolar epithelial cells. Importantly, HOCl can directly oxidize alpha$_1$-antitrypsin, as well as convert prometalloproteinases into active enzymes, which can then proteolytically inactivate alpha$_1$-antitrypsin (40). As a result, oxidants can further alter protease-antiprotease imbalance. Superoxide anion generated by activated neutrophils can also perpetuate tissue injury be reacting with nitric oxide released by neutrophils, macrophages, endothelial cells, and fibroblasts to form the highly reactive species peroxynitrite and peroxynitrous acid (41). These metabolites can induce lipid peroxidation in vitro, and enhanced peroxynitrite activity has been observed in the lungs of patients with IPF (42). Furthermore, oxidants produced by neutrophils and other cells have been shown to mediate crosslinking of extracellular matrix proteins that are central to the remodeling process (43). Finally, reactive oxidant species can further amplify neutrophilic inflammation and injury by activating key cellular transcription factors, including nuclear factor κB (NF-κB), activator protein-1 (AP-1), and janus kinase signal transducers and activators of transcriptions (JAK-STAT), signaling pathways required for the activation of proinflammatory and profibrotic genes (44,45).

Although neutrophils are classically considered to be a terminally differentiated effector cell capable of a limited repertoire of cellular responses, more recent evidence suggests that these cells can also participate in the afferent limb of the immune response. For instance, activated murine neutrophils have been shown to express HLA-DR, which correlates with the ability of neutrophils to present antigen (46). In addition, these cells can synthesize and release a variety of immunoregulatory eicosanoids, such as leukotriene B_4, platelet-activating factor, and prostaglandin E_2 (47), as well as cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, chemokines, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-12 (17,38,48). In fact, neutrophils have been shown to be instrumental to the development of protective type 1 cellular immune responses in response to a variety of intracellular pathogens (48). Given that type 1 phenotype cytokine responses generally inhibit the development of fibrosis in IPF and animal models of lung fibrosis (49,50), one could speculate that the toxic effects of neutrophil protease release and oxidant production might be partially offset by the ability of neutrophils to direct the development of antifibrotic type 1 immune responses. However, an integral role for neutrophils

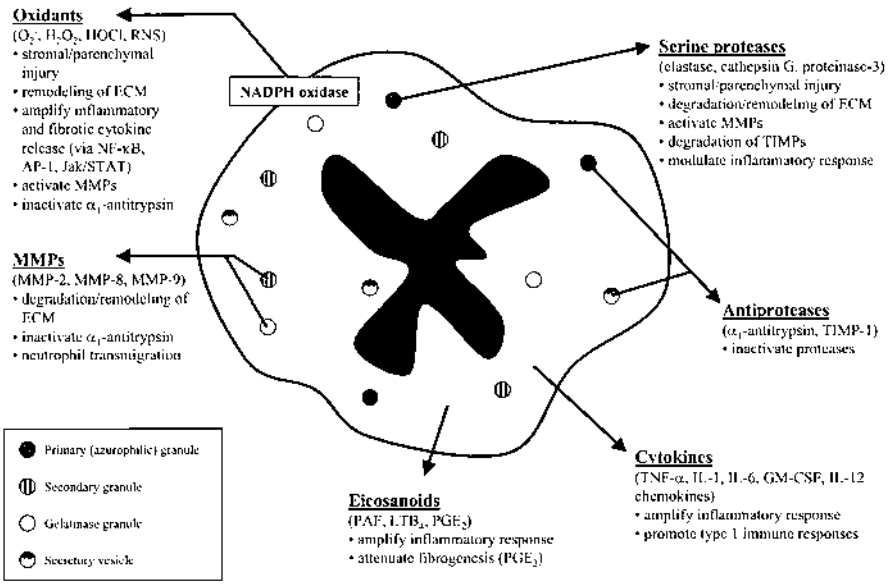

Figure 1 Schematic depicting the myriad functions of neutrophils in the inflammatory response. Local factors in the microenvironment drive the production of mediators that may then promote neutrophil accumulation, matrix degradation, or tissue injury. See text for details. O_2, superoxide anion; H_2O_2, hydrogen peroxide; HOCl, hypochlorous acid; RNS, reactive nitrogen species; ECM, extracellular matrix; NF-κB, nuclear factor-κB; AP-1, activating protein 1; JAK/STAT, Janus kinase/signal transducers and activators of transcription; MMP, matrix metalloproteinase; PAF, platelet-activating factor; LTB, leukotriene B_4; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor α; IL, interleukin; GM-CSF, granulocyte-macrophage colony stimulating factor; TIMP, tissue inhibitor of metalloproteinases; NADPH, nicotinamide adenine dinucleotide phosphate.

in skewing of type 1 and type 2 cytokine responses in pulmonary fibrosis has yet to be established experimentally. The potential role of neutrophil-derived products in pulmonary fibrosis is depicted in Fig. 1.

IV. Neutrophils in Experimental Pulmonary Fibrosis

Numerous models of experimental pulmonary fibrosis exist; however, no one model replicates all salient features of IPF in humans. Of the various models of experimental pulmonary fibrosis, bleomycin-induced lung injury is probably the best characterized. The common approach is to administer a single dose of bleomycin to rodents or rabbits intratracheally. In this model, acute inflammation occurs within hours, which is followed over subsequent weeks by the deposition of collagen and formation of fibrotic areas of lung. However,

unlike IPF, which tends to be slowly but inexorably progressive, bleomycin-induced fibrosis decreases over time, such that the lung collagen content decreases to levels at or slightly above baseline by 8 weeks after bleomycin challenge (51). Nonetheless, the bleomycin-induced pulmonary fibrosis model is a valuable tool for examining early events in fibrosis formation (52,53).

Neutrophilic recruitment to the lung occurs early after intratracheal instillation of bleomycin. Within the first 24 h after the administration of bleomycin, a marked increase in neutrophils is noted within bronchoalveolar lavage (BAL) fluid of affected animals (54). BAL fluid from bleomycin-treated rats contained approximately 40% neutrophils at 24 h after bleomycin treatment (as compared to $< 1\%$ in control animals), peaking at 72 h, and numbers of BAL neutrophils remaining elevated for greater than 2 weeks after bleomycin treatment (54). Neutrophil counts in BAL fluid returned to control levels approximately 30 days after bleomycin, demonstrating that the presence of neutrophils in alveolar spaces of bleomycin-treated animals precedes the development of fibrosis in these animals, which begins within 7 days and continues for a minimum of 30 days after bleomycin treatment (55). A representative experiment depicting the time course of neutrophilic influx and collagen deposition in a murine bleomycin-induced pulmonary fibrosis model is shown in Fig. 2.

Haslett and colleagues demonstrated marked neutrophilic influx into lungs of bleomycin-treated rabbits, as determined using ^{111}In–labeled neutrophils delivered intravenously (56). In this series of experiments, animals were treated with intratracheal bleomycin or saline followed by the intravenous injection of ^{111}In–labeled neutrophils. These investigators found that compared to saline-treated animals, bleomycin-treated animals demonstrated neutrophil recruitment to the lungs that persisted for at least 7 days (56). Additionally, the scintigraphic findings correlated with the recovery of ^{111}In–labeled neutrophils in BAL fluid ($r = 0.81$), suggesting that the scintigraphic findings represented intra-alveolar neutrophils and not neutrophils found in the vasculature or interstitial spaces. Taken together, these two studies illustrate the temporal relationship between intratracheal bleomycin administration and neutrophilic influx into the lungs, a finding that is observed in other models of pulmonary fibrosis, such as the intratracheal silica model (57) and the intratracheal asbestos model (58).

Although the presence of neutrophils in BAL fluid following intratracheal bleomycin administration is well established, the role these cells play in mediating inflammation and fibrosis remains controversial (59). Thrall and colleagues first demonstrated that neutrophilic depletion in bleomycin-treated rats resulted in increased net total lung collagen (55). In this study, rats received intraperitoneal injections of neutralizing antineutrophil serum 24, 12, and 2 h prior to and 3 days after the administration of intratracheal bleomycin. Quantification of total lung hydroxyproline at both time points revealed that,

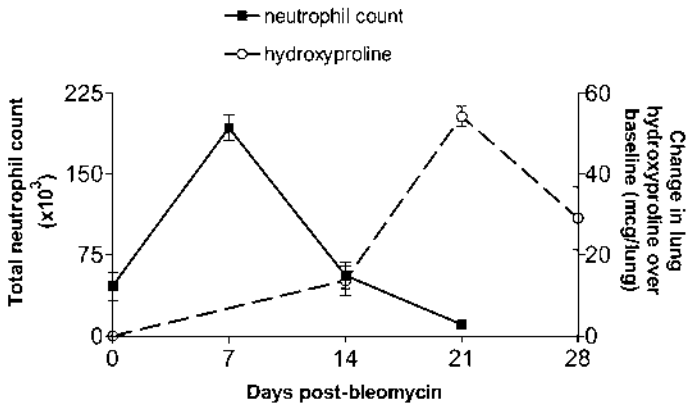

Figure 2 Time course of neutrophil recruitment (left axis) and collagen deposition (right axis) in murine bleomycin-induced pulmonary fibrosis. Collagen deposition was measured by total lung hydroxyproline content. Neutrophil influx was assessed by bronchoalveolar lavage at the designated time points.

in comparison to control animals, neutrophil-depleted rats developed more intense fibrosis 1 week after bleomycin administration. However, at 30 days after bleomycin administration, both control serum-treated and antineutrophil serum-treated animals displayed similar degrees of fibrosis. These data demonstrate that early collagen deposition is attenuated by the presence of neutrophils within the first week after bleomycin administration, but the influence of neutrophils does not significantly alter the overall extent of fibrosis.

In a similar study, Clark and Kuhn induced pulmonary fibrosis in hamsters with intratracheally administered bleomycin (60). Hamsters were then treated with antineutrophil antiserum twice daily from the fourth to the seventh day following bleomycin administration, and collagen synthesis was assessed at 8 and 12 days after bleomycin administration by assessment of total lung hydroxyproline concentration. These investigators demonstrated that at days 8 and 12 after bleomycin administration, lung collagen synthesis was significantly increased in hamsters depleted of neutrophils as compared to hamsters given control serum or no therapy. Interestingly, at 14 days after bleomycin administration, lung collagen synthesis was similar in hamsters given antineutrophil serum and those given control serum (60). Collectively, these studies illustrate a potential protective role for neutrophils in the early stages of bleomycin-induced pulmonary fibrosis formation. However, it remains uncertain whether continued neutrophilic depletion would alter collagen deposition at later time points.

To determine whether lung neutrophilic influx itself could induce pulmonary fibrosis, Harris and coworkers performed serial intratracheal administration of the human anaphylatoxin C5a, a known potent neutrophil

chemoattractant, in Golden Syrian hamsters (61). In this study, human recombinant C5a was instilled intratracheally to hamsters, with the procedure being repeated at 7 and 14 days after the initial treatment. Control animals received intratracheal saline, and subsets of the animals were sacrificed both 24 h before and 24 h after each of the three instillations. The investigators observed a significant increase in neutrophils in BAL fluid in C5a-treated animals compared to saline-treated animals after each dose. However, no difference was observed in total lung hydroxyproline content between C5a- and saline-treated animals. Additionally, histological examination performed 6 days after the final treatment revealed neither evidence of injury to epithelial cells nor the presence of fibrosis in C5a- or saline-treated lungs. Finally, transmission electron microscopy of lung sections at various time points after C5a administration revealed the presence of inflammatory foci predominated by neutrophils, but no significant fibrosis was observed (61). These data suggest that although neutrophilic inflammation may be associated with experimental fibrosis, it is not necessarily causative.

The functional role of neutrophils in bleomycin-induced pulmonary fibrosis has been explored further in mice with the beige mutation (62). Neutrophils from these animals are unable to degranulate or release hydrolytic enzymes (63), but have intact superoxide anion production. Interestingly, the administration of bleomycin to beige mice resulted in increased total lung collagen synthesis compared to heterozygous control mice (64). These studies are of significant interest, since they suggest that neutrophil-derived proteases might, in fact, attenuate the fibrotic process in bleomycin-induced pulmonary fibrosis (presumably by decreasing the accumulation of collagen and other extracellular matrix components seen in this disease).

In contrast to the potentially protective effects of neutrophils, other investigators have demonstrated that neutrophils may contribute to the pathogenesis of bleomycin-induced pulmonary fibrosis. It has been demonstrated that neutrophils isolated from BAL fluid of bleomycin-treated rats are activated, as evidenced by their greater capacity to produce superoxide anion when compared to peripheral blood neutrophils from the same animals (65). In addition to the production of potentially harmful oxygen free radicals, activated neutrophils also produce neutrophil elastase, lysozyme, cytokines, and arachidonic acid proinflammatory mediators (66,67). To further evaluate the role of neutrophils in bleomycin-induced pulmonary fibrosis, several investigators have targeted the toxic products of activated neutrophils in various model systems. Using a specific neutrophil elastase inhibitor, Taooka and coworkers demonstrated that the proinflammatory cytokines IL-1β and macrophage inflammatory protein (MIP)–2 mRNA levels in BAL cells were significantly lower in neutrophil elastase inhibitor–treated animals compared to control animals after intratracheal bleomycin instillation. Additionally, histological evaluation of bleomycin-treated mice revealed patchy areas of

subpleural fibrosis in control treated animals, whereas animals treated with the neutrophil elastase inhibitor demonstrated no evidence of fibrosis (68). Likewise, the systemic administration of a truncated form of secretory leukoprotease inhibitor (SLPI) attenuated bleomycin-induced pulmonary fibrosis in hamsters (67). Thus, it is apparent that neutrophils play a role in the pathogenesis of experimental pulmonary fibrosis. However, given the conflicting observations made in various model systems, it is also clear that further study will be required to define the contribution of neutrophils and their cellular products to experimental pulmonary fibrosis.

V. Neutrophils in Human Idiopathic Pulmonary Fibrosis

The role of neutrophils in the pathogenesis of human idiopathic pulmonary fibrosis is largely unknown. A confounding factor is the fact that IPF has only recently been redefined as strictly corresponding to the histopathological lesion usual interstitial pneumonia (UIP) (69). Therefore, older studies evaluating the presence of neutrophils in BAL fluid and histological sections of lung biopsy specimens from patients with idiopathic interstitial pneumonia included other histological diagnoses, such as desquamative interstitial pneumonia (DIP), nonspecific interstitial pneumonia (NSIP), and acute interstitial pneumonia (AIP) (69), and therefore may not be representative of UIP. In addition, patients with UIP often come to medical attention after symptoms have been present for an extended period of time. In most patients, symptoms are reported to have been present for greater than 6 months prior to seeking medical attention (2). Therefore, by the time patients are evaluated and subsequently undergo surgical lung biopsy, acute neutrophilic inflammation may have subsided. Finally, until recently, many studies have not required surgical lung biopsy for diagnosis of UIP. Although intense neutrophilia can be seen in BAL fluid of patients with UIP (70), this may not accurately reflect the degree of interstitial neutrophilia. Evaluation of interstitial and alveolar neutrophilic inflammation has been made somewhat more difficult by the relatively recent reports suggesting that in the absence of alternative diseases, a diagnosis of UIP may be presumptively made in the appropriate clinical setting and when classic changes on high resolution CT scan are observed (71), thereby obviating the need for surgical lung biopsy.

The majority of data implicating an active role for neutrophils in the pathogenesis of IPF are largely indirect. The earliest investigations showed that gallium 67 ^{67}Ga-scanning was able to identify areas of significant neutrophil activity in both the interstitium and alveoli of patients with IPF (72). These observations were confirmed by lung biopsy, and suggested that neutrophilic influx could accurately be detected using ^{67}Ga scanning. However, neither the functional role of neutrophilic inflammation nor the response to therapy was

assessed in this study. To address the role of ^{67}Ga scanning in predicting response to therapy, Gelb and associates performed ^{67}Ga scans in 16 patients with pulmonary fibrosis (13 patients with UIP, 3 patients with DIP) prior to instituting therapy with corticosteroids (13 patients) or corticosteroids plus D-penicillamine (3 patients) (73). ^{67}Ga scans were positive in 75% of patients in this group, and duration of follow-up was 3.5 ± 1.0 years. During follow-up, only four patients remained stable or improved. The investigators concluded that positive ^{67}Ga scans might correlate with the degree of neutrophilic inflammation, but were not useful in predicting the response to therapy in these patients (73). Similarly, Watters and coworkers correlated pretreatment BAL fluid cellular constituents with histopathology and clinical response to therapy (74). These investigators assessed BAL fluid from 26 untreated patients with IPF, and demonstrated that BAL fluid neutrophilia did not correlate strongly with any specific histological abnormality. Furthermore, pretreatment BAL fluid neutrophilia did not correlate with the frequency or magnitude of clinical change either before or after 1 year of corticosteroid therapy.

Like most studies, Haslam and colleagues also demonstrated that BAL fluid obtained from patients with CFA (i.e., IPF) ($n = 51$) contained significantly higher percentages of neutrophils ($P < .01$) than control subjects ($n = 15$), which is consistent with neutrophilic inflammation (75). Contrary to the aforementioned studies, however, these investigators found that increases in BAL neutrophils over 4% of total cells predicted a poor clinical response to corticosteroids, suggesting that neutrophils might play a pathogenic role in the development and/or progression of CFA. A shortcoming of this study is that the investigators did not draw a distinction between "lone" CFA and CFA associated with systemic disease. In a subsequent study, O'Donnell and coworkers evaluated a subset of patients with IPF who demonstrated greater than 10% neutrophils in BAL fluid prior to therapy (76). This group of patients had no significant suppression of BAL neutrophilia in response to corticosteroid therapy. However, these investigators did not evaluate the correlation between response to corticosteroid therapy and clinical course.

To further evaluate the role of neutrophils in the pathogenesis of IPF, Obayashi and colleagues evaluated 22 patients with IPF (77). These subjects had no history of environmental exposures that could cause interstitial lung disease, evidence of extrinsic allergic alveolitis, chronic pulmonary infection, or left ventricular failure. Subjects underwent open lung biopsy, which demonstrated interstitial pneumonia without granulomas, vasculitis, or inorganic polarizing material. However, a diagnosis of UIP was not necessary for enrollment. Subjects were evaluated by BAL and plasma levels of elastase-α_1-proteinase inhibitor complexes, as a measure of neutrophil activation, and BAL neutrophil counts. The investigators observed that there was no significant difference between subjects with IPF and normal, nonsmoking controls with respect to BAL neutrophil percentages or absolute neutrophil

counts. However, plasma and BAL levels of elastase-α_1-proteinase complexes were significantly higher in subjects with IPF compared to nonsmoking controls, suggesting that although absolute numbers of neutrophils in IPF may not be different compared to nonsmoking controls, the neutrophils present in the alveolar space in IPF were activated.

It remains a distinct possibility that neutrophils do not play an active role in the pathogenesis of IPF, but might instead be an "innocent bystander" cell recruited in response to mediators released during the fibrotic response. For instance, many mediators can exert a broad array of biological effects. Most notably, a family of chemotactic cytokines, referred to as CXC chemokines, exert potent neutrophilic chemoattractant activity and are also critical mediators of angiogenesis (78). In both experimental and human pulmonary fibrosis, the CXC chemokine family, which consists of both angiogenic and angiostatic molecules, is known to be important in influencing angiogenesis in pulmonary fibrosis, as well as other pathological conditions (79–84).

It has been shown that the expression of the neutrophilic chemotactic CXC chemokines IL-8 (IL-8/CXCL8) and epithelial neutrophil-activating peptide (ENA)-78/CXCL5 is markedly increased in the BAL fluid and cells isolated from patients with UIP as compared to patients with sarcoidosis or from normal volunteers (85–87). IL-8/CXCL8 mRNA expression positively correlated with degree of BAL neutrophilia. However, there was a distinct lack of neutrophilic infiltration in areas of lung where ENA-78/CXCL5 was expressed, suggesting a role of ENA-78/CXCL5 in UIP that is distinct and separate from neutrophilic chemotactic effects (86). Similarly, in a murine model of bleomycin-induced pulmonary fibrosis, these investigators demonstrated that MIP-2, the murine functional homologue of IL-8 (88), mediates angiogenesis but not neutrophilic recruitment (80). Specifically, neutralization of MIP-2 activity resulted in decreased angiogenesis and attenuated pulmonary fibrosis, but had no effect on the influx of neutrophils into the lung; again suggesting that the neutrophilic chemoattractant properties and angiogenic properties of the CXC chemokine family may be quite distinct. Thus, it remains possible that neutrophils do not play an active role in the pathogenesis of pulmonary fibrosis, but rather are recruited in response to other mediators.

VI. Conclusions

Although the role of neutrophils in the pathogenesis of UIP remains unknown, some experimental and human data support the historically accepted hypothesis that neutrophils may indeed play an active role in initiating and promoting pulmonary fibrosis. However, there are equally compelling data to suggest that neutrophils and/or their products may actually protect against the

development of progressive pulmonary fibrosis. Furthermore, data exist demonstrating that fibrosis in the lung can occur independent of neutrophilic recruitment and/or activation. The disparity in these data likely reflects the complex nature of the lung microenvironment during fibrosis, in which a myriad of cell-derived mediators are expressed. It is the balance of these mediators that influence the local milieu that drives an inflammatory or fibrotic response. The heterogeneity of responses in experimental models also reflects differences in methods of inducing lung injury and conditions under which they are studied. Our understanding of mechanisms of inflammation and fibrosis formation in the lung has greatly improved in the past few years. Through this understanding and further investigation, we will likely gain further insights into the role that neutrophils play in the evolution of these responses in the lung. As our comprehension of these processes improves, our ability to identify and deliver specific therapeutic agents to interrupt the relentless progression of IPF may also improve. It is only in this manner that we will be able to prevent the development of end-stage pulmonary fibrosis.

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14

Integrins and Pulmonary Fibrosis

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I. Introduction

Integrins are a family of cell surface receptors that are expressed on virtually every cell in complex multicellular organisms. Each member of this family is a heterodimeric transmembrane protein made up of an α and β subunit that assemble noncovalently (1,2). In humans, there are at least 18 α and 8 β subunits that have been shown to associate to form at least 24 receptors (Fig. 1). Integrins share in common the ability to detect and respond to ligands that are spatially restricted to the extracellular space, but there is a remarkable biochemical diversity of integrin ligands. Some $\alpha\beta$ complexes, such as the epithelial-restricted integrin, $\alpha v\beta 6$, or the platelet integrin, $\alpha IIb\beta 3$, are unique to specific cell types, whereas other $\alpha\beta$ complexes are widely expressed (3,4).

The combination of specific α and β subunits determines ligand specificity, but there is considerable overlap of ligand recognition within the family. Thus, for example, at least eight integrins can bind to the same core recognition sequence in the extracellular matrix protein fibronectin (5,6). The complexity of integrin-ligand interactions is suggested by the fact that each of these eight integrins binds to the same linear tripeptide motif, RGD (Arg-Gly-Asp), but the relative avidity of interaction of each of these integrins with the RGD-containing ligands (fibronectin, vitronectin, thrombospondin, fibrinogen, and tenascin C) are quite distinct. To a limited degree, overlapping ligand-binding recognition can be predicted based on sequence similarity of integrin α subunits (Fig. 2). Using this approach, the integrin family can be divided into five subfamilies: RGD-binding integrins (including the α subunits, $\alpha 5$, $\alpha 8$, αIIb, and αv); collagen-binding integrins ($\alpha 1$, $\alpha 2$, $\alpha 10$, and $\alpha 11$); laminin-binding

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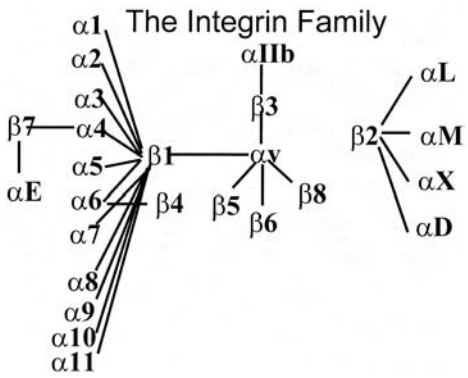

Figure 1 Organization of the integrin family. Connecting lines identify all currently known integrin heterodimers.

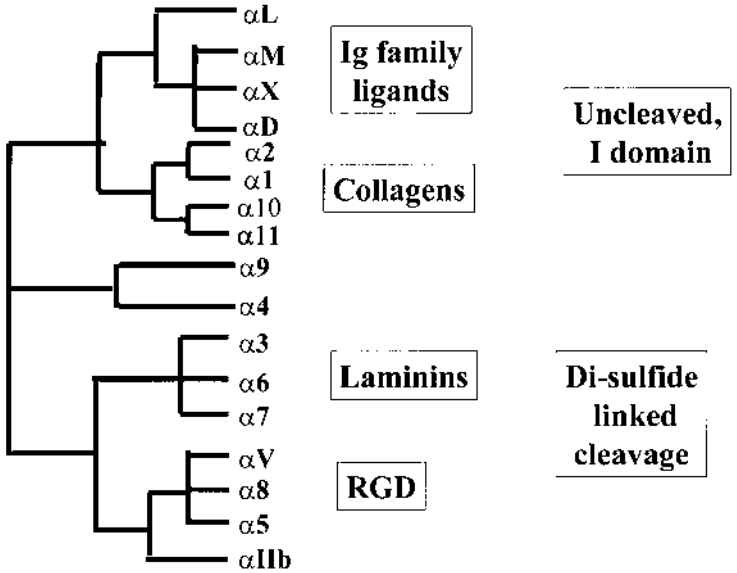

Figure 2 Integrin α subfamilies based on sequence similarity.

integrins (α3, α6, and α7); immunoglobulin family binding–integrins (αL, αM, αX, and αD); and the small subfamily of α4 and α9 that recognize a diverse group of ligands (4,6).

In addition to mediating cell adhesion, integrins have diverse effects on cell behavior by activating a wide array of intracellular signaling pathways

(outside-in signaling). Integrin signaling is further complicated by the ability of these receptors to change dramatically their avidity of ligand binding in response to signals initiated by other receptor families (inside-out signaling) (4). More recently, it has also become clear that integrins have the capacity to activate additional signaling pathways through their role in activating latent ligands for nonintegrin receptors in the extracellular space (e.g., transforming growth factor-β (7). Therefore, integrins have many potential ways to affect cellular homeostasis. This chapter reviews potential roles of integrins in various processes involved in the development of pulmonary fibrosis.

As suggested by its name, the mechanisms underlying the development of idiopathic pulmonary fibrosis (IPF) in patients are poorly understood. For this reason, much of what is known about potential mechanisms has been inferred from a combination of observational reports based on tissue from affected patients and experimental models that recapitulate one or more features of the disease. This body of work has led to the current view that epithelial injury, death of epithelial cells, abnormal epithelial repair, angiogenesis, and an imbalance between extracellular matrix production and matrix turnover all contribute to the development of pulmonary fibrosis. This chapter will outline the established and potential roles of integrins in each of these steps. Historically, lung inflammation has also been thought to play a central role in pulmonary fibrosis, but the role of inflammation has become more controversial in view of the disappointing effectiveness of anti-inflammatory drugs in treating patients with IPF and the unimpressive histological evidence of inflammation in tissue sections demonstrating advanced and progressive disease (8,9). Nonetheless, several disorders that lead to pulmonary fibrosis are associated with excessive influx of inflammatory cells, so we have chosen to include a discussion of inflammation in this chapter.

II. Mechanisms of Tissue Fibrosis

A. Epithelial Injury and Repair

In both human and experimental lung fibrosis, injury to the alveolar epithelial layer is well described (9,10). Findings include epithelial cell drop out and disruption of the basement membrane. In experimental models of pulmonary fibrosis, apoptosis of alveolar epithelial cells appears to play a critical role, since mice lacking the death receptor, fas, or its cell surface counterreceptor, fas ligand, are protected from pulmonary fibrosis induced by the cancer chemotherapeutic drug bleomycin (11). The observation that ligating fas directly by intratracheal instillation of a cross-linking antibody induces both alveolar epithelial apoptosis and pulmonary fibrosis provides further support for an important role of this pathway in the pathogenesis of pulmonary fibrosis (12). Because of the critical importance of the normal repair process in

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preventing abnormal scarring in most epithelial organs, dysregulation of the repair process has also been suggested as a potential cause of pulmonary fibrosis. Lung epithelial cells express at least eight members of the integrin family, and these are likely to contribute to the maintenance of normal epithelial integrity, to the survival of epithelial cells at sites of injury, and to the cell migration, proliferation, and differentiation that are each essential in the repair of injured epithelia (5,6).

Programmed cell death, or apoptosis, may occur when appropriate extracellular signals are not present (13). Frisch and coworkers observed that integrin–extracellular matrix interactions comprise one of these extracellular signals that prevent apoptosis. They coined the term *anoikis* to describe the apoptosis induced in many cell types when detached from their extracellular matrix (14). Frisch studied this process in epithelial cells by showing that Madin-Darby canine kidney (MDCK) epithelial cells and human keratinocytes became apoptotic when prevented from attaching to substrate (14). Similar findings of integrin-dependent survival were observed for endothelial cells (15). One significant physiological consequence of loss of anoikis may be cell survival and growth in the absence of appropriate environmental cues (e.g., malignant transformation) (16–18). However, in the case of pulmonary fibrosis, anoikis may be inappropriately stimulated by destruction of the basement membrane and loss of cell contact with components of the extracellular matrix at sites of epithelial injury.

In addition to the role that members of the integrin family play in cell survival, two integrins expressed on lung epithelial cells, $\alpha 3\beta 1$ and $\alpha 6\beta 4$, play important roles in maintaining the normal attachment to the laminin 5 that is a central component of the epithelial basement membrane (19,20). By analogy to more extensively studied epithelial organs (e.g., the breast) (21–24), reconstitution of a normal basement membrane is likely to be essential for normal differentiation of lung epithelial cells following injury. In this regard, $\alpha 3\beta 1$ could be especially important, since studies of epithelial cells from mice homozygous for a null mutation of the $\alpha 3$ subunit suggest that $\alpha 3\beta 1$ is essential for proper organization of the basement membrane (25,26).

Denuded epithelium is a feature of both clinical and experimental pulmonary fibrosis. To repair areas of epithelial denudation, epithelial cells need to proliferate and migrate across the remaining extracellular matrix. Both of these processes almost certainly depend on members of the integrin family. The potential roles of individual integrins in epithelial repair have been most extensively studied in the skin, but thus far, mice lacking one or even two of the integrins expressed on epithelial cells have not shown consistent defects in repair of cutaneous wounds (27). Because the matrix at sites of injury contains ligands for all of the integrins expressed on epithelial cells, there is likely to be considerable redundancy in these steps.

Recently, it has become apparent that the epithelium, as the primary target of injury, also plays important roles in directly regulating tissue responses to injury, including vascular leak, recruitment, and activation of inflammatory cells and fibrosis, itself. Members of the integrin family are also important participants in these more active roles of epithelial cells. One example especially pertinent to pulmonary fibrosis, integrin-mediated activation of the cytokine transforming growth factor-β (TGF-β), will be discussed in more detail below.

B. Inflammation

Although, as noted above, the role of lung inflammation in the late stages of IPF is controversial, alveolar inflammation is a prominent feature in other lung conditions where chronic pulmonary fibrosis develops (e.g., radiation injury, chronic hypersensitivity pneumonitis, or following asbestos exposure) (9). Leukocyte integrins are critical to the development of inflammation in their role as reversible adhesion receptors that inflammatory cells use to migrate to sites of cellular injury. For at least one subfamily of leukocyte integrins (the α4 subfamily) there is experimental evidence suggesting that integrins could be a therapeutic target for intervention. Since drugs targeting several leukocyte integrins are currently under development, it is worthwhile reviewing the roles these receptors could play in leukocyte recruitment and leukocyte-mediated tissue injury in the lung.

β2 Integrins

The β2 family of integrins is specifically expressed on leukocytes. This family plays an important role in leukocyte adhesion to a wide variety of substrates and in leukocyte migration across activated endothelial cells at sites of injury (28). The β2 subunit can associate with either αL, αM, αX, or αD. Although there are some differences in ligand binding preference, all members of this subfamily can recognize intercellular adhesion molecules (ICAMs) (29,30) on endothelial cells. Mice genetically altered for complete loss of β2 function demonstrate abnormal neutrophil recruitment to some but not all tissues, suggesting possible redundancy in this mechanism of cellular recruitment (30,31). However, the importance of this integrin family for normal host defense in humans is demonstrated by the disease leukocyte adhesion deficiency (LAD), in which affected individuals lack a functional β2 subunit. The disease is characterized by absent or reduced accumulation of neutrophils at extravascular sites and recurrent life-threatening bacterial infections (32–34).

Two other leukocyte integrins, αLβ2 and αMβ2, have been evaluated for their role in lung fibrosis. The administration of blocking antibodies against αLβ2 in a murine model of lung inflammation (intranasal exposure to the

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fungus *Faeni rectivirgula*) decreased histological evidence of inflammation, tissue damage, and lung fibrosis (35). Similar results were observed using antibodies against either $\alpha L\beta 2$ or $\alpha M\beta 2$ in bleomycin- and silica-induced pulmonary inflammation and fibrosis (36). Again, in both bleomycin- and silica-induced models of fibrosis, there was decreased histological evidence of inflammation and lung fibrosis after administration of blocking antibodies. These data suggest that the recruitment of inflammatory cells into the lung parenchyma can contribute to fibrosis, at least in these experimental models, and that in fibrotic lung diseases characterized by excessive inflammation inhibition of leukocyte recruitment could have therapeutic potential.

α4 and α9 Integrins

The $\alpha 4$ subunit is also expressed on all leukocytes and, like $\beta 2$, is critical for leukocyte emigration to sites of inflammation. Studies utilizing chimeric integrins have demonstrated that amino acids within the $\alpha 4$ cytoplasmic domain specifically enhance the rate of cell migration (37), suggesting that this subunit might be uniquely important for leukocyte migration. There are two known $\alpha 4$-containing integrins, $\alpha 4\beta 1$ and $\alpha 4\beta 7$, that preferentially bind to the endothelial ligands, vascular cell adhesion molecule-1 (VCAM-1) and mucosal addressin cell adhesion molecule-1 (MadCAM-1), respectively. However, recent evidence has identified a large and growing list of ligands for $\alpha 4$ integrins, including fibronectin, osteopontin, factor XIII, von Willebrand's factor, tissue transglutaminase, and several members of the a disintegrin and metalloproteinase (ADAMs) family of transmembrane metalloproteinases (38–41). There is at least one study specifically examining the role of $\alpha 4$ in pulmonary fibrosis using blocking antibodies to $\alpha 4$ (PS2) in the murine model of bleomycin-induced pulmonary fibrosis (42). Wang and coworkers found a significant decrease in both the total lung hydroxyproline content (an indirect measure of total lung collagen) and total bronchoalveolar lavage fluid (BALF) cell count (although no difference in neutrophil count) in mice given $\alpha 4$ antibody before intratracheal bleomycin. In addition to decreased lung hydroxyproline content, treatment with PS2 led to a decrease in the number of myofibroblasts, as demonstrated by immunohistochemistry staining of alpha smooth muscle actin (proposed to be a specific marker of myofibroblasts). These results suggest that $\alpha 4$ integrins may contribute to fibrosis through their known role in leukocyte emigration, and that the recruited leukocytes might be required for initiation of a process that ultimately leads to expansion of myofibroblasts and fibrosis. However, it is important to keep in mind that $\alpha 4$ integrins are not restricted to leukocytes, having been reported on a variety of other cells, including muscle cells (43), endothelial cells (44), and fibroblasts (39). It is thus conceivable that $\alpha 4\beta 1$ contributes to the development of pulmonary fibrosis through effects on resident lung cells.

The $\alpha 9$ integrin subunit is structurally similar to $\alpha 4$, and the $\alpha 9\beta 1$ integrin binds as well or better to several $\alpha 4\beta 1$ ligands (38,45). $\alpha 9\beta 1$ is highly expressed on neutrophils, and like $\alpha 4\beta 1$, $\alpha 9\beta 1$ has been shown to mediate transendothelial migration of neutrophils via binding to VCAM-1 on activated endothelial cells (45). Furthermore, the $\alpha 9$ cytoplasmic domain enhances cell migration to the same extent as the $\alpha 4$ cytoplasmic domain, although the biochemical mechanisms underlying $\alpha 4$- and $\alpha 9$-mediated enhancement of migration appear to be different (46). Therefore, the $\alpha 9\beta 1$ integrin may contribute to inflammatory responses in a manner similar to $\alpha 4$ and $\beta 2$.

$\alpha 1$ and $\alpha 2$ Integrins

The $\alpha 1$ and $\alpha 2$ integrin subunits are generally known for their interactions with collagens, but they have also been linked to inflammation in various models of disease. For example, interleukin-2 (IL-2) has been shown to induce both $\alpha 1$ and $\alpha 2$ expression in splenocytes, and antibodies to both integrins resulted in inhibition of leukocyte binding to collagen substrate (47). Further experiments were conducted using antibodies to $\alpha 1$ and $\alpha 2$ in models of delayed hypersensitivity to sheep red blood cells, contact hypersensitivity to fluorescein isothiocyanate (FITC) and anticollagen monoclonal antibody–induced arthritis. These studies showed that anti-$\alpha 1$ and anti-$\alpha 2$ monoclonal antibodies significantly inhibited inflammation as measured by swelling and leukocyte infiltration. However, no significant differences were observed after antibody administration in a model of nonspecific irritant dermatitis, suggesting that these integrins may be more important in antigen-specific immune responses. Possible mechanisms to explain how inhibition of $\alpha 1$ and $\alpha 2$ may block the inflammatory response include disrupted migration, activation, or apoptosis of inflammatory cells in peripheral tissues. As these integrins are important mediators of the inflammatory response in these models of hypersensitivity, they may be relevant to hypersensitivity diseases in the lung that can ultimately progress to fibrosis.

C. Angiogenesis

In some reports, pathological descriptions of human pulmonary fibrosis reveal diffuse abnormal vascular remodeling (48). The beneficial effects of blocking proangiogenic chemokines in the bleomycin-induced pulmonary fibrosis model provide further support for the hypothesis that neovascularization enhances fibrosis (49,50). The generation of new blood vessels, or angiogenesis, involves invasion and proliferation of smooth muscle and endothelial cells. Vascular endothelial cells stimulated by angiogenic growth factors show increased expression of a number of integrins including $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 5\beta 1$, $\alpha v\beta 3$, and $\alpha v\beta 5$ (51,52). In particular, the role of αv integrins and $\alpha 5\beta 1$ integrin in mediating angiogenesis have been extensively studied. Although the

mechanisms of new blood vessel formation are complicated, the observations that blockade of $\alpha 5\beta 1$ (53), $\alpha v\beta 3$ (54), and/or $\alpha v\beta 5$ (55) can dramatically inhibit angiogenesis in different models suggests important roles for integrins in the neovascular response. Although initial reports suggested that each of these integrins might be essential for angiogenesis, results from studies with integrin knockout mice make this interpretation unlikely (27,56–58). Although $\alpha 5$ subunit knockout animals die during embryonic development with substantial defects in vascular development (59,60), vasculogenesis is perfectly normal in mice lacking the $\beta 3$ subunit (57), the $\beta 5$ subunit (27), or both of these αv partners (58). Furthermore, angiogenesis in response to tumors or growth factors is either normal or, in fact, enhanced in these animals. These results suggest that when αv integrins are present, inhibition of their function can inhibit angiogenesis, perhaps through an effect of the unligated integrin in inducing cell death (61). However, angiogenesis can clearly occur normally in the absence of αv integrins.

D. Fibrogenesis

Over the past several years, disappointment over the ineffectiveness of anti-inflammatory drugs in treating IPF have led to an increasing interest in understanding and directly intervening in the process of fibrogenesis itself. Simplistically, increased tissue fibrosis could occur as a result of increased extracellular matrix (especially collagen) production, impairment of normal mechanisms for matrix degradation, or some combination of these two effects. In most tissues, including the lung, myofibroblasts are the principal source of the excess matrix that is deposited after injury (62). These specialized cells, which can be induced from quiescent fibroblasts in vitro, have dramatically enhanced capacity to synthesize and secrete collagen and other matrix components and also express several contractile proteins that may contribute to their ability to cause scar tissue to contract and compress adjacent unaffected areas (63). Although several cytokines have been shown to have the capacity to induce resting fibroblasts to adopt a myofibroblast phenotype, one of the most potent is TGF-β (64). There are three mammalian isoforms of TGF-β (1, 2, and 3) that each have the capacity to bind to the same receptors and induce the same biological effects (65). Nearly every human cell type produces and has receptors for TGF-β. Its actions are numerous, including regulation of cell proliferation and differentiation (66), wound healing, angiogenesis, and embryonic development (67). TGF-$\beta 1$ knockout mice die from widespread tissue inflammation (68,69), suggesting that TGF-$\beta 1$ is important as a negative regulator of inflammation. Especially pertinent is the role of TGF-β, alluded to above, in promoting fibrosis. There are numerous examples both in human disease and animal models that demonstrate a critical role for TGF-β in the regulation of

the extracellular matrix (ECM), scar tissue, and fibrosis, including pulmonary fibrosis (70–72).

TGF-β is secreted from cells as a functionally inactive complex (small latent complex) that requires activation. The small latent complex is formed by furin-based cleavage of the TGF-β gene product into an amino-terminal fragment called the latency-associated peptide (LAP) and a smaller carboxyl-terminal fragment that is the mature, biologically active cytokine (73). Each fragment is disulfide linked into a homodimer in the endoplasmic reticulum, and the two homodimers are noncovalently associated. In this form, the active cytokine is unable to bind to its receptor. In most cells, this latent complex is further modified by disulfide linkage to another gene product called latent TGF-β binding protein (LTBP). Upon secretion from the cell, LTBP is covalently cross-linked to components of the ECM through the action of the extracellular enzyme tissue transglutaminase. Thus, most tissues, including the lung, contain substantial amounts of TGF-β protein, but show little evidence of a TGF-β effect. In response to injury or inflammation, when TGF-β action is required to induce scar formation and turn off local inflammatory responses, these inactive stores must be activated. Ideally, this process should be under tight spatial and temporal control to prevent excessive scar formation and tissue destruction. In vitro, latent TGF-β can be activated through a wide variety of mechanisms, including proteolytic cleavage by plasmin and matrix metalloproteinases, denaturation by extremes of temperature or pH, and through direct binding to the ECM protein thrombospondin-1 (74). Studies with thrombospondin-1 knockout mice have suggested that binding to thrombospondin-1 is important for the developmental effects of TGF-β in vivo (75), but the relative importance of various mechanisms for TGF-β activation in injured and inflamed tissues is poorly understood. Recent data with mice homozygous for a null mutation of the β6 integrin subunit have suggested that the integrin αvβ6 may play an important role in this process in the injured lung.

αvβ6 Integrin

The αvβ6 integrin is expressed principally in epithelial cells and, although it is constitutively expressed at low levels, it is significantly induced by tissue injury and inflammation (76). We have identified several ligands for αvβ6, including fibronectin, tenascin-C, and vitronectin, which bind the integrin through the linear tripeptide sequence arginine–glycine–aspartic acid (RGD). A clue to the function of this integrin was provided by β6-deficient mice, which manifest significant inflammation in the lungs and skin (usually in sites of minor repetitive trauma). The lung inflammation consists of activated macrophages and lymphocytes as well as neutrophils and eosinophils. The effect on inflammation was specifically linked to β6, since reconstitution of β6 in lung

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epithelial cells was sufficient to prevent the inflammatory response in the lung (77). Further investigations revealed that the β6-deficient mouse was resistant to bleomycin-induced pulmonary fibrosis. Interestingly, the blunted fibrotic response was not accompanied by a decrease in inflammation, but in fact by an increase in the number of inflammatory cells in the lung. This combination of effects, enhanced inflammation and protection from fibrosis, was remarkably similar to the expected effects of a loss of TGF-β action, and suggested that the αvβ6 integrin might be acting through TGF-β in vivo. One possible link between the integrin and TGF-β would be if αvβ6 ligation regulated expression or secretion of TGF-β protein. However, in the strain of mice used in these studies (129svems$+/-$ter), there was no effect of bleomycin on TGF-β protein in the lungs, and no effect of the integrin knockout on TGF-β protein levels (7). The critical clue to how the integrin and TGF-β were linked came from examination of the sequence of the latent associated peptide of TGF-β1 (and TGF-β3). As shown in Fig. 3, TGF-β1 (and TGF-β3) LAP contain the linear RGD sequence we had previously identified as a critical site in all αvβ6 ligands. Furthermore, this site had been shown to mediate binding of the

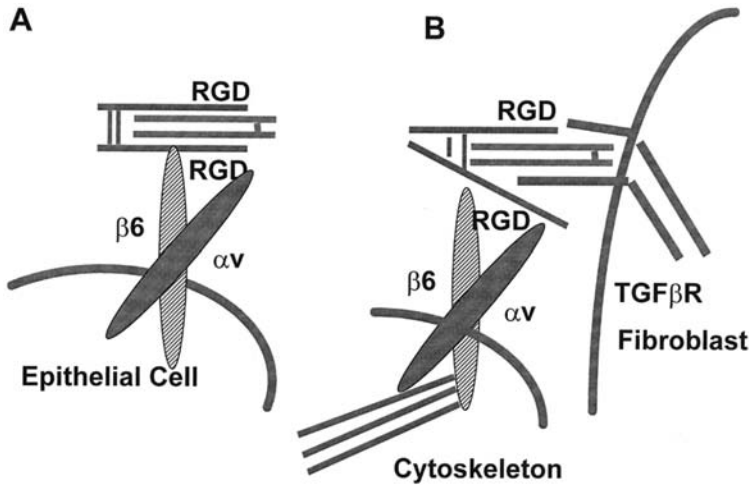

Figure 3 Proposed model of integrin β6–mediated TGF-β activation. (A) TGF-β activation can be regulated in part by regulation of expression of the integrin on the cell surface. αvβ6 binds to the arginine-glycine-aspartic acid (RGD) site in the latency associated peptide (LAP) of latent TGF-β, but binding of the integrin to LAP is not sufficient to cause TGF-β activation. (B) Activation of TGF-β is regulated by cytoplasmic signals that involve an organized actin cytoskeleton. TGF-β activated by this mechanism can be presented to TGF-β receptor (TGFBR) on adjacent cells, but does not freely diffuse away from the surface of the integrin-expressing epithelial cell.

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closely related integrins $\alpha v\beta 1$ and $\alpha v\beta 5$ to TGF-β1 LAP (73). Indeed, by cell adhesion assays and affinity chromatography, LAP was found to be the most effective ligand for $\alpha v\beta 6$ yet identified.

Although the precise mechanism(s) by which $\alpha v\beta 6$ interaction with latent TGF-β complexes leads to TGF-β activation remain to be determined, several aspects of this interaction are interesting and relevant to the pathogenesis, and possibly treatment, of pulmonary fibrosis. One important feature is that this interaction does not lead to the release of free active TGF-β from the surface of the integrin-expressing cell. Based on experiments culturing $\alpha v\beta 6$-expressing cells and TGF-β reporter cells on the same or on opposite sides of microporous filters (7) and on experiments plating cells simultaneously expressing the integrin and a TGF-β reporter at different densities (unpublished observations), it is clear that $\alpha v\beta 6$-mediated activation of TGF-β receptors requires direct cell-to-cell contact (see Fig. 3). Furthermore, binding of the integrin to latent TGF-β complexes is not sufficient to activate these complexes. This point was definitively shown utilizing a series of $\beta 6$ truncation mutants containing deletions of portions of the $\beta 6$ cytoplasmic domain (7). One such mutant was fully capable of binding to LAP but completely unable to activate latent complexes, suggesting that this process can be regulated through cytoplasmic proteins that interact with the $\beta 6$ cytoplasmic domain. One critical step appears to be interaction with the actin cytoskeleton, since cytochalasin D, which inhibits actin assembly, has no effect on LAP binding, but completely abolishes $\alpha v\beta 6$-mediated TGF-β activation (7). Taken together, these results suggest the potential to intervene in this process (and potentially in patients with IPF) by blocking expression, function, or activation of $\alpha v\beta 6$. Since the phenotype of $\beta 6$ knockout mice is quite mild compared to the phenotype of TGF-β1 knockout mice, such interventions have the potential to be considerably better tolerated than interventions targeting TGF-β itself.

$\alpha 1\beta 1$ Integrin

There are currently four integrins known to be collagen receptors: $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 10\beta 1$, and $\alpha 11\beta 1$ (78–80). (see Fig. 2) The major cell surface receptors for collagens on lung cells are $\alpha 1\beta 1$ and $\alpha 2\beta 1$. $\alpha 1$ Preferentially binds type IV collagen, whereas $\alpha 2$ preferentially binds type I collagen. $\alpha 1$ Is widely expressed primarily in mesenchymal and endodermal tissues in the adult, including fibroblasts, liver (81), microvascular endothelium (82), and smooth muscle (83,84). $\alpha 2\beta 1$ Is also expressed on epithelial cells (85). The expression of $\alpha 1$ is altered in various disease states, being induced in lymphocytes in rheumatoid arthritis (86), in graft versus host disease (87), and on chrondrocytes in osteoarthritis (88).

Evidence for a role for the $\alpha 1$ integrin subunit in tissue fibrosis comes from both phenomenological and gene deletion studies. In a phenomenological

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study using skin fibroblasts from scleroderma patients, collagen synthesis was found to be upregulated, whereas $\alpha 1$ expression was downregulated (89). Other in vitro studies have found that $\alpha 1\beta 1$ appears to negatively influence collagen synthesis (90,91). In vivo studies using the $\alpha 1$ integrin deletion mouse began in 1996 (92). Although the mice appeared to develop normally without obvious defects, in vitro studies found that embryonic fibroblasts (EFs) deficient in $\alpha 1$ were unable to spread on or migrate into collagen IV substrate. Further in vivo and in vitro experiments using $\alpha 1$-deficient dermal EFs revealed that these cells had a marked reduction in proliferative index compared to wild-type control cells (93). This reduction in proliferation was observed only when the $\alpha 1$ null cells were grown on a substratum of collagen type I or type I + IV. Furthermore, the $\alpha 1$ null EF cells not only have retarded growth, but demonstrate a 50% increase in apoptosis compared to wild-type EF cells when grown on type I or I + IV collagen. Examination of the Shc- growth-signaling pathway which promotes fibroblast cell proliferation (94–96) in these cells revealed a lack of activation of the adaptor protein Shc and associated Grb2 and mitogen-activated protein kinase activation, suggesting that $\alpha 1\beta 1$ integrin may uniquely affect both fibroblast proliferation and survival via this pathway.

These $\alpha 1$-deficient mice were also examined for abnormal collagen regulation (97). Although there was increased steady-state levels of collagen synthesis in $\alpha 1$-deficient mice as measured by tritiated proline incorporation, there was no appreciable difference in dermal thickness between $\alpha 1$ null and WT mice. This finding is thought to be due to increased collagenase expression in the $\alpha 1$ null animals as increased message for the murine collagenase-1 matrix metalloproteinase-13 (MMP-13) was found in the $\alpha 1$-deficient mice, and this effect of the collagenase could be partially overcome by crossing the $\alpha 1$ null mice into a collagenase-resistant collagen background. Examination of dermal thickness in $\alpha 1$-deficient mice crossed with collagenase-resistant mice showed an increase in dermal thickness. Therefore, these results suggest that $\alpha 1\beta 1$ regulates a feedback mechanism in collagen synthesis as well as MMP production.

Additional evidence for $\alpha 1$ playing a role in matrix regulation was provided by studies involving the mouse model of Alport's syndrome. This human disease is characterized by progressive renal failure and high frequency hearing loss, retinopathy, and lens abnormalities (98). The molecular mechanism of Alport's syndrome involves mutations in type IV collagen genes and pathologically involves expansion of the mesangial matrix, podocyte effacement, and alterations in the glomerular basement membrane (GBM). Given that $\alpha 1\beta 1$ is the most abundant integrin on mesangial cells, Cosgrove and coworkers produced a mouse deficient in both the collagen $\alpha 3$(IV) gene and the $\alpha 1$ integrin subunit and found that these double-knockout mice have a delayed progression of glomerular disease which

includes attenuated expansion of the mesangial matrix and markedly improved foot process architecture. Although blockade of TGF-β is also partially effective in this model, the effects of loss of $\alpha1\beta1$ and blockade of TGF-β are distinct, suggesting that the beneficial effects of loss of $\alpha1\beta1$ are not directly due to any interaction between this integrin and TGF-β activation or signaling. Although, to our knowledge, no studies examining the role of $\alpha1\beta1$ in pulmonary fibrosis have been published, $\alpha1\beta1$ is also expressed on mesenchymal cells in the lung and could thus participate in the development of pulmonary fibrosis.

III. Conclusions

There are many potential mechanisms whereby members of the integrin family may contribute to the development of pulmonary fibrosis. As reviewed above, the blockade of $\beta2$ integrins, $\alpha4$ integrins, $\alpha1\beta1$, and $\alpha v\beta6$ have each shown promise in blunting the fibrotic response to various insults. Based on the established functional roles of integrins in mediating cell survival, proliferation, and migration, it is certainly possible that additional members of this family could play important roles in pulmonary fibrosis. In our view, further investigation of the mechanisms by which specific members of the integrin family contribute to pulmonary fibrosis is likely to be both scientifically and therapeutically fruitful.

One subfamily of integrins that is clearly of interest is the $\alpha4$ subfamily. As noted above, the beneficial effects of blocking $\alpha4$ on bleomycin-induced pulmonary fibrosis could be due to effects on leukocyte recruitment, but could also be due to roles of $\alpha4$ integrins on resident lung cells. Future studies should elucidate which cells in the lung express $\alpha4$, and whether the in vivo effects observed by $\alpha4$ blockade are mainly due to inhibition of cellular recruitment, inhibition of resident lung cell activation, or a combination of both. Currently, $\alpha4$ inhibitors are undergoing clinical trials for treatment of asthma, so these drugs could be available for investigational treatment of patients with pulmonary fibrosis.

Based on studies in models of scarring in the skin and kidneys, $\alpha1\beta1$ could also be an attractive target for inhibition of pulmonary fibrosis. As a first step, it will be important to study directly the role this integrin plays in models of pulmonary fibrosis, and the availability of viable knockout mice and effective blocking antibodies should make these studies straightforward.

Finally, the possibility that the TGF-β activation that plays a central role in the progression of pulmonary fibrosis is critically dependent on dynamic regulation of the integrin $\alpha v\beta6$ makes this pathway an exciting area for future investigation. Because blockade of TGF-β itself may have unacceptable long-term toxicity, targeting the $\alpha v\beta6$ integrin or the upstream steps involved in its

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expression and activation have obvious appeal. However, much still needs to be learned about how $\alpha v\beta 6$ expression and activation are regulated and about how important this pathway is in patients with pulmonary fibrosis.

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15

Oxidants and Antioxidants in Idiopathic Pulmonary Fibrosis

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I. Introduction

According to the lungs' physiological function for gas exchange, high oxygen partial pressures are normally present in the alveolar spaces. Consequently, the lungs are usually exposed to a higher oxidative burden than any other organ. Therefore, the lungs are equipped with a complex antioxidant defense system, which normally protects the lung from injury. However, it has also been recognized that oxygen toxicity may occur under certain circumstances. Prolonged hyperoxia (> 60%) may result in acute pulmonary edema, which is believed to be induced by formation of oxygen-derived radicals which ultimately surmount all defense mechanisms (1). In recent years, oxidative injury has been identified to contribute significantly to a number of bronchopulmonary diseases (Table 1). Oxidative pathomechanisms have been intensively studied in fibrotic lung diseases and in the clinical setting of idiopathic pulmonary fibrosis (IPF). The available evidence suggests that oxidative stress and antioxidant deficiency mutually support the evolution of the disease process in IPF. Therefore, antioxidative therapy offers a promising new treatment strategy in this usually fatal disease.

II. Biology of Reactive Oxygen Species

Reactive oxygen species (ROS) are highly reactive electrophilic molecules. Most of them like superoxide anion ($O_2^{\bullet -}$) or nitric oxide (NO) are radicals but there are also potent oxidants without radical structure (i.e., without a free

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Table 1 Examples for Bronchopulmonary Diseases Driven by Oxidative Stress

Acute diseases
Acute lung injury (ALI)
Acute respiratory distress syndrome (ARDS)
Paraquat intoxication
Bleomycin lung
Hyperbaric oxygen
Chronic diseases
Chronic obstructive pulmonary disease
Asbestosis
Amiodarone lung
Cystic fibrosis
Idiopathic pulmonary fibrosis

single electron) like hydrogen peroxide (H_2O_2) or the hypohalides (e.g., HOCl). A major source for oxidants is the membrane-bound NADPH system of phagocytic cells (i.e., neutrophils, eosinophils, and macrophages), which is capable of producing high amounts of superoxide anions which are metabolized to form a number of different and even more toxic oxidants (2). Oxidants can also be generated by several other endogenous mechanisms involving xanthine oxidase, cytochrome P450 oxidases, mitochondria, and arachidonic acid metabolism (3,4). Moreover, nitric oxide (NO) is a radical produced by the NO synthetases (NOS) which exist in three distinct isoforms: NOS I is a constitutive enzyme found in neurons; NOS II is an inducible isoform expressed after gene induction in activated phagocytes, epithelial and smooth muscle cells; and NOS III is a constitutive enzyme located in endothelial cells (5,6). Only the inducible NOS II is capable of generating micromolar quantities of NO which may be cytotoxic, probably by formation of the more toxic compound peroxinitrite ($ONOO^-$) (7). Major pathways of oxidant metabolism in the lung are summarized in Figure 1.

ROS appear to be principal mediators of inflammatory and immunological lung injury. In this context, oxidants are capable of causing oxidative damage to a variety of different substrates (Fig. 2) which will ultimately result in alterations or destruction of cells and constituents of the extracellular matrix of the lungs.

A special relationship exists between oxidants and the protease/antiprotease balance within the lungs because of the oxidative inactivation of important antiproteases like $alpha_1$-proteinase inhibitor ($alpha_1$-PI) and secretory leukoprotease inhibitor (SLPI) (8–11). Conversely, some proteases like gelatinase and collagenase are oxidatively activated (12,13). Taken

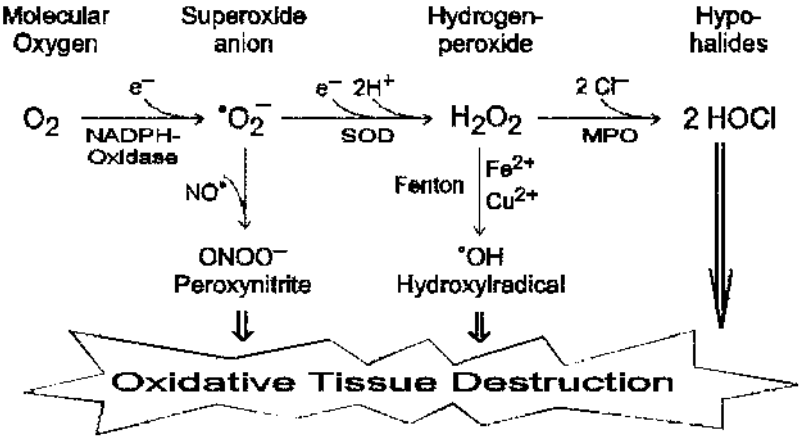

Figure 1 Metabolism of reactive oxygen species.

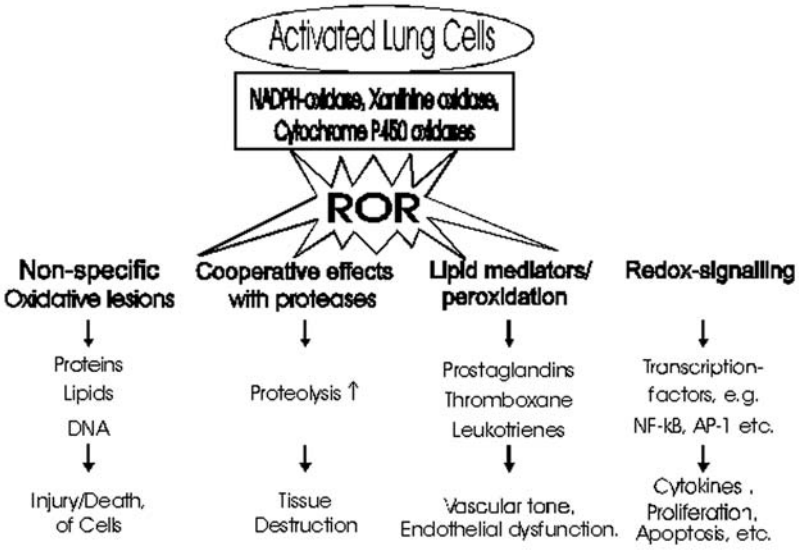

Figure 2 Mechanisms of oxidative lung injury.

together, these oxidative effects will result in an protease/antiprotease imbalance in favor of proteolytic activity, which also induces tissue damage and inflammation. The interaction between oxidants and the protease/antiprotease system is referred to as the "cooperative effect" (see Fig. 2).

Moreover, oxidants have been shown to promote arachidonic acid release via phospholipase-sensitive pathways, thereby eventually increasing the production of prostaglandins and leukotrienes with proinflammatory and profibrotic properties (14,15). Finally, oxidants are also involved in complex cell signaling pathways by activating oxidant-sensitive transcription factors like nuclear factor kappaB (NF-κB) and activator protein-1 (AP-1), which induce transcription of various proinflammator/fibrotic cytokines (Fig. 2) (16–22).

III. Antioxidant Defense

Physiologically, oxidants are completely counterbalanced by antioxidants within the lungs, so that oxidative damage does not occur or is at least minimized. The presence of numerous and highly active antioxidants has been described in the lungs, including scavengers, enzymes, and enzyme-systems (see Table 2) (23,24). Nonspecific scavenging effects are provided by serum proteins (e.g., albumin), whereas molecules like transferrin and ceruloplasmin also inhibit specific oxidative pathways like the Fenton reaction by binding catalytic metal ions (Fe^{2+}, Cu^{2+}). The antioxidatively active vitamins C and E also act as scavengers for ROS with preference for hydrophilic (vitamin C) and lipophilic (vitamin E) environments, respectively. Superoxide dismutase (SOD) and catalase (CAT) are antioxidatively active enzymes which promote degradation of superoxide anions to water and oxygen by a two-step reaction (Figure 3).

Glutathione is quantitatively the most important antioxidant of the lung in the extracellular and intracellular compartments (25). Its concentration in the epithelial lining fluid (ELF) of healthy lungs approximates 0.5 mM, which is about 100–150 times higher than the concentration found in plasma (25–27).

Table 2 Antioxidants of the Lung

Scavengers
Serum proteins, albumin, transferrin, coeruloplasmin, and others
Lactoferrin, taurin,
Vitamins C and E
Glutathione
Enzymes
Superoxide dismutase
Catalase
Enzyme systems
Gamma glutamyl cycle
Glutathione redox cycle

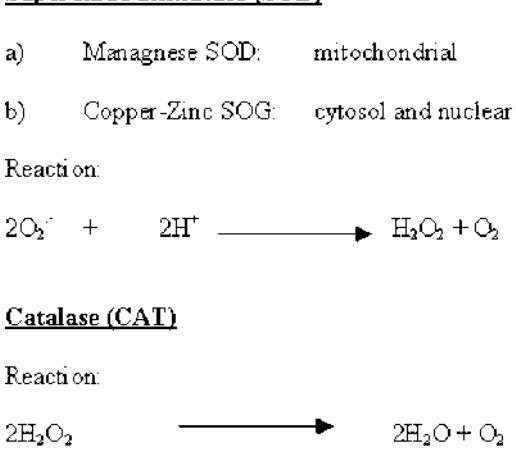

Figure 3 Antioxidant Enzymes of the lung: ***Superoxide dismutase (SOD)*** (a) Managnese SOD: mitochondrial (b) Copper-Zinc SOG: cytosol and nuclear Reaction: $2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$ ***Catalase (CAT)***, Reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$.

Intracellularly, glutathione levels of 1–10 mM are present in most mammalian cells (25). As a scavenger, reduced glutathione (GSH) is oxidized to glutathionedisulfide (GSSG). Importantly, however, glutathione is not only a scavenger for most biologically relevant oxidants but is also recycled intracellularly by the glutathione redox cycle and the gamma glutamyl cycle, respectively (25,28–30). The latter allows for de novo biosynthesis of glutathione using degraded extracellular glutathione or glutathionedisulfide as substrates (25,30) (Fig. 4). Owing to the high efficiency of these enzyme systems more than 95% of the total glutathione are in the reduced form (GSH) intra- as well as extracellularly under physiological conditions (25–27).

IV. Oxidant/Antioxidant Imbalance in IPF

In clinical IPF, Cantin and coworkers were the first to describe an exaggerated release of superoxide anions and hydrogen peroxide from cells obtained by bronchoalveolar lavage (BAL) (31). Moreover, myeloperoxidase concentration in BAL fluid was increased in IPF patients as compared to healthy controls (31). Consequently, the fundamental finding of an increased oxidant release from BAL cells in IPF patients but also in fibrosing alveolitis associated with connective tissue diseases was reproduced by several investigators (27,32,33). In view of the known cytotoxic effects of these oxidants in vitro, the hypothesis that oxidative stress is a pathogenetically relevant factor in IPF was generated.

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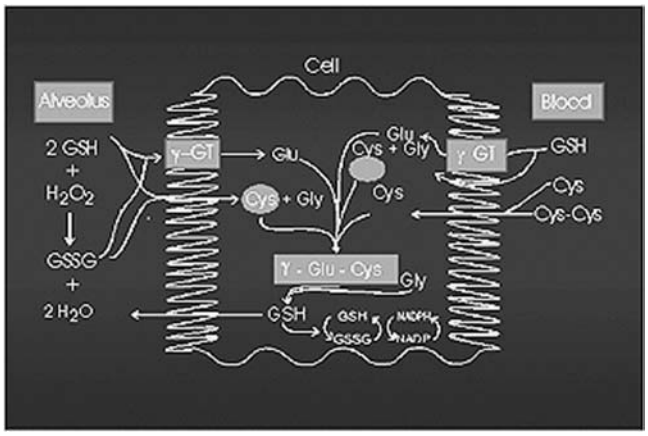

Figure 4 Glutathione biosynthesis and metabolism in the lung. Legend: γ-GT = gamma-glutamyl-transpeptidase; GSH = reduced form of glutathione; Cys = cysteine; Cys-Cys = cystin; Glu = glutamin; Gly = glycin; GSSG = oxidized form of glutathione (i.e. glutathionedisulfide).

Additional support for this notion came from studies showing that free catalytic iron and products of lipid peroxidation indicating oxidative stress were elevated not only in BAL fluid but also in plasma from IPF patients, suggesting an enhanced systemic oxidative burden in these patients (34). Moreover, an elevated methionine sulfoxide content of BAL-derived proteins was reported in IPF and fibrosing alveolitis but not in sarcoidosis, indicating exaggerated oxidative stress and protein oxidation in fibrotic but not in granulomatous lung disease (35,36).

An important additional finding in support of the hypothesis that an oxidant/antioxidant imbalance is involved in the pathophysiology of IPF was provided by Cantin et al. showing a significant lack of glutathione in the ELF of patients with IPF (37). Moreover, a slight but significant increase of the GSSG/GSH ratio was reported, suggesting a more oxidated state of the alveolar milieu in pulmonary fibrosis (27). However, this finding was not sufficient to assume that consumption by oxidants could be the cause for the decrease of extracellular glutathione levels. Based on experimental studies using lung epithelial cells, a more reasonable explanation for the lack of glutathione could be the downregulation of the gammaglutamylcysteine synthetase (gamma-GCS), the rate-limiting enzyme of glutathione biosynthesis, by transforming growth factor β1 (TGF-β1), a profibrotic cytokine abundantly present in IPF lungs (38). In recent studies, it has been demonstrated that gamma-GCS activity in alveolar epithelial cells is upregulated by oxidants and tumor necrosis factor-α(TNF-α)–mediated AP-1 activation,

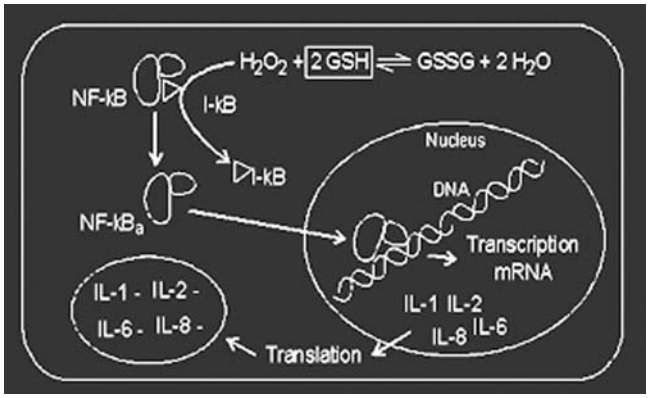

Figure 5 Glutathione-dependence of oxidative NF-kappaB activation. NF-kB = quiescent form of nuclear factor kappaB; I-kB = inhibitory subunit of NF-kB; NF-kBa = activated form of NF-kB; GSH = reduced form of glutathione; GSSG = oxidized form of glutathione; IL = interleukin; H_2O_2 = hydrogen peroxide.

whereas dexamethasone causes downregulation of gamma-GCS expression (39–41). The available data suggest that gamma-GCS activity and consequently glutathione levels are closely regulated by a number of cytokines and mediators, which in IPF patients results in a downregulation of glutathione production. As a consequence, exaggerated oxidant release from inflammatory cells is not adequately counterbalanced by glutathione, the major antioxidant of the lung, and oxidative cell and tissue damage ensues. This view is also supported by recently published data showing a reduction of intracellular glutathione content in BAL cells obtained from patients with fibrotic lung disease (42). Intracellular glutathione deficiency, however, does not only increase susceptibility of cells to oxidative damage but also enhances activation of oxidant-sensitive transcription factors like NF-κB and AP-1, consecutively resulting in transcription of proinflammatory cytokines like interleukin-1, TNF-α, and interleukin-8, which perpetuate inflammatory reactions in the lower respiratory tract (Fig. 5). Accordingly, restoration of intracellular glutathione levels has been demonstrated to reduce inflammatory cell activation and cytokine release (42,43).

V. Oxidant/Antioxidant Imbalance and Fibroproliferative Response

According to recent histological studies, the fibroproliferative response has been claimed to be the leading feature in IPF, whereas inflammation is

regarded as being a secondary phenomenon (44–46). Consequently, fibroblast foci have been identified to be the hallmark lesion of IPF (44,46).

In this context, the link between oxidative stress and fibroproliferative acitivity is of special interest. There are several findings indicating a close relationship between fibroblast activity and oxidative stress. In cell culture experiments, proliferation of human fibroblasts has been shown to be stimulated by hydrogen peroxide (47). Moreover, lung fibrolast growth is inhibited by high glutathione levels and increased in the presence of low extracellular glutathione concentrations as observed in IPF (37). In addition, TGF-β1, a profibrotic cytokine abundantly present in IPF, suppresses glutathione biosynthesis by inhibiting gamma-GCS gene expression, the rate-limiting enzyme in the gamma glutamyl cycle (38). More recently, the critical role of intracellular GSH for lung epithelial cell apoptosis was highlighted (48,49). In the context of IPF, apoptosis may be of signifcant importance for the outcome of the repair process following initial lung injury. Moreover, remodeling of the extracellular matrix (ECM) is influenced by intracellular glutathione levels and oxidants (50,51). Low intracellular glutathione levels have been shown to activate matrix metalloproteinases (MMPs), which are involved in the formation of fibrotic lesions (51,52). Consequently, supplementation of glutathione or *N*-acetyl-cysteine, a glutathione precursor, resulted in inhibtion of MMP activation (51,52). Furthermore, collagen gene expression and synthesis is stimulated by oxidants and can be inhibited by antioxidants (38,53,54). Taken together, there is ample evidence demonstrating that oxidative stress and glutathione deficiency mutually support firboblast proliferation and consecutive ECM formation.

VI. Oxidative Stress and Activity of IPF

There is only indirect evidence linking oxidative stress to severity and activity of IPF. Jack et al. reported that the concentration of lipid peroxidation products in the plasma of IPF patients was significantly higher in actively deteriorating disease as compared to stable IPF (34). Strausz et al. observed a decrease of oxidant release from BAL cells in patients responding to corticosteroid therapy, whereas in nonresponders, oxidant release remained unaltered (33). Finally, in patients with IPF and collagen vascular disease–associated fibrosing alveolitis, a significant inverse correlation between vital capacity and oxidative stress as measured by the methionine sulfoxide content of BAL proteins was reported (Fig. 6) (36). These data suggest, but do not prove, that in patients with fibrosing alveolitis or IPF, a high oxidative burden is associated with more severe and active disease.

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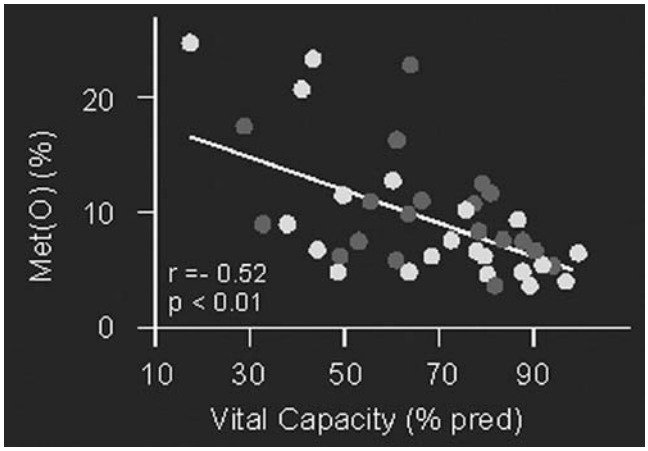

Figure 6 A link between oxidative stress and severity of IPF: Inverse correlation between vital capacity and oxidative stress in fibrosing alveolitis and IPF (reference 36). Met(O) = methionine sulfoxide content of BAL derived proteins; r = correlation coefficient; p = significance niveau; light gray = IPF; dark gray = collagen vascular disease associated fibrosing alveolitis.

VII. Antioxidative Treatment Strategies

The available evidence supports the hypothesis that in IPF-enhanced oxidative stress which is not adequately counterbalanced by antioxidants results in a sustained oxidant/antioxidant imbalance leading to oxidative damage, inflammation, and fibroproliferative response. Consequently, inhibition of oxidant production and release or restoration of an antioxidant defense are reasonable strategies to treat IPF. Different approaches for an antioxidative therapy of IPF are shown in Figure 7. However, cytotoxic therapies which may reduce oxidative stress by decreasing the number and activation of phagocytic cells have shown only limited success in clinical IPF and pose a significant risk of infection. Therefore, enhancement of an antioxidant defense may be a more promising treatment option. In view of the crucial role of the glutathione system within the complex antioxidant screen of the lung, restoration of intracellular and extracellular glutathione levels could turn out to be beneficial in IPF patients.

In line with this concept, Borok et al. used glutathione aerosol (600 mg twice daily) to augment pulmonary glutathione levels in IPF patients. A significant increase of total glutathione (GSH + 2 GSSG) concentration in the ELF was observed after inhalation of GSH (55). However, a closer analysis of the data presented reveals that most of the glutathione was present in its oxidized form (GSSG) (55). This finding suggests that GSH was rapidly

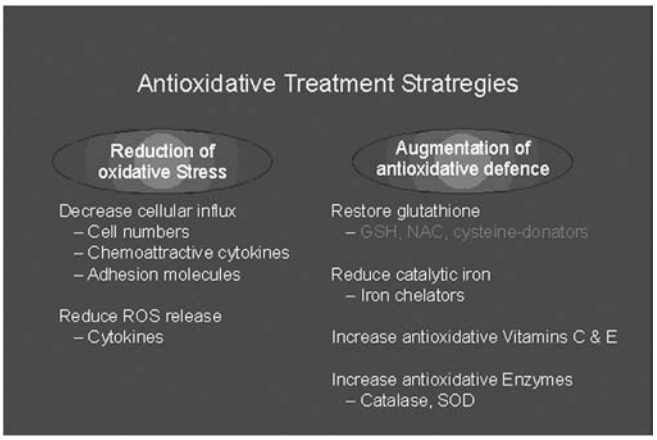

Figure 7 Different approaches to antioxidant therapy.

oxidized in the lungs and the luminal gamma glutamyl transpeptidase actvity of the bronchial and alveolar epithelial cells was insufficient to degrade the extracellularly accumulating GSSG for intracellular recycling. Consequently, this approach was not further studied.

Alternatively, *N*-acetyl-L-cysteine (NAC) is a well-known compound which acts as a glutathione precursor. After oral application, NAC is almost completely deacetylated in the gut mucosa and liver, where it contributes to elevated glutathione levels (56–61). This effect has proven to be beneficial in paracetamol intoxication where redox cycling metabolites of paracetamol lead to glutathione deprivation of liver cells and consecutive liver cell necrosis. Oral or intravenous NAC in very high doses (up to 300 mg/kg lean body weight in 24 h) has proven to be effective as an antidote for paracetamol intoxication, since it restores liver cell glutathione levels and precludes liver cell necrosis (62,63). Analogously, oral application of high-dose NAC was thought to increase circulating levels of cyteine and cysteine compounds (e.g., cystin), which could be utilized by lung parenchymal cells to restore glutathione biosynthesis.

VIII. Antioxidative Therapy with NAC in Animal Models of Oxidant Lung Injury and Fibrosis

Hyperoxia is a commonly used model for oxidative lung injury. In dogs exposed to 80–100% of oxygen protective effects of NAC have been shown with regard to inflammatory response, edema formation, increase in pulmonary vascular resistance, and ventilation-perfusion mismatch (64).

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Similar protective effects of NAC against hyperoxia have also been demonstrated in guinea pigs (65). A widely used model for pulmonary fibrosis is the bleomycin model. Again, NAC has been shown to inhibit inflammatory and fibrotic reactions induced in experimental animals after exposure to bleomycin (66). Moreover, protective effects of NAC have been demonstrated in paraquat-, phosgene-, and perfluorisobutin-induced pulmonary edema as well as in immune complex alveolitis and in amiodarone induced lung toxicity (67–71).

However, there have also been reports of increased toxicity of either hyperoxia or bleomycin with NAC treatment, and the effects of NAC on glutathione levels have not been unequivocal (72,73). Formation of thioyl radicals and the possibility of pro-oxidative effects of thiols in certain environments have been claimed to be responsible for adverse effects of NAC (73,74). Species differences in pro-oxidant and antioxidant enzyme equipment of different cell populations may also be responsible, at least in part, for these controversial findings.

Taken together, NAC has shown protective effects against oxidant-mediated acute lung injury and formation of lung fibrosis in a reasonable number of animal models. However, there are also controversial experimental results, and formation of thioyl radicals may be of clinical relevance.

IX. Antioxidative Therapy with NAC in Clinical IPF

Based on the above-described experimental studies, NAC was employed as a glutathione precursor in several clinical studies in IPF and fibrosing alveolitis. Meyer et al. administered a dose of 600 mg NAC tid orally to IPF patients and performed BAL before and after 1 week of therapy (75). They described an increase of total glutathione concentration which was significant in native BAL fluid but did not reach statistical significance when total glutathione concentrations in ELF were calculated (75). Moreover, the GSSG concentrations were not measured in this study. Therefore, the antioxidative effect of oral NAC therapy could not be assessed from the data presented (75). In another study, NAC was administered intravenously to IPF patients and healthy controls in three different doses: 600, 1800, and 4800 mg (76). BAL was performed before and after NAC infusion and total glutathione (i.e., GSH + 2xGSSG) concentration in ELF was evaluated. Meyer et al. found no significant change of glutathione concentrations after 600 mg of NAC, but a significant increase was observed after infusion of 1800 mg NAC in both healthy controls and IPF patients (76). A further increase of the NAC dose to 4800 mg did not result in an additional increase of glutathione levels in the ELF (76). From these data, it can be concluded that 1800 mg NAC is an optimal dose with regard to its effect on extracellular glutathione levels in the lungs.

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Moreover, GSSG concentrations did not change significantly in this study, indicating that systemic administration of NAC preferentially increases the antioxidatively active GSH concentration in the ELF. This finding is in concordance with the concept that oral NAC improves the availability of cysteine, which is the rate-limiting substrate of the gamma glutamyl cycle, resulting in an increased rate of GSH biosynthesis (30). Moreover, the observation that increasing the NAC dose to 4800 mg did not further enhance pulmonary glutathione levels also complies with this concept, because increasing substrate concentrations will not result in unlimited GSH production provided that the enzyme equipment of the cells is constant. The available data, therefore, support the notion that a daily dose of 1800 mg NAC may be sufficient to optimize the availability of cysteine as a substrate for pulmonary glutathione biosynthesis.

Based on these results, a small prospective open-label clinical trial was conducted in 20 patients with IPF ($n = 10$) or fibrosing alveolitis secondary to collagen vascular disease ($n = 10$) (77). After a run-in period of 4 ± 1 months, a baseline lung function test and BAL were performed and treatment with 600 mg NAC tid was started. After 12 weeks, 18 patients completed the protocol and underwent another lung function test and BAL. A significant increase of GSH concentations in the ELF was observed, whereas GSSG concentrations remained unaltered. Moreover, the methionine sulfoxide (Met[O]) content of BAL-derived proteins was measured as an indicator of oxidative stress (77). Interestingly, Met(O) levels were significantly reduced after 12 weeks of NAC treatment, thus providing for the first time biochemical evidence of antioxidative efficiency of high-dose oral NAC in patients with parenchymal lung disease, at least for the ELF (Fig. 8) (77). Moreover, glutathione deficiency was not restricted to the extracellular compartment in this cohort of patients, but the glutathione content of BAL cells was also decreased as compared to healthy controls (42). After the 12-week course of oral high-dose NAC, intracellular glutathione levels increased significantly, and this increase was paralleled by a fall in the sponatnous oxidative activity of BAL cells (42). In addition, the intracellular glutathione content was inversely correlated with the interleukin-8 concentration in the ELF, indicating a potential anti-inflammatory effect (42). In summary, the findings of this pilot study confirm that oral high-dose NAC using 600 mg three times per day improves the pulmonary antioxidative defense, decreases oxidative damage as measured by lowered Met(O) levels of BAL proteins, and has a mild inhibitory effect on inflammatory BAL cell activation, probably by partially restoring intracelluar glutathione levels. In addition to these biochemical and cell biological findings, follow-up of lung function revealed that there was a significant improvement of lung function during the 12 week NAC treatment period as compared to the run-in period (Fig. 9) (77). This result for the first

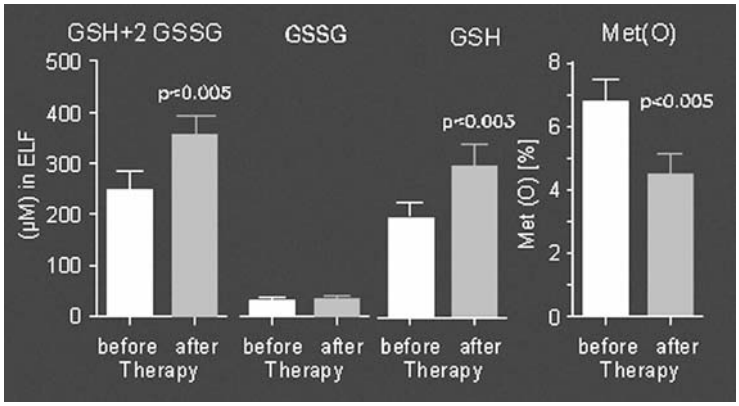

Figure 8 Biochemical evidence of antioxidant efficiency of oral high-dose NAC in patients with diffuse fibrosing alveolitis and IPF (reference 77). GSH = reduced form of glutathione; GSSG = oxidized form of glutathione; GSH + 2 GSSG = total glutathione; Met(O) = oxidized methionine sulfoxide content of BAL derived proteins; ELF = epithelial lining fluid.

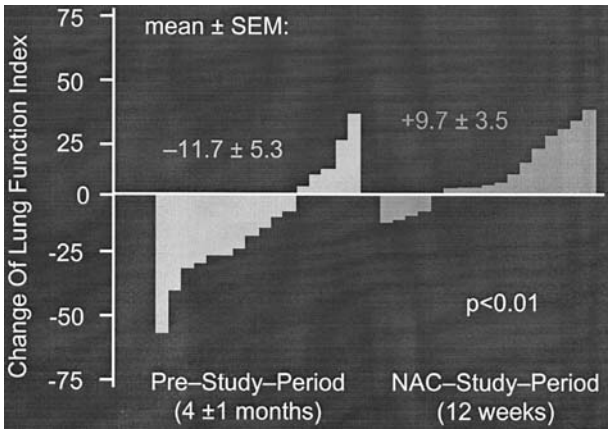

Figure 9 Effect of high-dose NAC therapy on lung function in diffuse fibrosing alvoelitis and IPF (reference 77). Lung function index = change of vital capacity (%pred.) + change of diffusing capacity (% pred.) + change of arterial oxygen partial pressure during steady state exercise.

time indicated that high-dose NAC supplementation may also be clinically beneficial in fibrotic lung disease.

In summary, data from preliminary clinical trials suggest that oral NAC application at an optimal dose of 3×600 mg per day enhances pulmonary

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glutathione levels in the intracelluar and extracellular compartment, thus improving antioxidative defense, inhibiting oxidative damage, and lowering inflammatory cell activation. Consequently, lung function as an indicator of clinical efficiency was also influenced favorably in patients with IPF and collagen vascular disease–associated fibrosing alveolitis. Based on these positive findings a prospective, randomized, placebo-controlled multicenter clinical trial was started in seven European countries (Idiopathic Pulmonary Fibrosis International Group Exploring NAC I Annual, **IFIGENIA**) investigating the clincal efficiency of NAC 3×600 mg per day or placebo on top of a standard immunosuppressive treatment regimen consisting of prednisone and azathioprine according to the recommendations of the American thocacic Society/European Respiratory Society consensus statement on IPF (45). Duration of the treatment period is 1 year, and primary endpoints are the lung function parameters vital capacity (VC) and diffusing capacity (DLco). The results of this trial will be available in the year 2003.

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16

Fibrin Turnover in Pulmonary Fibrosis

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I. Introduction

Recent studies support the involvement of disordered pathways of fibrin turnover and extravascular fibrin deposition in the pathogenesis of pulmonary fibrosis. Alveolar fibrin deposition is commonly associated with alveolar injury. The fibrinous neomatrix undergoes progressive remodeling and scarring after acute lung injury and in the interstitial lung diseases. Changes in local fibrin turnover favor increased fibrin formation and delayed fibrin clearance under these circumstances. These changes are predicated on the responses of alveolar macrophages, the lung epithelium, and lung fibroblasts to inflammatory mediators. In all, the events associated with scarring in the lung parenchyma recapitulates the central events that characterize wound healing. Recent interventional strategies designed to reverse basic derangements of local fibrin turnover have been successful in sepsis and pleural injury, raising the possibility that similar approaches could be extrapolated to prevent scarring in the injured lung itself.

II. Role of Fibrin Turnover in Tissue Injury and Repair

The hypothesis that abnormal fibrin turnover plays a central role in tissue inflammation and fibrotic repair is based on extensive supporting information. It is now well recognized that abnormalities of coagulation and fibrinolytic pathways and pathological fibrin deposition contribute to the pathogenesis of a broad range of disorders. These include remodeling following injury to a variety of organs, the growth and spread of neoplasms, and systemic responses to sepsis (1–4).

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Although extravascular fibrin deposition is not found in the normal lung, alveolar and interstitial fibrin is commonly found in the lung during evolving acute respiratory distress syndrome (ARDS) or interstitial lung diseases, including idiopathic pulmonary fibrosis (5,6). The morphological changes associated with acute and chronic lung injury therefore provide strong evidence that fibrin turnover is disrupted during the course of lung inflammation. Additional evidence derives from careful immunohistochemical analyses in preclinical models, which likewise confirm that alveolar fibrin deposition characterizes most forms of lung injury (2,7). Bleomycin-induced lung injury is one such commonly used example and is characterized, like ARDS, by an early exudative phase followed by accelerated pulmonary fibrosis. Alveolar fibrin deposition is extensive over the 2- to 3-week interval after induction of bleomycin-induced lung injury in marmosets or rats (8). The chronicity of alveolar fibrin may be a determinant of subsequent fibrotic remodeling. Acute alveolar fibrin deposition also occurs as part of the exudative alveolitis resulting from administration of intravenous administration of oleic acid in rats or sheep (8,9). Oleic acid–induced lung injury resolves with restoration of normal lung architecture, and alveolar fibrin rapidly resolves under these circumstances.

Florid alveolar fibrin deposition is likewise observed in baboons with evolving diffuse alveolar damage induced by septic challenge (10). Diffuse alveolar damage likewise is the histological constellation commonly observed in the lung tissue of patients with ARDS, and extensive alveolar deposition is typical of this histopathology (11). The fibrinous neomatrix appears to undergo rapid reorganization with collagen deposition in severe ARDS associated with accelerated pulmonary fibrosis. Alveolar fibrin is seen in idiopathic pulmonary fibrosis associated with active alveolitis (6); further supporting the hypothesis that a mechanistic linkage exists between transitional alveolar fibrin and subsequent pulmonary fibrosis.

Aberrant fibrin deposition is not strictly limited to the alveolar compartment during the course of acute lung injury. Thrombi can occur in the pulmonary arteries in severe ARDS (12,13) and have been associated with poor prognosis (13). The occurrence of these thrombi is associated with impaired oxygenation, atelectasis, and increased pulmonary vascular resistance. Disseminated intravascular coagulation can also occur in association with ARDS (14). Intravascular fibrin formation, coagulation proteases, and by-products of coagulation can potentiate exudative lung injury (15–17) and may also contribute to multiorgan failure associated with ARDS.

Extravascular fibrin deposition in the injured lung follows a progression similar to that associated with wound healing (2,18). In both situations, the permeability of the microvasculature is initially increased. As a result, plasma coagulation substrates enter the tissue parenchyma. Increased tissue

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factor (TF) procoagulant expression is concurrently stimulated by a variety of locally elaborated stimuli, including cytokines. TF associates with factor VII to form the extrinsic activation procoagulant complex, which initiates coagulation local fibrin deposition. Macrophages, fibroblasts, and polymorphonuclear leukocytes invade and remodel the transitional fibrin neomatrix. The remodeling process is also influenced by cytokines and proteases elaborated by these cells. Several of these mediators can induce expression of plasminogen activator inhibitors (PAIs) as well as plasminogen activators (PAs). Although tissue PA (tPA) and urokinase (uPA) are induced, the major PA effecting extravascular remodeling in the lung is uPA. In the injured tissue, local fibrin clearance can be impeded by the relative overexpression of PAIs, mainly PAI-1, which favors maintenance of transitional fibrin (2). As fibroblasts progressively invade the fibrin gel, collagen progressively increases within the fibrin neomatrix and eventually results in scarification.

Tissue fibrin as well as locally disordered coagulation or fibrinolytic pathways can influence local inflammation and tissue repair in a variety of ways, suggesting that these abnormalities are integral to these processes rather than incidental. Fibrin and its products can promote directed migration of macrophages and fibroblasts (19,20). In addition, fibrin can disrupt organization of endothelial cells (21) and suppress lymphocyte proliferation (22). Proteolytic fragments of fibrin(ogen) are also proinflammatory, causing increased vascular permeability (21,23). Fibrin formation is promoted by increased expression of tissue factor expression, which is stimulated by a variety of cytokines (2,7,24). Coagulation and fibrinolytic intermediates also interact with components of other inflammatory pathways, including the complement and kinin systems, to amplify the local inflammatory response (2). Plasmin also activates transforming growth factor $= \beta$ (TGF-β) and promotes fibrotic repair, in part, by induction of PAI-1 (25,26). The presence of fibrin and disordered pathways of fibrin turnover in the injured lung are therefore implicated in the potentiation of the inflammatory response and alveolar repair.

Information from recent studies of transgenic animals further supports the inference that fibrin turnover is involved in the pathogenesis of fibrotic repair after acute lung injury. The evidence derives from experiments in which fibrinolytic capacity in the lungs was altered in transgenic mice. Alveolar fibrin did not develop in mice deficient in plasminogen activator inhibitor-1 (PAI-1) that were exposed to hyperoxia (27). In a study that extends these observations, pulmonary fibrosis was increased when PAI-1 was upregulated in bleomycin-treated mice, whereas fibrosis conversely decreased when PAI-1 was knocked out (28). Presumably, these effects were due to augmented fibrinolytic capacity of the lungs in animals deficient in PAI-1 and decreased fibrinolytic activity in animals overexpressing PAI-1.

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III. Abnormalities of Coagulation in the Injured Lung

Information from the morphological, biochemical, and transgenic animal models reviewed above support the hypothesis that disordered fibrin turnover is integral to remodeling of transitional fibrin and fibrotic repair in the injured lung. Analyses of lower respiratory tract fluids from animals and patients with various forms of lung injury confirm that pathways of fibrin formation are activated. Consistent increases in local expression of procoagulant activity occurs in acute lung injury, accelerated pulmonary fibrosis, and interstitial lung diseases (7,18,29,30).

Based upon immunological and functional analyses the major procoagulant in the lower respiratory tract fluids of the lung is related to tissue factor (2,3). Relatively low levels of tissue factor procoagulant activity occur in the bronchoalveolar lavage (BAL) fluids of normal individuals (31,32). In the normal lung, coagulation substrates are also restricted from the alveolar compartment because of the relative impermeability of the pulmonary microvasculature and the lung epithelium. The low representation of tissue factor and distal coagulation substrates in the alveolar lining fluids of the normal lung limits the capacity to form alveolar fibrin, which is not found in the histologically normal lung.

In acute lung injury, increased tissue factor–related procoagulant activity occurs as part of the inflammatory response. Distal coagulation substrates can likewise enter the alveolae because of increased vascular and epithelial permeability in response to inflammation. This scenario is characteristic of virtually all forms of lung injury, including those used to induce lung injury in various preclinical models. The assessment of coagulation pathways in the lung has been done by analysis of the constituents of lung lavage or BAL fluids (2,33). For example, tissue factor–factor VII complexes are increased in BAL from rats or sheep with oleic acid– bleomycin-induced lung injury (9,34,35). Under these circumstances, these procoagulant complexes activate the distal coagulation cascade. Coagulation continues to be activated in the lung during protracted inflammation leading to alveolar fibrin formation. Along these lines, diffuse alveolar damage induced in adult baboons by sepsis or nonseptic insults is characterized by increased tissue factor–related procoagulant activity in BAL fluids (10,36). Lung lavage of premature baboons with evolving respiratory distress syndrome and diffuse alveolar damage is similarly characterized by increased procoagulant activity due to tissue factor associated with factor VII (37). In preclinical models of lung injury, the major procoagulant observed in lower respiratory tract fluids is therefore tissue factor complexed with factor VII in zymogen or activated form.

Tissue factor–related procoagulant activity is likewise increased in patients with acute and chronic lung injuries (2,7,32). A profound increase of tissue factor and overall procoagulant activity is detectable in BAL fluids of

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patients with ARDS (29,32). Similar abnormalities occur in BAL of patients considered to be at risk for ARDS by virtue of sepsis, multiple transfusions, aspiration, or a variety of other factors (32). In evolving bronchopulmonary dysplasia in infants with RDS, increased procoagulant activity is generally observed in lung lavage fluids (38). Consistent increases in BAL procoagulant activity are also seen in patients with interstitial lung diseases, although the increments are relatively less than those seen in ARDS BAL. Patients with sarcoidosis, idiopathic pulmonary fibrosis, and other interstitial lung diseases all exhibit increased BAL procoagulant activity that is mainly attributable to tissue factor associated with factor VII or VIIa (3,32,39,40). Changes in local procoagulant activity are therefore characteristic of acute or chronic lung injury and promote alveolar local fibrin formation.

IV. Abnormalities of Fibrinolysis in Lung Injury and Repair

The components of the urokinase plasminogen activator (uPA)–PAI-1–urokinase receptor (uPAR) system are all represented in the injured lung. Plasminogen is present in alveolar lining fluids in baboons with evolving diffuse alveolar damage and patients with ARDS (10,32). Plasminogen can be converted to the active endopeptidase plasmin by uPA or tissue-type PA (tPA). uPA is mainly involved in extravascular proteolysis and tissue remodeling, whereas tPA promotes intravascular thrombolysis (25). At the cell surface, localized generation of plasmin by uPA is facilitated by the interaction of this protease with its specific receptor, uPAR. The uPA-uPAR interaction at the surface of epithelial cells, alveolar macrophages, and lung fibroblasts promotes the degradation and remodeling of the extracellular matrix (ECM). uPA is also a potent chemotaxin and facilitates proliferation of epithelial cells and lung fibroblasts (41,42). Expression of uPA and uPAR also influences the release and activity of several cytokines (25). The regulation of cell traffic and cytokine-mediated cell-to-cell signaling also involves uPA and uPAR (43–45). The uPA-uPAR system therefore appears to be integrally involved in inflammation and matrix remodeling in lung repair.

Cells express uPA as an inactive single polypeptide chain proenzyme, prouPA, also called single-chain uPA or scuPA. Limited proteolysis converts scuPA into the more active two-chain uPA, tcuPA, which for purposes of this discussion, is defined as uPA. Both scuPA and uPA bind avidly to uPAR and both bound forms retain PA activity (46,47). Plasmin can cleave receptor-bound scuPA to form more active uPA, and receptor-bound scuPA or uPA can activate plasminogen to form plasmin (46–48). Interestingly, there is a difference in susceptibility of uPAR-bound scuPA versus uPA to inhibition by PAI. scuPA bound to uPAR resists irreversible inactivation by PAI, whereas

uPA remains susceptible. Remodeling of the lung during inflammation and fibrotic repair therefore integrates contributions of cellular and fluid-phase uPA. In both circumstances, expression of uPA-related proteolytic activity is regulated by interactions with PAI, and internalization and recycling of trimeric uPA-PAI-1-uPAR complexes can also regulate expression of cellular proteolytic activity (49).

A profound defect in alveolar fibrinolytic activity occurs in acute lung injury and fibrosing alveolitis due to a variety of underlying causes. Whereas alveolar fibrin formation is favored by increased expression of procoagulant activity, a concurrent decrement in fibrinolytic activity favors persistence of alveolar fibrin. In bleomycin-induced lung injury, the decrement in fibrinolytic activity is most profound when lung lavage procoagulant activity reaches its peak (8), and these changes parallel the greatest increase of alveolar fibrin deposition (35). Decreased fibrinolytic activity also occurs in association with increased local procoagulant activity in oleic acid–induced lung injury in rats and sheep (8,9). Similarly decreased fibrinolytic activity occurs with procoagulant activity in diffuse alveolar damage and evolving bronchopulmonary dysplasia (BPD) in baboons (10,36,37). Profound decrements of lung lavage fibrinolytic activity likewise occur in severely ill premature infants with evolving BPD (38). Over the course of ARDS, decreased BAL fibrinolytic activity is consistently observed. The alveolar procoagulant response gradually falls, whereas the fibrinolytic defect remains profound in BAL sequentially harvested for 2 weeks after clinical recognition of ARDS (50). These persistent abnormalities are likely responsible for the maintenance of alveolar fibrin during the course of evolving ARDS.

The decrement of alveolar fibrinolytic activity is predicated on inhibition of PA and plasmin by PAI-1 (and PAI-2) and antiplasmins, respectively (7). Interestingly, the presence of PAI-1 appears to be of particular importance (32,51). This situation is similar to that observed in pleural inflammation where PAI-1 antigen levels and activity are markedly increased and contribute to the downregulation of local fibrinolysis (52). In addition, plasma levels of PAI-1 in ARDS usually exceed those of other critically ill patients. The increment in circulating levels of PAI-1 likely results in large part from the composite effect of several inflammatory mediators on parenchymal lung cells. Expression of PAI-1 appears to play a pivotal role in the potentiation of intravascular thrombi that can contribute to multiorgan failure (53).

V. Regulation of Cellular Responses that Alter Fibrin Turnover in Lung Injury and Repair

Cells of the injured lung respond to inflammatory mediators by expressing increased levels of tissue factor and inhibitors of fibrinolysis. Alveolar

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macrophages as well as lung epithelial cells and fibroblasts all constitutively express tissue factor (3,31,54–56). By contrast, the expression of tissue factor by endothelial cells is inducible in the microvasculature of the injured lung. These cells respond to locally elaborated cytokines such as tumor necrosis factor = α (TNF-α) and TGF-β by expressing increased levels of this procoagulant. For example, lung epithelial cells and fibroblasts increase expression of tissue factor in response to treatment with TNF-α or TGF-β (57,58). Tissue factor released from cells stimulated by these and other cytokines likely contributes to the overall increment of procoagulant activity of BAL fluids in lung injury and repair (55). Parenchymal lung cells also express and secrete uPA and PAI-1 as well as PAI-2 (59–62). Local elaboration of these molecules, in addition to extravasation due to increased micovascular permeability, can influence alveolar fibrinolytic capacity. The same cytokines that induce tissue factor in these cells can increase expression of PAI versus that of PA, resulting in downregulation of overall fibrinolytic activity (57,58).

VI. Novel Pathways by Which Lung Epithelial Cells Regulate Fibrinolytic Activity

Expression of uPA, uPAR, and PAI-1 by lung epithelial cells and other cell types is altered by cytokines (26,58,63) or proteases, such as thrombin (64–66). The regulation of these molecules is complex, based upon emerging basic studies. Activation of these genes occurs at the transcriptional level but regulation occurs at multiple levels (46,67–71). Posttranscriptional regulation of uPA, uPAR, and PAI-1 have also been described (46,72). Recent studies have expanded our understanding of the mechanisms by which expression of uPA, uPAR, and PAI-1 are regulated at the posttranscriptional level by the lung epithelium (Fig. 1).

A. Posttranscriptional Regulation of uPA

Precedents in the literature show that posttranscriptional regulation of uPA occurs in epithelial cell systems. In renal epithelial cells, the stability of uPA mRNA is modulated by protein synthesis inhibition, phosphotyrosine kinase downregulation (73–75) and calcium ion fluxes (76). Chimeric gene analysis confirms that the regulation of uPA mRNA involves instability determinants, including one so-called AU-rich sequence, that are contained in the 3′ untranslated region (UTR) (77). Posttranscriptional regulation is involved in increased uPA gene expression in selected breast cancer cells (78).

As is the case in other solid neoplasms, uPA overexpression occurs in several lung carcinoma–derived cells compared to primary cultures of lung small airway epithelial cells (79). Overexpression of uPA in these cells involves posttranscriptional regulation. The regulatory mechanism involves the specific

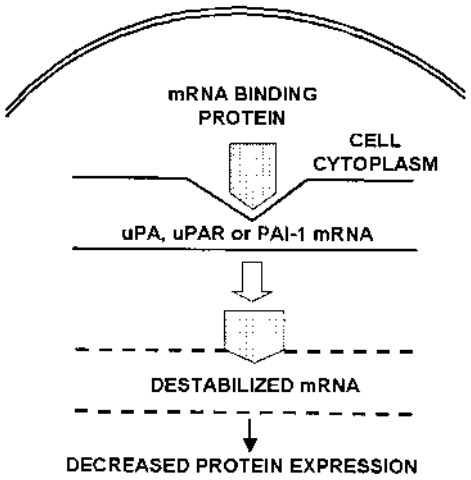

Figure 1 Posttranscriptional regulation of uPA, uPAR, and PAI-1 expression.

interaction between a uPA mRNA binding protein (uPA mRNBp) with a 66-nucleotide sequence within the uPA mRNA. 3′ UTR (79) (see Fig. 1). By ultraviolet (UV) cross-linking experiments, the uPA mRNABp was characterized as a 30-kD protein. This protein is found in the cytoplasm but not in the nucleus of nonmalignant lung small airway epithelial cells. The distribution of this protein is altered in lung carcinoma–derived cells. In these cells, the uPA mRNABp is found in the nuclear extracts, indicating that posttranscriptional regulation of uPA mRNA expression involves the nuclear-cytoplasmic distribution of the uPA mRNABp. Interaction of the uPA mRNABp and its binding sequence in the 3′ UTR of uPA mRNA in the cytoplasm is destabilizing, and the nuclear distribution of the binding protein in lung carcinoma cells stabilizes uPA mRNA. This mechanism presumably accounts for at least part of the increased expression of uPA observed in lung carcinoma–derived cells.

B. Posttranscriptional Regulation of the Urokinase Receptor (uPAR)

Expression of uPAR has been implicated in tumor invasion and metastasis (46,68,80). Like uPA, uPAR is regulated by a variety of stimuli at the transcriptional level (46). Recently, it has become clear that uPAR expression is also regulated at the posttranscriptional level in human and rabbit pleural mesothelial or mesothelioma cells (81,82), lung fibroblasts, or lung carcinoma cells (83). A variety of stimuli, including tumor promoters, cytokines, and translation inhibitors increases uPAR mRNA stability in these cells. These

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results suggest that uPAR gene expression is regulated at the posttranscriptional level.

The posttranscriptional regulatory mechanism involves the interaction of a uPAR mRNA binding protein (uPAR mRNABp) and a specific sequence within the coding region of uPAR mRNA (see Fig. 1). A 50-kDa uPAR mRNABp was identified by UV cross-linking analyses. This protein selectively interacts with a 51-nucleotide (nt) sequence of the uPAR mRNA coding region (81). Insertion of the 51-nt uPAR mRNA binding sequence into β-globin cDNA destabilized the chimeric β-globin/uPAR/β-globin mRNA, whereas a control sequence of uPAR mRNA did not change the stability of the chimeric β-globin/control/β-globin mRNA. The interaction between this 51-nt protein binding sequence of uPAR mRNA with uPAR mRNA therefore regulates uPAR mRNA stability. Agents that stabilize uPAR mRNA, such as tumor promoters, cytokines, and translational inhibitors, increase uPAR mRNA stability (81) and stabilize uPAR mRNA by inhibiting the uPAR mRNA-uPAR mRNABp interaction. These responses are detectable in rabbit lung fibroblasts and pleural mesothelial cells (82) or human mesothelial cells as well as malignant mesothelioma and lung carcinoma cells (83). The mechanism by which interaction between the uPAR mRNABp and uPAR mRNA regulates uPAR mRNA stability remains to be defined.

Transfection strategies have been used to determine how this posttranscriptional mechanism influences cellular function. These experiments demonstrate that this pathway is involved in a range of cellular responses that influence remodeling of the extracellular matrix. For example, MS-1 malignant mesothelioma cells overexpressing the chimeric β-globin/uPAR/β-globin sequence containing the uPAR mRNABp binding sequence exhibit increased cell surface uPAR expression, uPA-mediated DNA cellular proliferation as well as increased cellular migration and invasion (84). These observations show that the uPAR mRNABp-uPAR mRNA interaction could influence organization and remodeling of the tumor neomatrix. This pathway could likewise influence these processes in the context of evolving pulmonary fibrosis. Along these lines, fibroblasts obtained from normal lung tissue express less uPAR compared to fibroblasts isolated from fibrotic lungs (41). Differential uPAR expression between fibroblasts isolated from normal and fibrotic lungs is due, at least in part, to differences in the stability of uPAR mRNA. The uPAR mRNABp is present in the cytosolic extracts of normal lung fibroblasts and binds uPAR mRNA, whereas this interaction is not detectable in fibrotic lung fibroblasts. Posttranscriptional regulation of uPAR mRNA similarly occurs in primary cultures of rabbit lung fibroblasts or mesothelial cells (82). Another posttranscriptional pathway regulating stability of uPAR mRNA involves AU-rich elements in the uPAR mRNA 3′ UTR (85), indicating that control at this level can involve alternate independent pathways. Direct evidence that modification of posttranscriptional pathways that govern

uPAR expression could favorably alter pulmonary fibrosis awaits analyses using transgenic animals.

C. Posttranscriptional Regulation of PAI-1

Like uPA or uPAR, expression of the PAI-1 gene is regulated at both the transcriptional and posttranscriptional levels (86). Differences in mRNA stability appear to account for the differential stability of the two forms of PAI-1 mRNA; the relatively labile 3.2-kb form and the more stable 2.2-kb form. PAI-1 mRNA stability is regulated by cyclic AMP, and the process involves binding of several different proteins with PAI-1 mRNA in rat cells (87). These findings were recently extended. PAI-1 is increased in selected lung carcinoma cells compared to normal lung epithelial cells (88). The clinical relevance of this observation is underscored by the correlation of increased cancer tissue PAI-1 expression with poor prognosis in patients with lung cancer (89,90). PAI-1 expression in lung epithelial cells and cells derived from lung carcinomas has recently been shown to be regulated at the posttranscriptional level (88). The mechanism involves the interaction between a PAI-1 mRNA binding protein (PAI-1 mRNABp) and the 3′ UTR of PAI-1 mRNA. Selected cytokines influence PAI-1 mRNA expression by altering the ability of the binding protein to bind PAI-1 mRNA.

In lung epithelial cells, a 60-kD protein specifically binds to the PAI-1 mRNA 3′ UTR (see Fig. 1) (88). Under basal conditions, the PAI-1 mRNABp–PAI-1 mRNA interaction is readily detectable in small airway epithelial cells. Cytokine treatment of these cells increases PAI-1 expression and decreases the PAI-1 mRNABp–PAI-1 mRNA interaction, which is likewise decreased in carcinoma-derived cells that overexpress PAI-1. It therefore appears that complex formation is inversely related to PAI-1 expression by these lung epithelial cells and that the interaction is destabilizing.

The destabilizing effect of the PAI-1 mRNABp–PAI-1 mRNA interaction on PAI-1 mRNA remains to be established by chimeric gene analyses, such as those described in the sections above. Interestingly, the regulation of PAI-2 also appears to involve a posttranscriptional component involving an AU-rich motif present in the 3′ UTR of PAI-2 mRNA (91).

D. Role of uPA in the Regulation of uPAR Expression by Lung Epithelial Cells

Recently, a new pathway by which uPA regulates uPAR expression was defined in lung epithelial cells (Fig. 2). In Beas2B lung epithelial cells, uPA enhances uPAR protein and mRNA expression as well as the binding of ^{125}I-uPA in a time- and concentration-dependent manner. Induction of uPAR by uPA in

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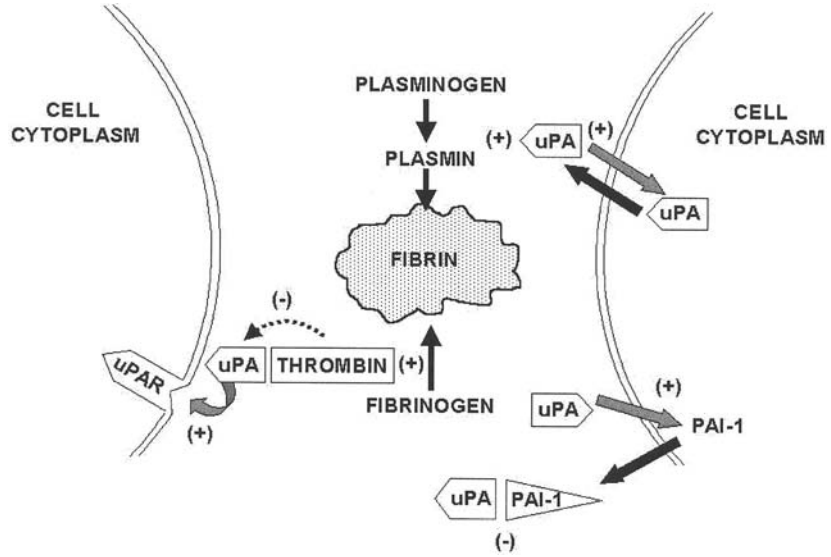

Figure 2 Regulation of the fibrinolytic system by uPA. (+) indicates facilitation. (−) indicates inhibition.

these cells requires uPA enzymatic activity but not binding of uPA to uPAR. The low molecular weight fragment of uPA which lacks the receptor binding domain can likewise induce uPAR expression by Beas2B cells. Thrombin inhibits the uPA-mediated induction of uPAR. It is therefore plausible that the lung epithelium could similarly regulate uPAR expression in lung injury or neoplasia. The ability of uPA to regulate uPAR expression in lung-derived epithelial cells raised the possibility that other components of the uPA-PAI-1–uPAR system might likewise be regulated through exposure of the cells to uPA. This possibility is under active investigation at this time. uPA has recently been found to induce its own expression as well as that of PAI-1 in Beas2B cells. (S. Shetty, unpublished observations, manuscripts submitted, Figure 2).

VII. Interventions for Pulmonary Fibrosis Based on Disordered Fibrin Turnover

The information reviewed above and, in particular, the studies in transgenic animals implicate disordered fibrin turnover in the pathogenesis of pulmonary fibrosis (1,4,18,27,28). This evidence supports the hypothesis that steps in disordered fibrin turnover and extravascular fibrin deposition could be targeted

to prevent pulmonary fibrosis. Recent clinical trials in sepsis suggest that such an approach might be effective.

The abnormalities of pathways of fibrin turnover in sepsis and the local derangements of fibrin turnover in the lungs in ARDS are similar (56). Increased tissue factor expression occurs in the lungs in ARDS and in the systemic coagulopathy that characterizes sepsis. As is the case in ARDS, activation of coagulation in sepsis is accompanied by concurrently decreased fibrinolysis, promoting intravascular or extravascular fibrin formation (2,7, 92–98). Intravascular thrombosis is associated with end-organ dysfunction and multiorgan failure in sepsis. In ARDS, extravascular fibrin and its derivatives is associated with surfactant dysfunction with resultant atelectasis, matrix remodeling, and eventual fibrosis (30,99–102). Anticoagulants have therefore been used to prevent organ dysfunction in sepsis. The anticoagulant strategies used in recent sepsis trials have employed selective anticoagulants: tissue factor pathway inhibitor (TFPI) and activated protein C (APC) (103,104). Apart from anticoagulant effects, these agents also have anti-inflammatory (105) properties that likely contribute to their salutary effects in sepsis patients. APC also exerts profibrinolytic effects (106). Whether the use of these agents could be extrapolated to the prevention of pulmonary fibrosis is strictly hypothetical at this time, but the results of recent interventional trials suggest the possibility.

TFPI has been used to inhibit the coagulopathic responses to endotoxin and was well tolerated in Phase I clinical trials (103). This inhibitor blocks the activity of the tissue factor VIIa procoagulant complex by forming a quaternary complex with TF, factor Xa, and factor VIIa. TFPI is present in BAL fluid of patients with ARDS, but endogenous levels are apparently insufficient to block fibrin deposition initiated by tissue factor VIIa (107). Preliminary results presented at the 2000 annual meeting of the American College of Chest Physicians in San Francisco suggest that TFPI was of benefit in the subgroup of septic patients with ARDS. There was a roughly 20% overall survival advantage for all septic patients treated with TFPI. The TFPI-treated patients in the ARDS subgroup enjoyed an approximately 35% survival advantage compared to patients receiving intravenous vehicle. TFPI treatment was also associated with improved pulmonary dysfunction scores, suggesting that the survival advantage was attributable to improvement of the underlying lung injury. Whether this intervention could be useful in the prevention of accelerated pulmonary fibrosis in protracted ARDS remains to be determined.

Recently, APC was shown to be of benefit for patients with sepsis (104). APC is an anticoagulant that inhibits coagulation factors Va and VIIIa. The anti-inflammatory properties of APC include the inhibition of TNF-α, interleukin-1 (IL-1), and IL-6 (105,108). In a recent multicenter trial, a 96-h intravenous infusion of recombinant APC significantly reduced overall mortality assessed 28 days later by about 20% (104). APC treatment reduced the mortality rate from 30.8 to 24.7%; a benefit that was highly significant. The

incidence of bleeding with APC treatment was slightly increased from 2.0 to 3.5%. Subgroup analysis of the effects of APC treatment in ARDS patients were not presented in this publication, and the ability of APC to prevent lung injury in such patients remains to be determined. However, the efficacy of this intervention in systemic sepsis suggests the possibility that lung injury and accelerated fibrosis, particularly that associated with sepsis-induced ARDS, might likewise be attenuated.

There is also preclinical evidence that interventions designed to reverse the local fibrinolytic defect could prevent lung injury associated with ARDS. A survival advantage was demonstrated in a porcine model of ARDS (109). In this model, intervention with either tPA or uPA conferred lung protection as assessed by histological and arterial blood gas analyses. In another approach, administration of plasminogen was used to reverse the deficiency of plasminogen and reduce the incidence of RDS in at-risk premature infants (110). Intervention with plasmin also reduced the mortality rate of infants with established RDS. Fibrinolytic agents were also used to reverse lung injury in a few small clinical trials. Treatment with streptokinase improved hemodynamics and oxygenation with angiographic clearance of pulmonary thrombi that occurred in association with acute lung injury (98). Treatment with either streptokinase or urokinase resulted in improved arterial oxygenation and no bleeding complications in another study of 19 ARDS patients (111). Whether this approach could be adapted to prevent accelerated pulmonary fibrosis associated with ARDS is currently unclear and remains to be studied.

Most recently, interventional trials have been initiated to determine if pathways of fibrin turnover can be used to block lung dysfunction in a baboon model of ARDS. In these trials, anticoagulants that block the activation of coagulation by the tissue factor–factor VII complex have tested in baboons with sepsis-induced ARDS. In the initial experiments, pretreatment with TFPI or active site–inactivated factor VII have been demonstrated effectively to protect the lungs (112). Animals treated with these agents did not develop impaired oxygenation and lung compliance remained near normal. Systemic acidosis, renal dysfunction, and lung or bowel edema were greatly attenuated. Rescue studies in which these agents will be introduced in the setting of established acute lung injury are now in progress. These trials suggest the intriguing possibility that accelerated pulmonary fibrosis could be prevented by selectively exploiting disordered fibrin turnover in the injured lung.

VIII. Conclusions

It now appears that disordered coagulation, fibrinolysis, and extravascular fibrin deposition are integral to inflammation and fibrotic repair that occur in association with evolving lung injury. In the injured lung, local procoagulant

pathways are upregulated and fibrinolytic pathways depressed. These conditions favor the appearance of alveolar fibrin, which organizes with pulmonary fibrosis. In ARDS, this progression is relatively rapid and can result in accelerated pulmonary fibrosis. In interstitial diseases, the same processes are protracted and appear to contribute to fibrotic repair. Recent studies using transgenic animals provide strong evidence that manipulation of pathways of fibrin turnover can favorably influence pulmonary fibrosis. Interventional trials targeting disordered fibrin turnover suggest new approaches by which the coagulopathy and mortality of sepsis can be attenuated. Ongoing preclinical studies are being conducted to determine if selective anticoagulants or fibrinolysins can specifically attenuate acute lung injury and anticoagulant approaches now appear promising. Completion of these studies and subgroup analyses of ongoing sepsis trials should soon allow us to determine whether these approaches will be successful. The relationship between disordered fibrin turnover and fibrotic repair suggests the hypothesis that anticoagulant or profibrinolytic strategies could prevent fibrosis after lung injury. Whether such interventions will ultimately prove to be of clinical value remains to be determined.

Acknowledgments

This work was supported by National Institute of Health grants RO-1 HL45018, RO-1 HL62453, and PO-1 HL42444 and the Temple Endowed Professorship.

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Arachidonic Acid Metabolites: Potential Mediators and Therapeutic Targets in Fibrotic Lung Disease

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I. Introduction

Arachidonic acid (AA) is a fatty acid constituent of the phospholipids of cell membranes. It is the precursor for a family of bioactive lipids collectively known as *eicosanoids*, the best known of which are leukotrienes (LTs) and prostaglandins (PGs). A low rate of eicosanoid synthesis occurs under basal conditions, but synthesis can be dramatically increased in response to a myriad of stimuli. Generation of eicosanoids and their interactions with cellular receptors result in a host of responses in target cells and tissues that are central to normal homeostasis as well as to the pathogenesis of many disease states. Because of the long-recognized roles of eicosanoids in pain, platelet aggregation, microvascular permeability, smooth muscle contraction, and inflammation, these mediators have been traditionally linked with diseases such as arthritis, ischemic cardiovascular disease, and asthma (1).

However, a contemporary view recognizes a broader spectrum of eicosanoid actions, including effects on immune responses, cellular proliferation, and apoptosis. These actions, in turn, reflect modulation of such fundamental cellular processes as signal transduction and gene expression. In this context, a growing body of evidence supports a potentially pivotal role for eicosanoids in the pathogenesis of fibrotic lung disease. Data from patients with idiopathic pulmonary fibrosis (IPF) as well as animal models suggest that *an imbalance favoring profibrogenic LTs over antifibrogenic PGs promotes the development of pulmonary fibrosis*.

This chapter will focus on (1) the actions of eicosanoids relevant to pulmonary fibrosis, (2) the evidence that abnormalities in eicosanoid

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production are found in fibrotic lung disease, and (3) the therapeutic potential and implications for interventions targeting these mediators. We will begin by briefly reviewing our current understanding of how eicosanoids are synthesized and how they exert their actions at the cellular level.

II. Eicosanoids and Their Synthesis

The initial step in the eicosanoid biosynthetic pathway (Fig. 1) (1) involves activation of a phospholipase A_2 (PLA_2) enzyme, which hydrolyzes AA from membrane phospholipids. This is generally a consequence of an increase in intracellular calcium, which can be triggered by exogenous substances such as antigen, endotoxin, particulates, oxidants, and xenobiotics, as well as by endogenous factors such as cytokines, proteases, kinins, hormones, and complement. Once liberated, free AA is converted to a variety of oxygenated metabolites by several parallel metabolic pathways. The two best-studied pathways are the cyclooxygenase (COX) and 5-lipoxygenase (5-LO) pathways. The former gives rise to the prostanoids (PGs, prostacyclin [PGI_2], and thromboxane [TxA_2]), whereas the latter yields the LTs and 5-hydroxyeicosatetraenoic acid (5-HETE). Other lipoxygenase pathways yield additional HETEs as well as lipoxins, whereas epoxides arise from the actions of cytochrome P450 enzymes.

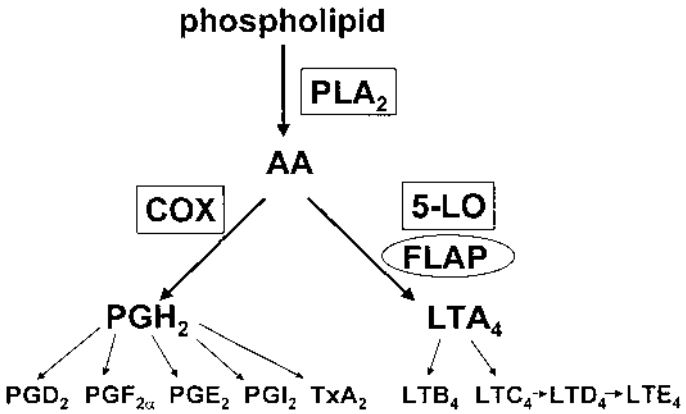

Figure 1 Biosynthetic pathways for the formation of LTs and prostanoids. Abbreviations are defined in the text. The distal enzyme responsible for converting PGH_2 to a given prostanoid (not shown) is termed the relevant prostanoid synthase. The distal enzymes responsible for converting LTA_4 to LTB_4 and LTC_4 (not shown) are termed LTA_4 hydrolase and LTC_4 synthase, respectively.

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The COX enzyme converts AA to an unstable intermediate, PGH_2. COX exists as two specific isoforms, the constitutive COX-1 isoform and the inducible COX-2 isoform. Subsequent metabolism to the bioactive prostanoids (PGD_2, $PFG_{2\alpha}$, PGE_2, PGI_2, and TxA_2) is carried out by the corresponding synthase (e.g., PGE synthase). Like COX, PGE synthase exists in both constitutive and inducible isoforms. The first committed step in LT synthesis is catalyzed by the enzyme 5-LO, acting in concert with its helper protein, 5-LO activating protein (FLAP); FLAP is thought to act as an AA-binding protein which optimally "presents" AA to 5-LO. The resultant intermediate LTA_4 can be hydrolyzed by LTA_4 hydrolase to LTB_4, or be conjugated with reduced glutathione to form LTC_4 by the glutathione transferase, LTC_4 synthase. Once secreted from the cell, LTC_4 can be metabolized to LTD_4 and then to LTE_4 by the sequential removal of glutathione's glutamic acid and glycine constituents. Because LTs C_4, D_4, and E_4 have similar biological actions and all retain the cysteine moiety of glutathione, they are often collectively referred to as cysteinyl LTs; it is these molecules that comprise the mytotropic activity previously known as slow-reacting substance that is implicated in the pathogenesis of asthma.

Cellular specificity in the profiles of eicosanoid generation exists. Although both leukocytes and structural cells are capable of prostanoid synthesis, substantial 5-LO expression is confined primarily to leukocytes. Furthermore, although PGE_2 is the major prostanoid product of epithelial cells, fibroblasts, and smooth muscle cells, PGD_2 is the major prostanoid of mast cells. Finally, eosinophils and mast cells synthesize primarily LTC_4, neutrophils synthesize primarily LTB_4, and macrophages synthesize both LTs.

Cells must have the capacity to modulate their potential for eicosanoid synthesis. In fact, most of the eicosanoid-forming enzymes can be regulated in three distinct ways. First, the level of expression of the protein can be enhanced at either transcriptional or translational steps; for example, by cytokines or growth factors. Second, the catalytic efficiency of existing enzyme molecules can be modulated either by posttranslational modification (such as phosphorylation by kinases) or by alterations in the level of essential enzyme cofactors such as calcium or glutathione. Finally, changes in the intracellular localization of some of these proteins can influence their function; for example, activation of PLA_2 and of 5-LO is associated with translocation from a soluble intracellular site to the perinuclear membrane (2).

One unique feature of eicosanoid synthesis is the rapidity with which it can occur. Since the requisite enzymes are generally expressed constitutively, and since stimulus-induced calcium transients necessary to activate AA release (and 5-LO, the only other enzyme in the pathway which requires specific activation) occur within seconds to minutes, eicosanoids can be synthesized and released from source cells within this same time frame. Their generation therefore lags behind that of a preformed mediator such as histamine only

slightly, but precedes that of typical protein mediators such as cytokines, whose elaboration requires new transcription and translation, by hours. However, the fact that eicosanoid synthesis can be further amplified by increases in expression of eicosanoid-forming enzymes also permits sustained generation or delayed bursts of synthesis. Eicosanoids are therefore unique among mediators in their ability to participate in both immediate and delayed phases of various responses.

III. Eicosanoid Receptors and Signal Transduction Mechanisms

The biological actions of eicosanoids are generally presumed to be mediated by their binding to specific receptors on target cells. Ligation of these receptors results in the generation of intracellular signals which, in turn, transduce the biological actions of the lipid mediator. Receptors for most of the major eicosanoids have been characterized using classic pharmacological techniques, and more recently, many have been cloned (3). These are G-protein–coupled transmembrane receptors similar to those for hormones and chemokines. They are coupled to signal transduction events including alterations in intracellular calcium and cyclic AMP concentrations. These, in turn, influence the activation of diverse protein kinases such as protein kinases A and C and tyrosine kinases. Although these receptors are assumed to be primarily distributed on the plasma membrane, receptors for PGE_2 have also been identified on the nuclear membrane (4). It has recently been discovered that certain eicosanoids can also bind to an alternative type of receptor known as peroxisome proliferator-activated receptors (PPARs) (5). These are intranuclear receptors that also act as transcription factors, regulating the expression of proteins involved in lipid metabolism, inflammation, and cellular proliferation. It is attractive to speculate that interaction with nuclear receptors of either the classic G-protein–coupled or of the PPAR classes may mediate some of the autocrine effects of eicosanoids formed locally at the nucleus.

It is now recognized that, as is the case for certain synthetic enzymes such as COX and PGE synthase, receptors for a given eicosanoid can be encoded by multiple genes. This is best exemplified by the receptors for PGE_2 (termed the E prostanoid, or EP, receptors); four distinct EP receptors are known, with some coupled to adenyl cyclase and others to calcium mobilization (3). More recently, two distinct receptors for cysteinly LTs (cysLT1 and cysLT2) have been identified that vary in their specificity for LTC_4 versus LTD_4 and in their tissue distribution (6,7). Likewise, two receptors for LTB_4 (BLT1 and BLT2) with different affinities and tissue distributions have been identified (8,9). It is likely that such complex receptor variety provides a means for functional and cellular specificity in the actions of

Table 1 G-Protein–Coupled Eicosanoid Receptors

Receptor	Ligand specificity	Signal transduction
cysLT1	$LTD_4 >> LTE_4 = LTC_4$	$\uparrow Ca^{2+}$
cysLT2	$LTC_4 = LTD_4 >> LTE_4$	$\uparrow Ca^{2+}$
BLT1	LTB_4	$\uparrow Ca^{2+}$, $\downarrow$ cAMP
BLT2	$LTB_4 >$ 12-HETE > 15-HETE	$\uparrow Ca^{2+}$, $\downarrow$ cAMP
EP1	PGE_2	$\uparrow Ca^{2+}$
EP2	PGE_2	$\uparrow$ cAMP
EP3	PGE_2	$\downarrow$ cAMP, $\uparrow Ca^{2+}$
EP4	PGE_2	$\uparrow$ cAMP
IP	PGI_2	$\uparrow$ cAMP
TP	$TxA_2 > PGD_2$	$\downarrow$ cAMP, $\uparrow Ca^{2+}$
DP(1)	PGD_2	$\uparrow$ cAMP
CRTH2 (DP2)	PGD_2	$\downarrow$ cAMP, $\uparrow Ca^{2+}$
FP	$PGF_{2\alpha}$	$\uparrow Ca^{2+}$

In general, ligation of receptors that increase intracellular cyclic AMP (cAMP) results in anti-inflammatory and antifibrotic effects. By contrast, ligation of receptors that increase intracellular Ca^{2+} and/or decrease cAMP results in proinflammatory and profibrotic effects. The intranuclear receptor PPAR-γ, which recognizes 15-deoxy-PGJ_2, 15-HETE, and perhaps other eicosanoids, is not listed here.

these potent mediators. The major eicosanoid receptors as well as their ligand specificity and signaling mechanisms are listed in Table 1.

IV. Effects of Eicosanoids on the Pathobiological Features of Fibrotic Lung Disease

It is generally thought that fibrotic lung disease represent an abnormal response to some inciting injurious event. The precise role and temporal sequence of the various component abnormalities involved in the pathogenesis of pulmonary fibrosis in general and IPF in particular are unclear. However, as determined from studies of both human disease and animal models, the key pathobiological elements are thought to include (10–12) (1) expansion of the population of inflammatory and immune effector cells (macrophages, lymphocytes, neutrophils, and eosinophils); (2) a helper T type 2 (Th2)–type of cellular immune response characterized by elaboration of cytokines such as interleukin-4 (IL-4), IL-5, and IL-13 in preference to Th1-type cytokines IL-2, IL-12, and interferon-γ (IFN-γ); (3) elaboration by activated macrophages and other effector cells of mediators including reactive oxygen intermediates, cytokines such as tumor necrosis factor-α (TNF-α), chemokines such as IL-8

and monocyte chemoattractant protein (MCP)-1, and fibroblast growth and differentiation factors such as platelet-derived growth factor (PDGF), basic fibroblast growth factor, and transforming growth factor-β (TGF-β); (4) acquisition by fibroblasts of altered phenotypes characterized by unchecked proliferation or differentiation to α-smooth muscle actin-positive myofibroblasts capable of excessive collagen synthesis; and (5) loss of the normally suppressive signals for fibroblasts elaborated by alveolar epithelial cells due to epithelial cell loss or injury.

The actions of arachidonate metabolites on these pathobiological components will now be considered. More is known about the effects of LTs and PGE_2 on these processes than about other eicosanoids. An important generalization that will be apparent is that *LTs promote these processes*, *while PGE_2 inhibits them* (Table 2). Although there is less information about the effects of PGI_2 in this context, its effects generally resemble those of PGE_2.

Table 2 Biological Actions of LTs and PGE_2 on the Pathobiological Features of Pulmonary Fibrosis

Feature	LTs	PGE_2
Leukocyte accumulation		
Myelopoiesis	↑	↓
Chemotaxis	↑	↓
Adhesion molecule expression	↑	↓
Leukocyte survival	↑	↔
Immune responses		
Dendritic cell function	↑	(↓)
Lymphocyte function	↑	↓
Th2 polarization	↑	(↓)
Leukocyte activation		
Reactive oxygen intermediates	↑	↓
Cytokines	↑	↓
Chemokines	↑	↓
Growth factors	↑	↓
NF-κB activation	↑	↓
Fibroblast activation		
Chemotaxis	↑	↓
Growth factor receptor expression	?	↓
Proliferation	↑	↓
Collagen synthesis	↑	↓
Collagenase activity	?	↓
Myofibroblast differentiation	?	?

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

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The nonenzymatic metabolite of PGD_2, 15-deoxy-PGJ_2, is a possible endogenous ligand for the PPAR isoform PPAR-γ, and the relevant information about this substance, albeit limited, will also be discussed.

A. Effects on Accumulation of Inflammatory and Immune Effector Cells

LTs increase the number of inflammatory cells in the lung (13,14) by acting on a variety of determinants of their accumulation. They stimulate granulocyte and macrophage colony formation in the bone marrow (15,16). LTB_4 acts as a direct chemoattractant for neutrophils and eosinophils (17). Cysteinyl LTs also upregulate endothelial cell expression of adhesion molecules that are necessary for leukocyte emigration into tissues (18). Finally, LTs are potent inhibitors of neutrophil (LTB_4) (19) and eosinophil (cysteinyl LT) (20) apoptosis, thereby prolonging the survival of cells that have been recruited. These mechanisms explain the ability of drugs that inhibit LT synthesis (zileuton) or block cysteinyl LT receptor-1 (zafirlukast, montelukast, and pranlukast) to reduce leukocyte numbers in the lung in asthmatics (21).

PGE_2 has directly opposite effects on leukocyte accumulation. It inhibits myelopoiesis (22) and also inhibits leukocyte recruitment by suppressing adhesion molecule expression (23) as well as chemotaxis (24).

B. Effects on Immune Responses

LTs have been shown to play a role in the generation of systemic immune responses (25). This may be explained by recent data indicating that cysteinyl LTs are required for the migration to lymph nodes of antigen-presenting dendritic cells (26), a process which is crucial for the initiation of such responses. Moreover, LTB_4 can enhance T-lymphocyte responses (27). Beyond their generalized stimulatory effect on immune responses, there is emerging evidence that cysteinyl LTs can promote a polarized Th2-type response both in vitro and in vivo. This reflects their capacity to selectively increase synthesis of IL-4 and IL-5 (28–30) without increasing synthesis of IFN-γ. In fact, a cysteinyl LT receptor antagonist increased IFN-γ production by T lymphocytes (29), and lung leukocytes from 5-LO knockout mice produced greater amounts of IFN-γ than did cells from wild-type animals (31).

PGE_2 has long been recognized to inhibit both B- and T-cell responses (32,33). However, it has been noted to have discrepant effects on dendritic cells, promoting their maturation (34) but inhibiting their ability to present antigen (35). The role of PGE_2 in the polarization of T-cell responses is equivocal. PGE_2 is known to selectively inhibit Th1 cytokine expression in lymphocytes

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in vitro (36), and is commonly thought to favor Th2 polarization. However, in vivo inhibition of PGE_2 synthesis during a period of antigen sensitization has been shown to enhance Th2 lung responses in the lung (37). This discrepancy could reflect the fact that in vivo responses involve cell types other than lymphocytes or differences in the profile of EP receptors on T lymphocytes in vitro versus in the context of an allergic response in vivo.

C. Effects on Inflammatory Mediators and Leukocyte Activation

5-LO metabolites activate alveolar macrophages (AMs) and other cells to elaborate a number of mediators implicated in the pathogenesis of fibrotic lung disease. These include reactive oxygen intermediates (38), IL-6 (39), IL-8 (40), TNF-α (41), fibroblast growth factor activity (42), endothelin-1 (43), and matrix metalloproteinases (44).

The predominant effect of PGE_2, by contrast, is to abrogate the elaboration of a wide range of inflammatory mediators. These include reactive oxygen intermediates (45), IL-8 (46), TNF-α (47), endothelin-1 (48), fibroblast growth factor activity (42), and LTs themselves (49). Most of these salutary effects appear to be explained by increases in intracellular cyclic AMP, and are likely mediated by PGE_2 actions at the adenyl cyclase–coupled receptors EP2 and EP4 (50). Many of these actions are therefore shared by PGI_2, which also signals via adenyl cyclase activation upon ligation of its receptor, IP. Some of the anti-inflammatory activities of PGE_2 may be exerted through its well-described capacity to upregulate expression of the suppressive cytokine, IL-10 (51), and this effect is also dependent on signaling via EP2/4 (50).

In some experimental systems, PGE_2 amplifies rather than inhibits inflammatory mediator generation. This has been most consistently observed with IL-6 generation (52). It is likely that the effects of PGE_2 in any given cell at any given point in time will ultimately be determined by the extant profile of EP receptors and their functional coupling to signal transduction pathways.

It is appealing to explain the opposing but equally pleiotropic actions of LTs versus PGE_2/PGI_2 by implicating directionally opposite effects on a centrally important regulator of inflammatory mediator generation. In this regard, LTs have been reported to activate (39,53,54) and PGE_2/PGI_2 to inhibit (55) activation of the transcription factor nuclear factor-κB (NF-κB). A variety of signal transduction pathways (protein kinase C, mitogen-activated protein kinase) are also the target for stimulation by LTs (56,57) and inhibition by PGE_2 (58,59). It should be noted that 15-deoxy-PGJ_2 exerts a generalized downregulatory effect on the generation of a variety of inflammatory mediators, also by inhibiting activation of NF-κB (60). It appears that this inhibitory effect on NF-κB can be mediated either through activation of

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PPAR-γ or in a PPAR-γ–independent manner that reflects direct interactions between the prostanoid and the transcription factor (61).

D. Direct Effects on Fibroblasts

In contrast to the well-known effects of LTs on inflammatory cells, it is not generally recognized that LTs can exert direct stimulatory effects on fibroblasts themselves. LTB_4 is chemotactic for fibroblasts (62), as it is for leukocytes. Cysteinyl LTs also stimulate fibroblast proliferation (63) and collagen synthesis (64). Proliferative effects have been linked to enhanced proto-oncogene expression (65).

Among those factors that downregulate fibroblast function, none has been more extensively investigated than PGE_2. This prostanoid inhibits fibroblast chemotaxis (66). It also potently suppresses fibroblast proliferation in response to a gamut of mitogens, including serum, TGF-β, PDGF, epidermal growth factor, insulin, and fibronectin (67–69). Similar inhibitory effects have been described for smooth muscle cell proliferation (70). This growth-inhibitory effect may be mediated by reduced cellular uptake of nucleotides and amino acids (67,71) or by reduced surface expression of growth factor receptors (72). PGE_2 selectively inhibits collagen synthesis (73) and also promotes its degradation (74); as a result, collagen accumulation is markedly attenuated. Most of these inhibitory effects are shared by PGI_2, and they appear to be mediated by increases in fibroblast cyclic AMP levels (75,76). For example, both PGE_2 (77) and PGI_2 (78) have been reported to inhibit TGF-β–induced production of connective tissue growth factor. Recent reports indicate that 15-deoxy-PGJ_2 also posseses suppressive activity toward fibroblasts. This prostanoid was shown to inhibit myofibroblast proliferation, procollagen mRNA expression, and α-smooth muscle actin expression (79) while also inducing apoptosis (80).

As is the case with effects on inflammatory mediators, there are some experimental systems in which PGE_2 either fails to inhibit (81) or actually stimulates (82) fibroblast proliferation.

There is little known about EP receptors on fibroblasts, and it seems likely that the effects of this prostanoid will be best understood in the context of an understanding of how the expression of and signaling by these receptors is regulated under normal conditions and in fibrotic lung disease. In this regard, it is interesting that proliferative responses of fibroblast lines from patients with IPF were inhibited by PGE_2 to a lesser extent than were lines from normal lung (83).

It is characteristic for mitogens to stimulate endogenous PGE_2 synthesis by fibroblasts via induction of COX-2 (84,85); this likely represents a negative feedback mechanism for controlling proliferation, which may be subject to derangement in fibrotic disease (see Sec. V).

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V. Abnormalities in Eicosanoid Synthesis in Pulmonary Fibrosis

LTB_4 levels in bronchoalveolar lavage fluid (BAL) have been reported to be greater in patients with idiopathic pulmonary fibrosis (IPF) than in normal volunteers (86,87). To "capture" LTs on both the alveolar surface as well as the interstitial compartment, their levels were recently quantitated in lung homogenates. Homogenates from IPF patients contained 15-fold more LTB_4 and fivefold more cysteinyl LTs than did homogenates from nonfibrotic lung (88). Interestingly, homogenate LT levels correlated significantly with the histological extent of fibrosis, which is consistent with the notion that LTs participate not merely in the inflammatory but also in the fibrotic phase of this disorder. The AM was suggested to be one source of these LTs on the basis of immunohistochemical analyses of lung sections that revealed a nuclear membrane distribution of 5-LO, indicative of an activated enzyme (2), in this cell type. Confirmation that the AM 5-LO pathway was constitutively activated was obtained by demonstrating that, in the absence of an exogenous stimulus, AMs obtained by BAL from IPF patients elaborated significantly more LTs than did AMs from normal volunteers (88). In asbestosis, a disease that shares many pathobiological features with IPF, increased LTB_4 content has likewise been demonstrated in both BAL fluid and AM-conditioned medium (89). Increases in lung LT levels have also been demonstrated in hamsters (90) and mice (31) following the intratracheal administration of bleomycin, the best-studied animal model of pulmonary fibrosis. In the mouse, overproduction of LTs persisted beyond the inflammatory phase to the fibrotic phase of the model (31), mirroring what has been observed in IPF patients. Although LT generation is primarily a property of bone marrow–derived cells, it is of interest that dermal fibroblasts from patients with the fibrotic skin disorder systemic sclerosis were recently shown to express 5-LO and to synthesize LTs (91).

The in situ stimulus for LT synthesis in the lungs of patients with IPF or other fibrotic diseases is unknown, but a number of possible candidates exist among those substances that are known to be both elevated in fibrotic lung and capable of stimulating 5-LO metabolism in vitro. These substances include immune complexes (92), IL-8 (93), IL-4 (94), IL-5 (95), endothelin-1 (96), MCP-1 (97), and TGF-β (98,99). It should also be noted that cytokines are capable not only of stimulating LT synthesis but also of increasing LT receptor expression (100). Therefore, an intricate web of interactions exists among LTs and proinflammatory cytokines: LTs amplify cytokine generation and cytokines amplify the synthesis of and responses to LTs.

Reduced PGE_2 levels in BAL fluid (101) and in AM-conditioned medium (102) have also been described in IPF. PGE_2 is the major eicosanoid produced by fibroblasts, and it has also been demonstrated that fibroblasts

isolated from the lungs of IPF patients have a reduced capacity to synthesize PGE_2 under basal conditions (103). Moreover, IPF fibroblasts exhibit a reduced capacity to upregulate their PGE_2 synthetic capacity in response to stimuli such as lipopolysaccharide, IL-1, TNF-α, and TGF-β; this defect is associated with impaired induction of COX-2 mRNA and protein (85,103,104). Impaired PGE_2 synthetic capacity has also been reported for fibroblasts isolated from the lungs of rats with bleomycin-induced pulmonary fibrosis; however, in this instance, deficient COX-2 expression could not be implicated, and the enzymatic mechanism for this defect remains unexplained (105).

Defective PGE_2 synthesis by fibroblasts in fibrotic lung disease may be important for two reasons. First, endogenous PGE_2 synthesized by fibroblasts appears to represent an important autocrine negative feedback regulator of fibroblast functions (84,85). COX inhibition invariably amplifies the mitogenic effects of growth factors on fibroblasts. An inability to upregulate PGE_2 synthesis in response to growth factors and proinflammatory cytokines present in situ in the injured lung may promote the development of an abnormal fibroblast phenotype characterized by excessive proliferation and collagen synthesis. Second, it is of interest that the capacity of cysteinyl LTs to stimulate lung fibroblast proliferation was most apparent under conditions where endogenous PGE_2 synthesis by fibroblasts was pharmacologically inhibited (63). Under conditions where fibroblast PGE_2 synthesis is indeed impaired in injured lung, the mitogenic actions of cysteinyl LTs may be unmasked.

There is a growing realization that dysfunctional interactions between alveolar epithelial cells (AECs) and pulmonary fibroblasts is an important determinant of fibrotic lung disease (12). Under normal circumstances, AECs suppress fibroblast proliferation (106). Since PGE_2 is the major and PGI_2 the secondary eicosanoid product of AECs (107), antifibrotic prostanoids are prime candidates to mediate the suppressive effects of AECs on fibroblasts. Indeed, fibroblast-suppressive activity in AEC-conditioned medium was eliminated when AECs were treated with the COX inhibitor indomethacin (106). AEC injury and loss is a universal phenomenon observed in lung injury, and in animal models, a failure of reepithelialization of the alveolar surface is a central determinant of fibrotic, rather than reparative, responses to injury (108,109). It is therefore quite plausible that impaired PGE_2 synthesis by AECs, as has been shown for AMs and fibroblasts, predisposes to pulmonary fibrosis. No information about prostanoid production by injured AECs is yet available. However, a possible salutary role for AEC PGE_2 production is suggested by preliminary data from our laboratory. AECs isolated from mice genetically deficient in the chemokine receptor CCR-2, already known to be protected from pulmonary fibrosis in response to bleomycin despite having no less lung vascular leak or inflammation (110), manifest a substantial increase in their capacity for PGE_2 synthesis (111).

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Although it is clear that various cell types from fibrotic lung manifest diminished PGE_2 synthesis, the underlying mechanisms remain obscure. As mentioned, AEC loss is a simple explanation for impaired AEC-derived prostanoids. In addition, just as was postulated above for LT overproduction, underproduction of PGE_2 might likewise be attributed to alterations in the injured lung in the levels of various substances that can modulate the expression or activity of PG-synthesizing enzymes. For example, a deficiency of glutathione in fibrotic lung is well known and is thought to be a reflection of oxidant stress (112). Since the PGE synthase enzyme is glutathione dependent (113), its activity may be impaired under conditions of glutathione deficiency. In this regard, it is conceivable that the ability of extracellular glutathione to suppress lung fibroblast proliferation (114) is related to enhanced PGE_2 generation. In addition, diminished granulocyte-macrophage colony-stimulating factor (GM-CSF) expression has been reported in bleomycin-treated lung (115). Since GM-CSF also promotes arachidonic acid release and PGE_2 synthesis in AMs by upregulating PLA_2 expression (116), it was not surprising that GM-CSF knockout mice developed exaggerated fibrosis in response to bleomycin in association with reduced PGE_2 levels in lung homogenates and AM-conditioned medium (117). Injured and fibrotic lung also contains mediators that may have inhibitory effects on PGE_2 synthesis. For example, IL-4 and IL-13 have been shown to inhibit COX-2 induction in vitro (118). Likewise, preliminary studies from our laboratory suggest that MCP-1, a key CCR2 ligand induced in fibrotic lung, inhibits PGE_2 synthesis when added directly to normal AECs (111). We speculate that AECs from CCR-2–deficient mice overproduce PGE_2 because they are protected from this inhibitory effect of MCP-1.

Possible alterations in AEC COX activity assume additional significance because of their ramifications for net eicosanoid synthesis in the distal lung. It is well accepted that different cell types can interact with each other to generate eicosanoids in ways not predictable from the simple sum of the products of the two cell types individually. One type of cell-cell interaction termed "trans-cellular metabolism" involves one cell donating an eicosanoid intermediate to a neighboring cell. For example, we have demonstrated that AECs and AMs in coculture can exchange free AA. In this scenario, AA released by AMs that would ordinarily be metabolized predominantly to 5-LO metabolites can instead be utilized by the AEC COX pathway for prostanoid synthesis. Alternatively, AA released by AECs that would ordinarily be metabolized predominantly to prostanoids can instead be utilized by the AM 5-LO pathway for LT synthesis. If the AEC COX pathway is blocked by the addition to cocultures of indomethacin, both AEC-derived as well as AM-derived AA are metabolized to LTs (119). Should COX activity be reduced in AECs from fibrotic lung, as it is in AMs and fibroblasts, a further amplification of production of profibrotic LTs would result from transcellular interactions such as these.

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VI. Effect of Eicosanoid Modulation on Experimental Pulmonary Fibrosis

Although the demonstration of LT overproduction in fibrotic lung disease in humans and in animal models is intriguing, such data fail to establish a *causal* role for LTs in the pathogenesis of these fibrotic disorders. More than a decade ago, the first-generation lipoxygenase inhibitor nordihydroguaiaretic acid was shown to markedly attenuate bleomycin-induced fibrosis and, concomitantly, the release of AM-derived fibroblast growth factor activity (120). However, this agent inhibits not just 5-LO but all lipoxygenase pathways, and it also possesses generalized antioxidant activity (121). More recently, dietary γ-linolenic acid was reported to suppress both bleomycin-induced fibrosis and bleomycin-induced elevations of lung LTB_4 content (90). In a similar manner, fatty acids such as γ-linolenic acid may exert anti-inflammatory actions by modulating the synthesis of non–5-LO–derived eicosanoids (122) and by activating PPAR-γ (123).

To more critically and specifically test the hypothesis that products of the 5-LO pathway are capable of playing a causal role in the pathogenesis of fibrotic lung disease, we have recently examined bleomycin-induced fibrosis in 5-LO knockout mice (31). Indeed, as determined both histologically and biochemically, LT-deficient mice exhibited substantially less fibrosis in response to bleomycin than did wild-type mice. Several potential mechanisms for this protection were identified. First, the knockout mice demonstrated a marked reduction in the pulmonary accumulation of all leukocyte subsets following bleomycin. Second, lung mononuclear cells from 5-LO knockout animals elaborated increased amounts of the antifibrotic cytokines IFN-γ and IL-10. Third, the protected LT-deficient mice synthesized increased levels of the antifibrotic eicosanoid PGE_2. These results therefore provide the strongest and most unambiguous evidence to date that 5-LO metabolites can play an important causal role in the pathogenesis of fibrosis.

Like parenchymal pulmonary fibrosis, airway remodeling complicating chronic asthma is characterized by mesenchymal cell proliferation and subepithelial fibrosis in the context of a Th2-type inflammatory process with dysregulated epithelial cell–mesenchymal cell interactions (124). It is of interest, therefore, that subepithelial fibrosis in a mouse model of chronic antigen-induced asthma was markedly attenuated by the continuous administration of a cysteinyl LT receptor antagonist (125).

Experience with prostanoid modulation in animal models of fibrotic lung disease has been conflicting. Continuous administration of the COX inhibitor indomethacin has been shown to protect against bleomycin-induced fibrosis in some studies (126,127). However, a significant worsening of fibrosis was recently observed when indomethacin was administered during the post-inflammatory fibrotic phase following bleomycin administration (117). This

discrepancy is consistent with data indicating that the effects of indomethacin on fibrosis initiated by butylated hydroxytoluene depended on the timing of its administration (128). Indomethacin has also been shown to exacerbate experimental hepatic fibrosis (129). Indomethacin can act in a COX-independent manner (130). Thus, targeted gene deletion may represent a more specific approach to investigating the role of COX metabolites. In this regard, it has recently been reported that COX-2 knockout mice developed an exaggerated fibrotic response to bleomycin (131).

Taken together, these data indicate that COX products have the capacity to either promote or suppress fibrotic responses to lung injury. This is not surprising, since this group of molecules includes not only the predominantly antifibrotic PGE_2 and PGI_2 but also metabolites such as PGD_2 and TxA_2 that may promote Th2 immune responses (132) (and, theoretically, perhaps fibrogenesis). Since it targets prostanoid synthesis nonspecifically, the effects of a COX inhibitor would depend on the specific profile of prostanoids being synthesized at the time it is administered. Moreover, since the actions of PGE_2 will depend on the ambient EP receptor profile on target cells, even a selective PGE synthase inhibitor could have pleiotropic effects. It seems clear that a full understanding of the contribution of specific prostanoids and their receptors to fibrogenesis will require pharmacological or genetic approaches that are much more selective than those that have been studied to date.

VII. Effects on Eicosanoid Synthesis of Commonly Used Therapeutic Agents for Pulmonary Fibrosis

Corticosteroids have long been the mainstay of therapy in IPF and other fibrotic lung diseases. Although no randomized placebo-controlled trials have ever been conducted, it is now quite clear that only 10–20% of patients with IPF demonstrate actual improvement following corticosteroid therapy (133). It is likely that most of those patients who do improve have the histological lesion termed nonspecific interstitial pneumonia; the larger subset of IPF patients with the histological pattern termed usual interstitial pneumonia rarely benefit from corticosteroid therapy (134). The efficacy of corticosteroids in animal models of fibrotic lung disease have generally been disappointing as well (135,136). This may reflect the fact that the effects of corticosteroids on the central pathobiological features of pulmonary fibrosis are mixed. For example, although they inhibit the synthesis of many cytokines, they fail to discriminate in this regard between profibrogenic (e.g., TNF-α) and potentially antifibrogenic (e.g., IFN-γ, IL-10, hepatocyte growth factor) cytokines (137). Moreover, they have conflicting effects on fibroblasts themselves, and actually stimulate fibroblast proliferation in some experimental systems (138).

How do corticosteroids affect eicosanoid biosynthesis? Importantly, corticosteroids are potent inhibitors of the induction of COX-2 (103,139). As COX-2 induction and PGE_2 synthesis in fibroblasts themselves or in neighboring cells represents an important negative-feedback regulator of fibroblast proliferation in response to growth factors and cytokines (84,85), a mechanism which may itself be impaired in pulmonary fibrosis (85,103,104), further interruption of this loop by corticosteroids might dramatically favor fibrogenesis. The inducible isoform of PGE synthase is regulated in a similar manner as is COX-2, and its induction is likewise inhibited by corticosteroids (140). It was recently shown that corticosteroids augmented collagen synthesis by lung fibroblasts via a mechanism that involved decreased endogenous PGE_2 synthesis (141).

With respect to the 5-LO pathway, there is a growing body of evidence indicating that corticosteroids generally fail to inhibit in vivo LT production in patients with asthma (142). This may reflect a release of the usual brake on LT synthesis provided by PGE_2 or the fact that, in vitro, corticosteroids have been shown actually to enhance gene expression of 5-LO and/or FLAP in monocytes (143). *In general, then, corticosteroids tend to exaggerate the imbalance between LTs and PGE_2 that already exists in pulmonary fibrosis*. The degree to which these potentially unwanted effects on eicosanoid biosynthesis limit the therapeutic utility of corticosteroids is unknown.

Immunosuppressive and cytotoxic agents are also commonly used in the treatment of fibrotic lung disorders. A limited amount of information is available regarding the effects of these agents on eicosanoid synthesis, but little of this is based on studies of lung cells. Azathioprine has been shown to inhibit prostanoid production by peritoneal macrophages (144), and methotrexate has been reported to have similar activity in both peritoneal macrophages (145) and synoviocytes (146). Cyclophosphamide has been reported to stimulate LTB_4 generation by lung fibroblasts (147) and to amplify peritoneal macrophage synthesis of cysteinyl LTs as well as PGE_2 by peritoneal macrophages (148). If applicable to relevant lung cells, these inhibitory effects on prostanoids and stimulatory effects on LTs might be expected, as suggested above for corticosteroids, to exaggerate the eicosanoid imbalance that already exists and that favors fibrogenesis. Colchicine is a microtubule-destabilizing agent that has been utilized in the treatment of IPF, albeit with limited evidence supporting a beneficial effect. It is nevertheless interesting that, in vitro, this agent has been shown to inhibit AM LT synthesis (149).

VIII. Role of Eicosanoids in the Pathogenesis of Pulmonary Fibrosis: An Integrated Model

Although our understanding of the pathobiology of pulmonary fibrosis is rudimentary at best, it is clear that it represents an aberrant response to injury

involving dysregulated cell-to-cell interactions. Although many types of molecules and mediators have been the focus of interest in investigations of fibrotic lung disease, eicosanoids have received comparatively little attention. However, the information presented in this chapter makes a case for eicosanoids being worthy candidates to participate in such interactions. A proposed model for their involvement in the pathogenesis of fibrotic lung disease is presented in Figure 2, and will be discussed briefly below. This model incorporates into a general pathobiological scheme those derangements in eicosanoid production by AECs, AMs, and fibroblasts that have been discussed previously.

Under normal homeostatic conditions, AMs and fibroblasts remain unactivated and a suppressive state is fostered by constitutive PGE_2 elaboration by AECs, as well as by AMs and fibroblasts. An injurious event results in dysfunction or loss of AECs and concomitant activation of AMs. An important consequence of AEC dysfunction/loss is diminished capacity for PGE_2 secretion, and this has a permissive effect on AM activation, promoting the generation of proinflammatory and profibrotic substances such as TNF-α, MCP-1, IL-8, TGF-β, and LTs and reducing AM synthesis of PGE_2. Injured AECs may themselves now secrete proinflammatory/profibrotic mediators, including MCP-1, IL-8, and TGF-β. A milieu characterized by the lack of PGE_2 along with the presence of potential activation signals results in fibroblast activation. If reconstitution of the epithelium (with a recovery of PGE_2 synthetic capacity), control of AM activation, and appropriate fibroblast induction of COX-2 (with resultant increased endogenous capacity for PGE_2 synthesis) occur, repair can take place. However, if there is an inadequate degree of epithelial recovery, of AM deactivation, or of fibroblast COX-2 induction, fibrosis ensues. The profibrotic effects of LTs would also be magnified under conditions of relative PGE_2 deficiency.

IX. Therapeutic Strategies to Alter Eicosanoid Synthesis or Actions

In view of the available data implicating eicosanoids in the pathobiology of fibrotic lung disease, modulation of their tissue concentrations or actions represent appropriate therapeutic targets in these disorders. Although no clinical trials of eicosanoid modulators have been performed in patients with pulmonary fibrosis, three generic strategies can be considered.

The *first generic strategy* is inhibition of the synthesis of a potentially profibrotic eicosanoid, and antagonism of its receptor is the *second generic strategy*. LTs are the obvious candidates here. The key question is whether the therapeutic goal in fibrotic lung disease ought to be inhibition of the synthesis or action of a single class of 5-LO metabolites or of all such products, including

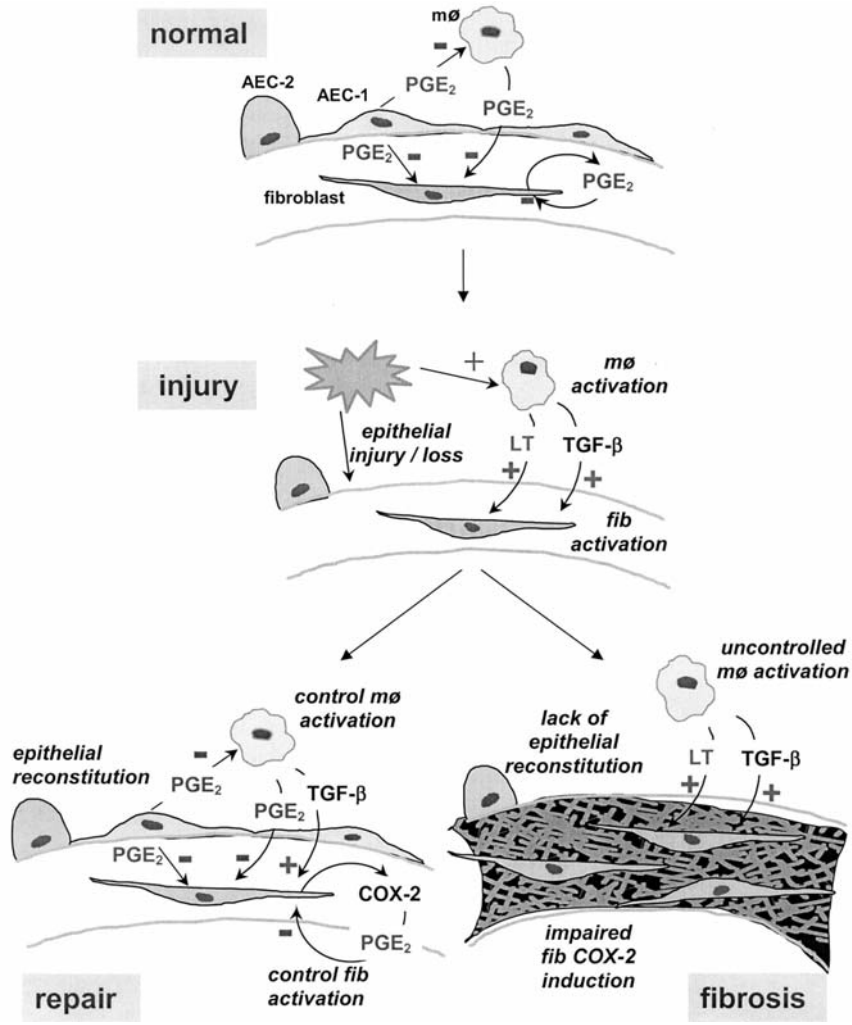

Figure 2 Model for the role of eicosanoids in the pathogenesis of pulmonary fibrosis. Under normal conditions, PGE_2 elaboration by type I (AEC-1) and type II (AEC-2) AECs, macrophages (mϕ), and fibroblasts (fib) contribute to the maintenance of lung suppression of fibrogenesis. Lung injury results in epithelial cell loss or damage, with impaired capacity for PGE_2 generation, as well as mϕ activation to produce LTs and growth factors; these events promote fibroblast activation. If the epithelium can be reconstituted and if fibroblast COX-2 can be appropriately induced, PGE_2 elaboration is sufficient to control mϕ and fibroblast activation, and repair can take place. If these critical responses are impaired, mϕ and fibroblast activation are unchecked and fibrosis develops. – indicates inhibition; + indicates stimulation.

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LTB_4 as well as cysteinyl LTs. Both cysteinyl LT receptor 1 antagonists (zafirlukast, montelukast, and pranlukast) as well as the 5-LO enzyme inhibitor, zileuton, are currently available for the treatment of asthma. LTB_4 receptor antagonists are currently under development. There are three theoretical reasons for favoring a 5-LO (or FLAP) inhibitor as a therapeutic candidate for the treatment of pulmonary fibrosis. First, both classes of LTs are overproduced in IPF, and both have biological actions that are relevant to the pathogenesis of fibrosis. This contrasts with the situation in asthma, where most of the evidence points to cysteinyl LTs as the predominant relevant 5-LO metabolite. Second, there are two receptors for both LTB_4 and cysteinyl LTs, and it is as yet unclear what the relative roles in fibrotic disease might be for any one of these multiple receptors. Third, in vivo inhibition of LT biosynthesis from AA would be expected to result in shunting of AA to COX-derived products, thereby increasing the generation of anti fibrotic prostanoids such as PGE_2, PGI_2, or 15-deoxy-PGJ_2. This approach, therefore, affords the potential for both a reduction in the synthesis of profibrotic eicosanoids and an increase in synthesis of antifibrotic eicosanoids, thereby reversing the eicosanoid imbalance that exists in pulmonary fibrosis. In fact, a trial of zileuton pharmacotherapy is currently underway at our institution for patients with newly diagnosed and previously untreated usual interstitial pneumonia.

Synthesis of profibrotic LTs could also be abrogated by blocking the initial release of AA from membrane phospholipids. This could be accomplished either by the inhibition of PLA_2-mediated deacylation or by reducing the arachidonate content of phospholipids via dietary modifications of fatty acid intake. The latter can be accomplished by supplementation with ''fish oil'' diets rich in n-3 fatty acids such as eicosapentaenoic acid. The theoretical limitation of a strategy which reduces the levels of available free AA is that synthesis of PGE_2 and other potentially protective prostanoids would be expected to be reduced along with potentially deleterious 5-LO metabolites. On the other hand, if the production of profibrogenic LTs greatly exceeds the production of antifibrogenic PGs, the nonspecificity of inhibiting this most proximal step may not be problematic. Indeed, dietary modification of fatty acids has been reported to inhibit bleomycin-induced fibrosis (90).

The *third*, and final, *generic strategy* involves administration of, or upregulating the synthesis of, a potentially beneficial eicosanoid with antifibrotic actions. The best candidate for such a strategy would be PGE_2 given its suppressive actions on multiple aspects of disease pathogenesis. Because systemic administration has adverse effects on vasoregulation, targeted administration to the lung is preferable. Such a strategy is feasible, since inhalation of exogenous PGE has been shown to increase the lung lavage levels of this prostanoid in animals (101). As discussed above, selective EP2 and/or EP4 agonists might confer more specificity of response than would

PGE_2 itself, since these receptors are coupled only to increases in cyclic AMP to the exclusion of potentially opposing signals such as increased intracellular calcium (EP1) or decreased cyclic AMP (EP3). This same inhalational strategy could be applied to other eicosanoids with potentially antifibrotic effects, such as PGI_2 or PPAR-γ agonists. Indeed, chronic administration of aerosolized PGI_2 or its more stable analogues is a well-established approach for the treatment of pulmonary vascular disease (150), in which mesenchymal cell proliferation and matrix protein deposition results in vascular remodeling. Finally, the desired increase in cyclic AMP consequent to administration of PGE_2 or PGI_2 could be amplified in duration and magnitude by coaerosolization of an inhibitor of phosphodiesterase, the enzyme responsible for cyclic AMP degradation. Coadministration of PGI_2 and the phosphodiesterase inhibitor rolipram resulted in synergistic beneficial effects of these two agents in an animal model of pulmonary hypertension (151).

Strategies which might result in increased expression of COX, PGE synthase, or EP receptor proteins represent potentially longer lived means of increasing PGE_2 synthesis or actions in vivo. These could entail either gene therapy approaches or administration of other agents known to upregulate gene expression of these enzymes. Finally, it is obvious that combination strategies consisting of inhibiting a profibrotic eicosanoid while increasing levels of an antifibrotic eicosanoid (or its receptor) might have additive benefit.

One further therapeutic question is worthy of consideration; namely, could the coincidental use of nonsteroidal anti-inflammatory agents for the treatment of other conditions, by inhibiting the synthesis of antifibrotic prostanoids, promote the development of, or increase the severity of, pulmonary fibrosis in an individual who is otherwise susceptible? Based on animal data with COX inhibitors or COX knockout mice, it is certainly plausible to consider that either nonselective or COX-2–selective inhibitors could have this undesired effect. It must be acknowledged, however, that no such data in patients are available. However, it is certainly the case that many patients with rheumatological diseases who are susceptible to developing pulmonary fibrosis take these agents, and no data are available on the relative incidence of fibrosis in those who do and do not take these agents. Also, many patients take over-the-counter COX inhibitors without ever disclosing this to their physicians. For these reasons, it would be premature to dismiss this possibility out of hand.

X. Conclusions

Although eicosanoids have long been implicated as important players in the pathogenesis of asthma, pulmonary hypertension, and other lung diseases, they

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have received little attention as possible participants in the pathogenesis of fibrotic lung disease. A growing body of information, reviewed in this chapter, indicates that an imbalance of eicosanoid synthesis (overproduction of LTs and relative underproduction of prostanoids, especially PGE_2) exists in pulmonary fibrosis and may contribute to the dysregulated cell-to-cell interactions thought to underlie fibrotic responses to injury. Particularly compelling is the fact that LTs promote and prostanoids inhibit so many of the key pathobiological elements in fibrogenesis — leukocyte recruitment and activation, a Th2-type immune response, and virtually every relevant aspect of fibroblast function. A central concept is that eicosanoids do not act alone, but interact with many other mediators implicated in the pathogenesis of fibrosis. Moreover, these interactions are bi-directional, as eicosanoids and other mediators (e.g., cytokines) each modulate the production of the other. Finally, because of pharmacotherapeutic advances in the eicosanoid field driven by investigations into other diseases, such as asthma and pulmonary hypertension, therapeutic agents and strategies are available that can be readily adopted for application to pulmonary fibrosis. This is a critical advantage in a disease that desperately requires novel pathogenetic insights that can rapidly be translated into new therapies.

The last decade had witnessed a remarkable series of basic advances in our understanding of the synthesis and actions of eicosanoids. Additional basic insights and creative translational efforts will pave the way for a greater appreciation of the importance of eicosanoids in fibrotic lung disease and their utility as therapeutic targets.

Acknowledgment

Research in the author's laboratory supported by National Institute of Health grants P50 HL56402 and ROI HL58897.

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18

Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Pulmonary Fibrosis

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I. Introduction

The extracellular matrix (ECM) is a dynamic structure that not only constitutes the tissue scaffold but, importantly, regulates cell behavior by modeling effective cellular environments and by instructing cellular phenotype. Moreover, the ECM also actively participates in the presentation of a wide variety of growth factors. In this context, the tightly controlled turnover of ECM is critical for maintaining lung structure and function. Lung fibrosis, a process characterized by an aberrant ECM remodeling, can be conceptualized as resulting from an imbalance in the equilibrium of synthesis and degradation of ECM molecules. A large number of enzymes are involved in extracellular matrix remodeling, primarily the family of matrix metalloproteinases (MMPs) which are able to cleave the various components of the ECM.

II. Matrix Metalloproteinases

Considerable evidence implicates MMPs as playing essential roles in many biological processes, yet the actual mechanisms underlying their influence are mostly uncertain. Importantly, mechanisms do not only include the degradation of ECM molecules, but also the modulation of the activity of growth factors and cytokines either by direct cleavage or releasing them from ECM bound stores. Therefore, the modification of the ECM microenvironment may result in a number of changes in cellular behavior; for example, facilitating cell migration, promoting cell growth, or inducing apoptosis. The comprehension

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of these processes should result in a more rational approach toward reducing the pathological effects of MMPs in disease while preserving their indispensable and beneficial functions.

A. MMP Family and Putative Substrates

Matrix metalloproteinases, or matrixins, are a family of extracellular matrix–degrading proteinases that share common functional domains. In the last years, it has become evident that in addition to their extracellular matrix substrates, MMPs also cleave cell surface molecules and other nonmatrix substrates, regulating cell behavior in several ways (1,2).

Currently, 22 human family members have been identified in addition to some other vertebrate members with no human homologues found until now (3,4). Matrix metalloproteinases receive progressing MMP numbers according to their discovery, and some are also known by their most used common names (1–4). According to structural and functional characteristics, human MMPs have been classified into six different subgroups of closely related members with rather distinctive but often overlapping substrate specificities: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs (MT-MMPs), and other MMPs (Table 1).

Almost all MMPs can cleave or degrade some ECM proteins; however, it is important to note that only a small fraction of more than 100 known macromolecular components of the ECM have been examined as potential substrates (3). Additionally, from the numerous candidates that have been tested and identified as substrates in vitro (see Table 1), only very few have been corroborated in vivo. Among them are the interstitial collagens where it has been demonstrated that the degradation products found in vivo have the exposed amino acid ends equivalent to those recognized in vitro for some MMPs (5). Collagenases, a subgroup that includes MMP-1, MMP-8, and MMP-13, can generate these cleavages in native fibrillar collagens (types I, II, III) within the triple helical domain. For many years, it was believed that interstitial collagen breakdown was an exclusive function of collagenases. However, a growing body of evidence has demonstrated that gelatinase A (MMP-2) and MT-1MMP (MMP-14) may also cleave fibrillar collagens (6–8).

On the other hand, MMPs can process a variety of nonmatrix proteins. For instance, osteopontin, a protein that is often colocalized with MMPs and that functions in wound healing, inflammation, and tumor progression is a substrate for stromelysin 1 (MMP-3) and for matrilysin (MMP-7) (9). The cleavage by these enzymes enhances the adhesive and migratory stimulus of this protein (9). In addition, several MMPs can modulate the activity of a number of growth factors by diverse mechanisms (1). For example, transforming growth factor-β (TGF-β), the typical profibrotic growth factor, has strong affinity for matrix components, and it has been demonstrated that decorin

Table 1 Human Matrix Metalloproteinases Family

Subgroup	MMP	Other names	Matrix and other substrates
Collagenases	MMP-1	Collagenase-1	Collagens I, II, III, VII, X, gelatin, entactin, aggrecan, tenascin,
	MMP-8	Collagenase-2	α1-proteinase inhibitor, pro–TNF-α, α_1-antichymotripsin
	MMP-13	Collagenase-3	
Stromelysins	MMP-3	Stromelysin-1	Proteoglycans,laminin, fibronectin, gelatin, fibrinogen, entactin,
	MMP-10	Stromelysin-2	pro–IL1-β, pro-EGF, pro–TNF-α, α_1-proteinase inhibitor, IGFB
	MMP-11	Stromelysin-3	
Gelatinases	MMP-2	Gelatinase A	Collagen IV,V, gelatin, elastin, decorin, fibronectin,
	MMP-9	Gelatinase B	pro–TGF-β, pro–IL1-β, pro–TNF-α
Matrilysins	MMP-7	Matrilysin-1	Laminin, prodefensin, osteopontin, decorin, gelatin, fibronectin,
Minimal domain	MMP-26	Matrilysin-2/ endometase	collagens III, IV, V, IX, X, XI, fibrinogen, proteoglycans
MT-MMPs	MMP-14	MT1-MMP	Gelatin, fibronectin, vitronectin, collagen IV, proMMP-2,
Membrane-type	MMP-15	MT2-MMP	cell surface–bound CD44, cell-bound tissue transglutaminase
MMPs	MMP-16	MT3-MMP	
	MMP-17	MT4-MMP	
	MMP-24	MT5-MMP	
	MMP-25	MT6-MMP/leukolysin	
Other MMPs	MMP-12	Metalloelastase	Elastin, plasminogen, fibronectin, laminin gelatin,
	MMP-19	RASI	tenascin, fibronectin Amelogenin, aggrecan
	MMP-20	Enamelysin	
	MMP-23	CA-MMP	
	MMP-27	Epylisin	
	MMP-28		

IGFB, Insulin growth factor beta; CA, cysteine array.

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cleavage by MMP-2, -3, or -7 can release matrix-bound TGF-β (10). But additionally, the gelatinases MMP-2 and MMP-9 can directly process TGF-β into its active form. In this context, it has been shown that MMP-9 localization to the surface of normal keratinocytes is CD44 dependent and can activate latent TGF-β (11).

By affecting matrix and nonmatrix substrates, MMPs can regulate apoptosis. For instance, degradation of basement membrane brings as a consequence the loss of cell survival signals which has been proposed to motivate apoptosis in the involuting mammary gland (12). Likewise, the generation of soluble Fas ligand by MMP-7 appears to be important for epithelial cell apoptosis during prostate involution (13).

There is general agreement that MMPs are necessary in the implementation of cell migration. This complex process involves cell-to-cell and cell-to-matrix attachments, matrix remodeling, and the presence of chemotatic factors to guide migration. Matrix proteolysis as MMP-2–dependent cleavage of laminin-5 has been shown to induce epithelial cell migration (14). Likewise, MT1-MMP (MMP-14) promotes cell migration and is localized at the migration edge in many migratory cells, including invasive cancer cells (15). MT1-MMP plays an important role during the process of neovascularization, because its activity can modulate endothelial migration, invasion, and formation of capillary tubes during the angiogenic response (16).

B. Domain Structures of MMPs

The primary structures of MMPs share in addition to their conserved zinc-binding site in the catalytic domain added stretches of sequence homology that gives them a relatively conserved structure (3,17). However, none of the MMPs has all the possible building compact units. As illustrated in Figure 1, starting from the N-terminus, the following features of domain organization are observed:

Predomain

The majority of the MMPs have an amino-terminal signal peptide containing 18–30 residues rich in hydrophobic amino acids that destines the synthesized polypeptide to the endoplasmic reticulum where this signal is removed. The majority of the MMPs are secreted to the extracellular space, although some of them are expressed as cell surface enzymes.

Propeptide Domain

The propeptide domain follows the predomain and keeps the zymogens inactive until they are activated by proteolysis. In general, the prodomain comprises about 80 residues, and in almost all MMPs, contains a highly

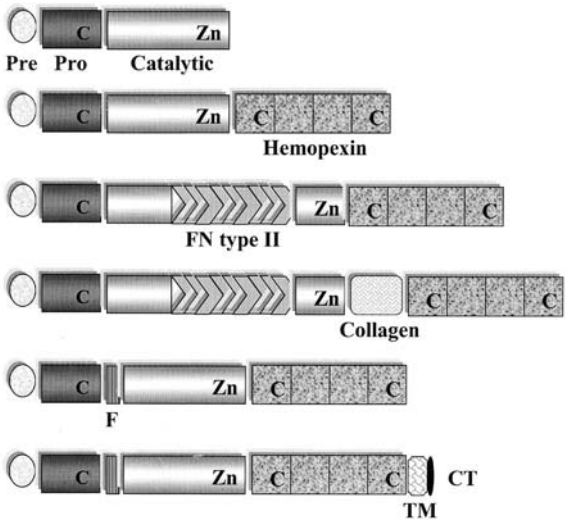

Figure 1 Domain structure of the MMPs: Pre, signal sequence; Pro, propeptide with a free zinc-ligating thiol group; Zn, zinc-binding site; F, furin-susceptible site; CT, cytoplasmic tail; TM, transmembrane domain; FN type II, fibronectin type II domain; collagen, type V domain; C, conserved cysteine.

conserved sequence PRCXXPD near the C-terminal end of this segment. A critical cysteine residue of this domain makes contact with the catalytic zinc atom and maintains the enzyme in its latent form. The cleavage of the propeptide domain produces a conformational change that modifies the proenzyme to its active form.

Furin Cleavage Site

The furin cleavage site is a domain containing about nine amino acids and it is present in 40% of the known MMPs. Its consensus sequence RXKR between their procatalytic and catalytic domains leads to activation by alternative intracellular cleavage by furin.

Catalytic Domain

The catalytic domain has a conserved zinc-binding site with the sequence HEXXHXXGXXH, where Zn binds three conserved histidine (H) residues. In an inactive state, the conserved cysteine residue in the prodomain provides the fourth coordination site for the catalytic zinc ion. This domain determines the specificity for the cleavage site, and data obtained at the structural and molecular level has been used to design synthetic inhibitors of MMP

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activity, some of which have been used in clinical trials for different human diseases (18,19).

Fibronectin and Type V Collagen Domain

Gelatinases A and B (MMP-2 and MMP-9) are distinguished by containing three type II fibronectin domains intercalated within their catalytic domains. Gelatinase B, the largest MMP, has also a unique type V collagen–like insert. It has been proposed that these structures may regulate enzyme interactions with substrates like elastin and gelatin (20,21).

Hemopexinlike Domain

The hemopexinlike domain is a domain of about 200 residues that contains four repeats with a Cys residue at either end. This C-terminal domain has homology to a serum protein called hemopexin and is connected to the catalytic domain by a linker region. It is present in almost all MMPs with the exceptions of matrilysins 1 and 2 (MMP-7 and MMP-26) and MMP-23 that contains a C-terminal domain of only 100 residues that lacks any significant similarity with hemopexin (22). Matrilysins 1 and 2 lack this domain, probably due to a deletion rather to an evolutionary origin prior to the addition to hemopexin (3).

Transmembrane and Cytosolic Domains

Four membrane-type (MT) MMPs (MT1, MT2, MT3, and MT5-MMP [MMP-14, -15, -16, and –24]) have a sequence motif that controls the insertion of these enzymes into the cell membrane. This domain ranges from 80 to 110 residues and includes a short segment within the cytoplasm. The other two currently known MT MMPs (MT4-MMP and MT6-MMP [MMP-17 and -25, respectively]) have C-terminal hydrophobic extensions that act as a glycosyl-phosphatidyl inositol membrane anchoring (24–26).

Besides to a common domain structure, the presence of gene clusters in some chromosomes suggests that they arise from duplications of an ancestor gene. Eight of the known human MMP genes (MMP-1, -3, -7, -8, -10, -12, -13, and -20) are clustered on chromosome 11 at 11q21-23. Other known MMP genes are dispersed along chromosomes 1, 8, 12, 14, 16, 20, and 22 (3,22).

C. Regulation of MMP Activity

MMP levels are usually low in normal adult resting tissues, and with some exceptions, their production and activity are maintained at virtually undetectable levels. By contrast, their expression becomes elevated when there is a challenge to the system, such as wound healing, repair or remodeling processes, in diseased tissues, and even in several cell types grown in culture (2).

MMPs are tightly regulated at the transcriptional and posttranscriptional levels as well as at the protein level through activators, inhibitors, and their cell surface localization.

D. Transcriptional Regulation

Most MMPs are strictly regulated at the level of transcriptional activation of the gene and promoter elements through a variety of stimulatory and suppressive factors that influence multiple signaling pathways (27). For example, the expression of various MMPs can be upregulated or down-regulated by integrin-derived signals, extracellular matrix proteins, physical stress, and changes in cell shape (2). Furthermore, MMP expression is modulated by numerous cytokines and growth factors, including interleukins and interferons, TGF-β, epidermal growth factor (EGF), keratinocyte growth factor (KGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), and the extracellular matrix metalloproteinase inducer EMMPRIN (basigin or CD147) (27,28). Many of these stimuli induce the expression and/or activation of c-*fos* and c-*jun* proto-oncogene products, which heterodimerize and bind activator protein-1 (AP-1) sites within several MMP gene promoters.

In some cases, a differential regulation has been found. For example, TGF-β, a growth factor widely assumed to be inhibitory for MMPs, suppresses the transcription of the MMP-1 (collagenase-1) and MMP-3 (stromelysin-1) genes, but induces the expression of MMP-13 (collagenase-3) in human fibroblasts (29).

Some *cis*-regulatory elements affect MMP gene expression depending on their proximity to one another in the gene promoter. AP-1 sites act synergistically with adjacent Ets-binding sites in genes such as MMP-1 but not in others such as MMP-13 (30). Other putative regulatory elements have been also identified within various MMP gene promoters. These include TGF-β inhibitory elements in several MMP genes, and AP-2, Sp1, Sp3, NF-B, CCAAT/enhancer-binding protein-β, or retinoic acid response elements that are also found in several MMP genes (2,27). Although important progress in the knowledge of the mechanisms involved in MMP gene regulation has been achieved, the cross-talk between the many signaling pathways, nuclear factors, and gene regulatory elements that modulate the expression of these enzymes are scarcely understood.

MMP gene expression can also be affected by genetic polymorphisms that may, in turn, influence the development or progression of several diseases. Recently, naturally occurring sequence variation has been detected in the promoter of a number of MMP genes. These polymorphisms have been shown to have allele-specific effects on the transcriptional activities of MMP gene promoters, and to be associated with susceptibility to a number of human

diseases including coronary heart disease, aneurysms, and cancers (31). For example, an MMP-1 single nucleotide polymorphism in the promoter generates a functional Ets-binding site immediately adjacent to an AP-1 site that increases transcriptional levels of this collagenase. (32).

E. Posttranscriptional Regulation

MMP expression can also be influenced by posttranscriptional mechanisms. For example, MMP-1 and MMP-3 mRNA transcripts are stabilized by EGF, whereas MMP-13 transcripts are stabilized by PDGF and glucocorticoids and destabilized by TGF-β (2,33). The turnover of MMP-1 mRNA is apparently regulated by AU-rich sequences in the 3′ untranslated region, and similar sequences may also regulate the stability of other MMP transcripts. In addition, alternative mRNA splicing of MT3-MMP generates a soluble and proteolytically active form of the enzyme, whereas the multiple transcripts of MMP-13, MMP-17, and MMP-20 probably result from alternative polyadenylation (2).

F. Activation of Latent Matrix Metalloproteinases

MMPs are first synthesized as inactive proenzymes or zymogens, and their latency is maintained by an unpaired cysteine sulfhydryl group near the C-terminal end of the propeptide domain (34). Once displaced after proteolysis, the thiol group is replaced by a water molecule that can then attack the peptide bonds of MMP targets.

Although most MMPs are secreted as latent zymogens, there are several exceptions. MMP-11 (stromelysin-3), MMP-27 (epilysin), and the MT-MMPs contain a furinlike motif between their procatalytic and catalytic domains and thus can be activated directly inside the cell by members of the proprotein convertase family.

On the other hand, it has recently been suggested that although activation of all known matrix metalloproteases in vitro is accomplished by proteolytic processing of the propeptide, in the case of gelatinase B, other mechanisms seem to operate. In this case, binding to a ligand or to a substrate may lead to a detachment of the propeptide from the active center of the enzyme causing its activation (35).

The extracellular activation of most MMPs can be initiated by other already activated MMPs or by several serine proteinases that can cleave peptide bonds within MMP prodomains (3). However, MMP-2 is unable to be activated by serine proteinases and is instead activated at the cell surface through a unique multistep pathway involving MT-MMPs and tissue inhibitor of MMPs 2 (TIMP-2). In the activation step, the N-terminal domain of TIMP-2 binds to MT1-MMP on the cell surface and the bound TIMP-2 acts as a receptor for proMMP-2. Subsequently, adjacent TIMP-2–free MT1-MMP

activates the proMMP-2 in a ternary complex. MT1-MMP forms a homophilic complex through the hemopexinlike domain that acts as a mechanism to keep MT1-MMP molecules close together to facilitate proMMP-2 activation (36).

Processing of proMMP-2 by MT1-MMP and claudin-5 without TIMP-2 involvement has recently been reported (37). Expression of claudin-5, a major component of endothelial tight junctions replaced TIMP-2 in proMMP-2 activation by MT1-MMP, and also promoted activation of proMMP-2 mediated by all MT-MMPs (37).

III. Tissue Inhibitors of Metalloproteinases

The control of MMPs once they are active either in the extracellular space or membrane bound depends on a number of general inhibitors including a specific and closely related family of homologous proteins referred to as tissue inhibitor of metalloproteinases (TIMPs). There are currently four members of this family, TIMP-1 to TIMP-4, which have in common their matrix metalloproteinases inhibitory action. However, they differ in expression patterns, and other properties such as proMMP activation, cell growth and cell survival–promoting activity, matrix binding, inhibition of angiogenesis, and induction of apoptosis (3,38–40).

A. Structure

The TIMPs are small secreted proteins of about 20–29 kD which present some common structural characteristics and that reversibly inhibit the MMPs in a 1:1 stoichiometric fashion. One of the common features is the presence of 12 similarly separated conserved cysteine residues. These invariant cysteines form intrachain disulfide bonds folding the proteins into two domains: the large N-terminal domain and the small C-terminal domain. Each domain contains three disulfide bridges, which make the TIMPs quite stable. The two regions of the TIMP molecules are separated by a two-residue isthmus at residue 125–126 in human TIMP-1 and a single residue at a position between 121 and 128 in the other three TIMP species (3). Overall, the primary structures of the four TIMPs show a 35–50% homology among them.

Their MMPs' inhibitory action is found in the amino-terminal domain. However, although the carboxy-terminal domain has no inhibitory activity, it appears to enhance the binding of some TIMPs to MMPs, as in the case of TIMP-1 with stromelysin, by providing further contact and by orientating the inhibitory end of the TIMPs toward the active center (41). Each of the two terminal domains is held in a relatively rigid conformation by three disulfide bridges, which are highly conserved across all species of TIMPs and organisms. The first cysteine after the signal peptide is critical for the binding of TIMPs to zinc (3).

B. Biological Roles

Although inhibition of proteolytic MMPs' activity appears to be the major task of TIMPs, a surprising feature of them is their multiplicity of functions. Furthermore, although TIMPs bind tightly to most MMPs, they exhibit some differences in their inhibitory properties. Thus for example, TIMP-2 and TIMP-3, unlike TIMP-1, are effective inhibitors of the membrane-type MMP subfamily. As mentioned, TIMP-2 has a specific role in the binding of progelatinase A to MT1-MMP as a prerequisite to the activation of MMP-2 (40). Likewise, TIMP-4, unlike TIMP-2, does not promote proMMP-2 activation by MT1-MMP. However, TIMP-4 binds to MT1-MMP, inhibiting its autocatalytic processing, and when coexpressed with TIMP-2, TIMP-4 competitively reduced proMMP-2 activation by MT1-MMP (42).

Non–MMP-Inhibitory Functions of TIMPs

In the last years, it has been evident that TIMPs have a number of effects on cell behavior, and interestingly, most of them seem to be mostly independent of their MMP-inhibitory activity.

The first TIMP associated with other functions than MMP inhibition was TIMP-1. Shortly after being cloned and sequenced, it demonstrated its potent growth effect on human keratinocytes and several other cell types, and similar findings were reported for TIMP-2 (38,40,43). This function may have different tissue consequences and may influence a number of physiological and pathological processes. Thus, for example, TIMP-1 expression that is increased in patients with malignant non-Hodgkin's lymphoma and correlates with its clinical aggressiveness is an autocrine and paracrine survival factor that inhibits induced cell death in Hodgkin/Reed-Sternberg cells (44).

TIMP-3 has further unique properties. First, it is the only TIMP that binds tightly to the extracellular matrix, probably through the interaction of the amino-terminal domain with heparan sulfate and chondroitin sulfate on the cell surface or with secreted proteoglycans (45). TIMP-3 is also capable of inhibiting members of two groups of enzymes within the adamalysin family: the ADAMs (*a d*isintegrin and *a m*etalloproteinase domain) and the ADAMTSs (ADAM with *t*hrombo*s*pondin–like repeats). Examples include the enzyme TACE (TNF-cleaving enzyme, ADAM-17, [46]), and the members of the ADAMTS group responsible for aggrecan degradation in cartilage (47).

In contrast to the effects of TIMP-1 and -2 that promote cell survival, one of the most interesting effects of TIMP-3 is its ability to initiate cell apoptosis. This effect is related to the inhibition of the shedding of TACE and the stabilization of TNF-α receptors on the cell surface (48). The apoptotic effect of TIMP-3 relies on the N-terminal domain, and interestingly, mutation of the Cys1 residue destroys this effect, indicating that the TIMP-3 must be in a form that can inhibit proteases (49).

TIMP-4 was cloned recently (50). Like other members of the TIMP family, the TIMP-4 protein is encoded by five exons and the exon-intron boundaries are at locations very similar to those of the other TIMP genes, demonstrating the high degree of conservation of gene structure in this family. The human and mouse TIMP-4 genes map to comparable locations in the respective genomes, localizing to human chromosome 3p25 and mouse chromosome 6.

TIMP-4 plays a role in extracellular matrix homeostasis in a tissue-specific fashion, and like TIMP-3, its upregulation induces programmed cell death (48). Evidence obtained in cardiac fibroblasts suggests that TIMP-4 controlled fibroblast transformation induced by polyomavirus and instigated apoptosis in transformed cells (51).

The local relationship between some MMPs, primarily MMP-1, MMP-2, and MMP-9, and TIMPs also plays a critical role in angiogenesis. Capillaries are surrounded and supported by a collagen-rich extracellular matrix, and the formation of new vessels requires a differential regulation of MMPs and TIMPs. Actually, some of the most convincing evidence supporting the requirement of MMP activity during the process of angiogenesis comes from the influence of TIMPs and other inhibitors. Thus, cartilage-derived TIMP was shown to be a strong inhibitor of embryonic neovascularization, whereas TIMP-2 was demonstrated to inhibit basic fibroblast growth factor–driven capillary endothelial cell proliferation (52,53).

In addition, the TIMPs differ in terms of their gene regulation and tissue-specific patterns of gene expression. Relevant for this discussion, a recent report evaluated the differential expression of all the four TIMPs in normal and aberrant wound healing, including chronic venous ulcers and ulcerative vasculitis (54). Expression of TIMP-1 and -3 was found in proliferating keratinocytes of normal healing wounds, whereas no epidermal expression was detected in chronic ulcers. Both TIMPs were also abundantly expressed by fibroblasts, macrophages, and endothelial cells, suggesting that they may be involved both in the regeneration of the epidermis by stabilizing the basement membrane zone and in the regulation of extracellular matrix remodeling and neovascularization. In normally healing wounds, TIMP-2 localized under the migrating epithelial tip more frequently than in chronic ulcers, suggesting that its lack might contribute to uncontrolled activity of MMP-2 in chronic ulcers. In general, this study suggests that TIMPs are temporally and spatially firmly regulated and that the imbalance between matrix metalloproteinases and TIMPs may lead to delayed wound healing.

In summary, MMPs are multifunctional and strictly regulated enzymes that appear to be implicated in numerous biological processes. However, their complex, various, and somehow overlapping effects often may be difficult to understand fully how MMPs function in vivo.

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IV. MMPs and TIMPs in Fibrotic Lung Remodeling

Several members of the MMPs and TIMPs families may participate in the pathogenesis of pulmonary fibrosis in at least four interrelated processes: extracellular matrix (ECM) remodeling, basement membrane disruption/epithelial cell apoptosis, cell migration, and angiogenesis. On the other hand, an additional and unexplored role of matrix remodeling by MMPs in lung fibrosis is through the release after matrix cleavage of matrix-bound growth factors (55). As mentioned, TGF-β, a prototype of profibrotic factor, is one of the mediators that can be released and/or activated by several MMPs (10,11).

A. Extracellular Matrix Accumulation and Remodeling: A Crucial Role for Interstitial Collagenases and TIMPs

After injury, a dynamic controlled remodeling of ECM, reflecting the net outcome of synthesis and degradation of matrix components, is essential for wound healing to provide strength and temporary structure. However, if this process is not properly regulated, it can be detrimental. In this context, the final common feature of any fibrotic lung disorder is the abnormal accumulation of ECM, including fibrillar (i.e., types I and III) collagens, fibronectin, elastic fibers, and proteoglycans (56,57). The ultimate consequence is an extensive structural disorganization in the lung microenvironment where alveolar-capillary units are lost and replaced by scarring, bronchiolization of alveoli, and honeycombing.

For many years, research in this area was mainly focused on the synthesis of the ECM molecules and on the topography of deposit. However, recent work performed in several types of human fibrotic lung diseases and in experimental models strongly supports that many of the mechanisms behind this severe architectural remodeling involve an uncoordinated regulation and expression of both the MMP and TIMP families (58–69).

It is important to emphasize, however, that studies on the expression of interstitial collagenases, the enzymes responsible for degrading fibrillar collagens, and TIMPs in experimental models and human fibrotic lung disorders have given contradictory results. In general, a progressive increase of TIMP-1 with no significant changes in MMP-13, the prevalent collagenase expressed in mice and rats, has been found in bleomycin- and silica-induced lung fibrosis (64–66). By contrast, in human lung fibrosis, studies regarding the transcriptional behavior of lung genes through cDNA microarray analysis as well as the local morphological expression have invariably shown a strong upregulation of the interstitial collagenase MMP-1 (59–60,70). These results should be interpreted cautiously and could indicate a number of differences including a diverse type of collagenases expressed in mammal species damage temporality (acute/chronic) and more importantly, type of lung disorders.

In this sense, it is important to emphasize that a bleomycin or silica experimental model is stereotypic and simplistic and does not represent the complexity of pulmonary fibrosis, primarily IPF. Moreover, we are far from understanding the biological consequences of the cleavage of matrix and nonmatrix substrates that result in an abnormal repair. In this context, the notion that an MMP is involved in any pathological event is generally based on the strength of its association and the existence of reasonable mechanisms related with its known in vitro functions.

Still, the finding of an overexpression of interstitial collagenase in IPF lungs where the main characteristic is the excessive accumulation of fibrillar collagens can be considered to be a paradox. Besides, this enzyme has been strongly implicated in diseases characterized by exaggerated ECM degradation, such as rheumatoid arthritis and lung emphysema (71,72).

However, an interesting observation and possible explanation for this paradox relates to the location of MMPs and TIMPs in the lung parenchymal microenvironment, which seems to be critical to understand the possible role of these enzymes in the pathogenesis of the disease. This complex process can be exemplified with our recent findings regarding the expression of the collagenases subfamily in IPF (60). Thus, although MMP-1 was highly expressed in IPF lung tissue, the localization of the enzyme was noticed in some free alveolar macrophages, but mainly in reactive alveolar epithelial cells as well as bronchiolar epithelial cells lining honeycomb cystic spaces. Importantly, this enzyme was practically absent in the areas of fibrogenesis, that is, the interstitial compartment, and fibroblastic foci. Likewise, MMP-8 was revealed in few neutrophils, whereas MMP-13 was not detected (60). In other words, the absence of MMP-1 expression by interstitial fibroblasts in vivo might explain in a simplistic way the presence of scars that do not undergo resorption.

B. TIMPs in Pulmonary Fibrosis

As mentioned, a major role of TIMPs in tissue remodeling is their ability to inhibit MMPs by binding in 1:1 enzyme-inhibitor complexes in a noncovalent fashion. Therefore, changes in their production and in their localization may contribute to modifications in the enzymatic activity of MMPs in the lung microenvironment. Actually, this seems to be the case, since regarding expression and location, a different picture emerges from the TIMP studies than the mentioned for interstitial collagenases. Thus, a noticeable interstitial presence of TIMPs -2, -3, and -4 compared with collagenases was found in vivo in human lung fibrosis, suggesting that a nondegrading fibrillar collagen microenvironment is present in IPF (60). Likewise, fibroblasts/myofibroblasts obtained from IPF lungs exhibited a marked upregulation of all four TIMPs in vitro as compared with fibroblasts from normal lungs (73). Interestingly, TIMP-1 is barely expressed in the extracellular matrix in human lung fibrosis,

whereas as mentioned is the main TIMP increased in experimental lung fibrosis (58,60,64–66).

Of particular interest is that TIMP-2 has been found primarily expressed by subepithelial fibroblast/myofibroblast foci in IPF lungs (59,60). These distinct clusters of mesenchymal cells likely represent microscopic areas of acute lung injury, and appear to play a crucial role in the pathogenesis of IPF (61). It is important to emphasize that some differences in the MMP/TIMP expression between cryptogenic organizing pneumonia (COP), a reversible fibrogenic process, and IPF, an irreversible one, have been noticed. Thus, myofibroblasts in intra-alveolar fibrosis of COP show predominantly MMPs, and they ultrastructurally appear to be phagocytosing collagen fibrils, whereas those of IPF exhibit a predominant reaction for TIMP-2 (59). The expression of TIMP-2 in these sites of active fibrogenesis may have several effects including collagenase activity inhibition, stimulation of fibroblast proliferation, and activation of latent gelatinase A. Actually TIMP-2 has been colocalized with nuclear markers of cell proliferation, suggesting a role in the expansion of the fibroblast/myofibroblast cell population (60). This process may explain, at least partially, the survival of mesenchymal cell populations in the fibroblast foci from the expected cell death as it is observed in a normal wound healing model (74). Supporting this point of view, it has been demonstrated that rats showing spontaneous recovery from experimental cirrhosis exhibit a rapid decrease of TIMP-1 and TIMP-2 together with an increase of collagenolytic activity and apoptosis of hepatic stellate cells (75). Likewise, in human liver fibrosis, serum levels of TIMP-1 and -2 are related to the histological degrees of both periportal necrosis and liver fibrosis; additionally suggesting that they may be useful in the assessment of liver fibrosis in chronic liver disease (76). No studies with this aim have been done in pulmonary fibrosis.

Research on TIMP-3 and -4 in lung fibrosis is scanty. In a study performed in IPF lungs, TIMP-3, the only TIMP that binds to the ECM, was found strongly staining the elastic lamina of vessels, and TIMP-4 was found in interstitial macrophages and plasma cells. Interestingly, TIMP-3 may induce apoptosis (39,40), and it is also capable of inhibiting members of the ADAMs family such as tumor necrosis-α (TNF-α)–converting enzyme (TACE) (46). It also inhibits the shedding of interleukin-6 (IL-6), L-selectin, and syndecans 1 and 4, which seem to be mediated by ADAM-type proteases (77–79).

Recently, an important finding regarding TIMP-3, ECM turnover, and lung disease was reported (80). When the functional effects of knocking out this gene was explored in mice, TIMP-3 null mice presented remarkable lung changes characterized by enlarged airspaces from 2 weeks of age that progress in time with no signs of inflammation or fibrosis. Lungs from aged null mice displayed a reduced amount of collagen, enhanced degradation of collagen in the peribronchiolar space, and disorganization of collagen fibrils in the alveolar

interstitium. Furthermore, TIMP-3 null fibroblast cultures demonstrated enhanced destruction of ECM molecules in vitro. Interestingly enough, no changes in total elastin content were observed in TIMP-3–deficient lungs. Moreover, there was no enhanced infiltration of metalloelastase-expressing macrophages and neither TACE nor soluble TNF-α levels were detectable in these lungs. These data imply that although the collagen matrix in null lungs may be susceptible to turnover in the absence of TIMP-3, elastin ultrastructure is relatively impervious to attack. The excessive deposition of TIMP-3 in IPF and the emphysematous changes provoked by its absence suggest that this inhibitor plays an essential role in lung ECM turnover.

What is clear when all the evidence is taken together is that the disruption of the proteolytic balance between MMPs and their endogenous inhibitors affects lung structure and function, and that a shift of the TIMP/MMP balance in the lung to favor ECM accumulation may culminate in fibrosis.

C. MMPs and the Loss of Integrity of Basement Membrane

The basement membrane is a specialized structure containing ECM components such as nonfibrillar type IV collagen, laminin, entactin, fibronectin, and heparin sulfate/chondroitin proteoglycans (81). It provides a stable substructure on which normal epithelial cells adhere, and it plays critical roles in epithelial cell polarity and function, thus maintaining the integrity and differentiation of the alveolar epithelium.

Disruption of the basement membrane is an early finding in pulmonary fibrosis, and it seems to be important in the pathogenesis of the disease. In a pioneer study performed by Raghu et al. (82) in adult respiratory distress syndrome and IPF lungs, it was found that the basement membrane was disrupted early in the disease course. Thus, numerous gaps in the distribution of type IV collagen and laminin were observed by indirect immunofluorescence together with invasion of the alveolar spaces by types I and III interstitial collagens. Furthermore, it has been postulated that migration of fibroblasts/myofibroblasts into the alveolar spaces occurs through partially disrupted and denuded epithelial basement membranes (60, 83, 84). Therefore, intraluminal fibrosis, a frequent process observed in a number of fibrotic lung disorders including IPF, may be at least partially mediated by basement membrane disruption (85).

On the other hand, the basement membrane is closely associated with epithelial cells displaying complex relationships that influence each other reciprocally to regulate epithelial growth, differentiation, and survival. In this context, the disrupted basement membrane may also contribute to the failure of an orderly repair of the damaged alveolar type I epithelial cells, affecting normal reepithelialization, and moreover it may have an additional deleterious role by inducing epithelial apoptosis. In fact, the integrity of the basement

membrane is required to suppress programmed cell death, as it has been demonstrated in mammary epithelium and other epithelia as well (86, 87). Studies in lungs are scanty, but some findings suggest that a similar process takes place. In an in vivo model of short-term hyperoxia, the culture of hyperoxic alveolar epithelial cells on various biological adhesion substrates, primarily laminin, reduced epithelial apoptosis and increased the ratio of expression of Bcl-2 to IL-1β–converting enzyme compared with culture on plastic (88). Laminin also restored glutathione levels and conferred improved optimal mitochondrial viability. In this model, protection against hyperoxia-induced damage was mediated by an increased activation of extracellular signal-regulated kinase.

The mechanisms involved in the disruption of the basement membrane in fibrotic lungs remain unknown, but seem to be related to the overexpression of some MMPs. In part, this hypothesis comes from studies performed in cancer. The ability to invade and to form metastasis, a characteristic of highly malignant cancers, depends on the distribution of the tumor cells throughout the basement membranes, and studies performed in both cell culture and animal models confirmed a role for MMPs (89). Furthermore, different studies have strongly supported the involvement of gelatinases A and B in cancer cell migration and invasion, and the mechanisms seem to include, at least partially, the breakdown of basement membrane components (90).

Gelatinases A and B are two members of the MMP family that can be synthesized by several lung cell types (91–94), and are the subgroup of MMPs most extensively studied in interstitial lung diseases. MMP-2 and MMP-9 present a broad matrix substrate specificity including different components of the basement membrane such as laminin, fibronectin, elastin, and type IV collagen, but also including nonmatrix substrates influencing the activation of a number of growth factors as pro–TGF-β, pro–TNF-α, and IL-1β (1,10,11,55).

Both gelatinases A and B have been found to be upregulated in acute and chronic lung injury, and their overexpression has been mainly associated with their capacity to degrade components of the basement membrane. Gelatin zymography of bronchoalveolar lavage fluid (BALF) samples as well as in situ hybridization and immunohistochemistry have shown a noteworthy increase of gelatinases A and B in rat lungs exposed to 100% oxygen (95). Furthermore, increased in vivo activity was demonstrated by in situ zymography where areas of intense activity were observed throughout the alveolar septa. Likewise, both enzymes are also upregulated in rat lungs by subacute hyperoxia (96) and are primarily localized in alveolar macrophages and epithelial cells.

In IPF, both gelatinases A and B are also elevated in BAL (Fig. 2). Importantly, they are additionally synthesized by subepithelial myofibroblasts, coinciding in some areas with denuded alveolar basement membranes (58–61). This finding suggests that myofibroblasts may play a role in the degradation of

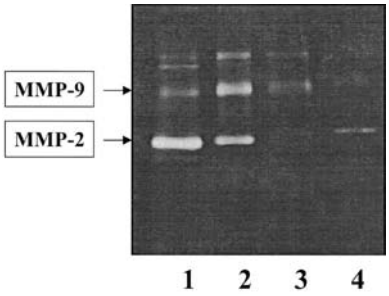

Figure 2 Identification of gelatinolytic enzymes in BAL by SDS-PAGE gelatin zymography. Lanes 1 and 2 represent BAL samples from IPF patients and lanes 3 and 4 BAL fluid from normal individuals. Zones of enzymatic activity appear as clear bands over a dark background. IPF patients reveal a marked increase of progelatinase B and progelatinase A activities. In both IPF samples, a slight band corresponding to the gelatinase B activated form of 86-kD is also evident. Likewise, IPF BAL fluid in lane 1 displayed the 68-kD active form of progelatinase A.

the epithelial basement membrane, facilitating their migration into the alveolar spaces. Furthermore, fibroblasts obtained from IPF lungs expressed MMP-9 transcript in vitro, and its expression seems to be closely related with the percentage of myofibroblasts (73). In one of these studies, MMP-2 showed focal colocalization in capillary endothelial and disrupted epithelial basement membranes (58).

Likewise, in bleomycin-induced pulmonary fibrosis, an increase of gelatinase B activity and disruption of the alveolar epithelial basement membrane have been found (97). In the same experimental model, macrophage metalloelastase (MMP-12), an enzyme that it is also able to degrade basement membrane components, was noticed to be strongly upregulated (66).

D. MMPs and Epithelial Cell Migration

The reestablishment of the alveolar surface is a mandatory event for successful repair of the alveolar-capillary barrier after lung injury. Lung reepithelialization is a complex and not-well-understood process that should involve a sequence of steps including detachment, migration, reattachment, proliferation, and differentiation of type II in type I pneumocytes. From all these steps, at least detachment and migration are facilitated by ECM cleavage. In skin wound healing, for example, MMP-9 can cut basal lamina type IV collagen and anchoring fibril type VII collagen, being responsible for releasing keratinocytes from their attachment to the basal lamina (98). Furthermore, the leading edge of alveolar epithelium may oftentimes penetrate underneath fibrous tissue, and appropriate reepithelialization in

such circumstances requires the denaturing capacity of a variety of MMPs. Supporting this point of view, some recent data suggest that migration of fetal alveolar epithelial cells and those derived from hyperoxic rats through gelatin correlates with MMP-2 and MMP-9 expression and synthesis (99). Likewise, the lungs of gelatinase B–deficient mice showed minimal alveolar bronchiolization after bleomycin instillation, suggesting that gelatinase B facilitates migration of Clara cells and other bronchiolar cells into the regions of alveolar injury (100). In this context, an intriguing finding was the strong expression of MMP-1 observed in the epithelial cells covering areas of intra-alveolar and interstitial fibrosis in IPF lungs (60), giving rise to the question of its functional role. An analogous situation has been described in migratory keratinocytes during skin wound healing. Actually, basal keratinocytes at the edges of the wound strongly express collagenase-1, and this expression is confined to periods of active reepithelialization, supporting the idea that this enzyme facilitates cell migration during wound repair (101). It has been demonstrated that collagenase-1 is induced by binding through $\alpha 2\beta 1$ integrin to native type I collagen, and then degradation of collagen initiates keratinocyte migration during reepithelialization (102).

In this sense, it can be speculated that a similar situation may occur in pulmonary fibrosis, and accordingly disrupted basement membrane exposes alveolar epithelial cells to a close contact to fibrillar collagen and then migrating pneumocytes overexpress collagenase-1. Actually it has been shown that alveolar epithelial cell locomotion on type I collagen is promoted by collagenases, and moreover in vitro wound healing of an alveolar epithelial cell monolayer appears to be enhanced in the presence of exogenous collagenases (103). These findings suggest that collagenases could modulate the repair process by decreasing cell adhesion and cell stiffness and by increasing cell migration on type I collagen.

The strong epithelial expression of MMP-1 in IPF lungs suggests that a similar process may occur in vivo in this disease, although reepithelialization seems to be unsuccessful, at least partially, because it appears to occur when alveolar structures have already disappeared. Naturally, several steps are necessary for the appropriate conclusion of this process. First, alveolar type II pneumocytes must migrate over a provisional wound matrix composed primarily of fibrin, fibronectin, and fibrillar collagens (an early event where collagenases play a role through collagen degradation and modification of cell adhesion sites). Then the provisional matrix should be replaced by normal basement membrane, and finally type II alveolar epithelial cells must differentiate into type I pneumocytes. It is possible that after migration, disrupted basement membrane, and/or fail in epithelial cell differentiation avoid successful repair in IPF. In addition, chronically induced epithelial cell apoptosis may also play a role (104,105).

A similar role in epithelial cell migration may be attributed to matrilysin (MMP-7). In the mentioned study of multiple gene expression by using cDNA microarray (70), it was found that MMP-7 was highly expressed in IPF lungs. This enzyme has a strong affinity for heparin and is able to degrade several matrix substrates such as proteoglycans, laminin, fibrin/fibrinogen, and others (3). Similarly to MMP-1, MMP-7 was primarily expressed by alveolar epithelial cells, and might also have a role in cell migration (106). Actually, in a similar way that MMP-1 facilitates migration of keratinocytes over the collagen-rich matrix of dermis, MMP-1 and MMP-7 may participate in alveolar and bronchiolar cell migration over different matrices during fibrotic lung remodeling.

E. Angiogenesis and Pulmonary Fibrosis

The formation of new blood vessels is a complex and fundamental process for tissue repair after injury and requires coordinated regulation of matrix proteolysis and endothelial cell migration.

Studies of angiogenesis in pulmonary fibrosis are scanty, but this process has been described in humans as well as in experimental models of fibrosis, and it may participate in the formation of precapillary systemic-pulmonary anastomoses found in fibrotic regions (107,108). However, its precise role in lung inflammation and fibrosis has not been elucidated. At least theoretically, angiogenesis may have a detrimental role, as it has been described in cancer, or it may have a protective role facilitating an appropriate repair as observed in myocardial infarction and in most skin lesions that heal rapidly.

Some studies in IPF and experimental fibrosis suggest that angiogenesis enhances the fibrotic response (109–112). Thus, in lung tissue from IPF patients, it was found that levels of IL-8, an angiogenic factor, were greater than in control lungs, whereas the opposite was noticed with the levels of the angiostatic CXC chemokine gamma interferon (INF-γ)–inducible protein-10 (IP-10) (109). Interestingly, when IL-8 or IP-10 was depleted from IPF lung samples, tissue-derived angiogenic activity was markedly attenuated or increased, respectively. In addition, fibroblasts isolated from IPF patients constitutively synthesized more IL-8 and less IP-10 than control fibroblasts. Moreover, conditioned media from IPF fibroblasts demonstrated constitutive angiogenic activity that was attributable, in part, to IL-8.

More recently, a significant increase of epithelial neutrophil-activating peptide-78 (ENA-78) levels was reported in a large number of IPF lung specimens compared with lung samples obtained from patients undergoing thoracic surgery for reasons other than interstitial lung disease. Alike the results observed with IL-8, when ENA-78 was depleted from IPF lung samples, tissue-derived angiogenic activity was markedly reduced (110).

Findings in experimental pulmonary fibrosis support a profibrotic role for angiogenesis. Thus, in bleomycin-induced lung fibrosis, neutralization of an angiogenic chemokine or administration of an angiostatic chemokine reduced the ECM accumulation (111,112). Thus, for example, systemic administration of IP-10 induced a marked attenuation of the lung fibrotic response in parallel with a reduction in angiogenesis and without changes in lung lymphocyte or natural killer (NK) cell populations. Furthermore, IP-10 had no direct effect on isolated pulmonary fibroblasts, suggesting that fibroplastic inhibition was regulated by controlling angiogenesis (111).

However, it is important to emphasize that neovascularization is a prominent feature in organizing pneumonia, a usually reversible fibrogenic disorder (59), and vascular endothelial growth factor, a potent angiogenic agent that is expressed by epithelial cells in the mature lung, is significantly depressed in the BAL of IPF patients (113).

MMP/TIMP Relationships and Angiogenesis

Neovascularization occurs by a series of tightly regulated sequential steps that begin when capillary endothelial cells are stimulated by an angiogenic stimulus. A critical event in this process is the production of proteolytic enzymes, including MMPs, which facilitates the endothelial cells lining the existing microvessels to move toward the angiogenic stimulus via the degraded basement membrane and continue to breakdown the interstitial ECM as they migrate (114,115).

From the large family of MMPs, an increasing body of evidence has been documented that gelatinases A and B play an essential role in angiogenesis by degrading the vascular basement membrane and also by cooperating with collagenases in the degradation of interstitial extracellular matrix. Thus, for example, the formation of tubular networks by endothelial cells in vitro is strongly increased by the addition of gelatinase A and significantly reduced in the presence of neutralizing antibodies or TIMP-2 (116). Likewise, studies performed in both gelatinase A–and gelatinase B–deficient mice have shown an important attenuation in angiogenic activity which affects either tumor progression or skeletal growth plate vascularization (117,118). In addition, membrane type 1 MMP (MMP-14), associated with gelatinase A, also appears to play a role in this process. Thus, endothelial cell–ECM interactions control the expression of both MMPs via matrix-induced signaling leading to transcriptional activation and subsequent formation of active multiprotease complexes on the cell surface (119).

On the other hand, migratory endothelial cells must also pass through the perivascular ECM primarily composed of type I collagen, and evidence supports that collagenase-1 activity is a fundamental requirement for angiogenesis (120). Furthermore, human endothelial cell stimulation by

vascular endothelial growth factor or basic fibroblast growth factor, both potent angiogenic molecules, induces the expression of interstitial collagenase (121,122). Additional evidence supporting a role for MMPs during the vascular remodeling associated with blood vessel formation has come from studies in which the blocking of MMP activity resulted in inhibition of angiogenesis (123,124). In summary, it has become apparent that at least four matrix metalloproteinases, MMP-1, MMP-2, MMP-9, and MT1-MMP, actively participate in the process of new capillary formation.

However, other MMPs may inhibit angiogenesis, and some data suggest that matrilysin (MMP-7), macrophage metalloelastase (MMP-12), and even gelatinase B may block angiogenesis by converting plasminogen to angiostatin, which is one of the most potent angiogenesis antagonists (114).

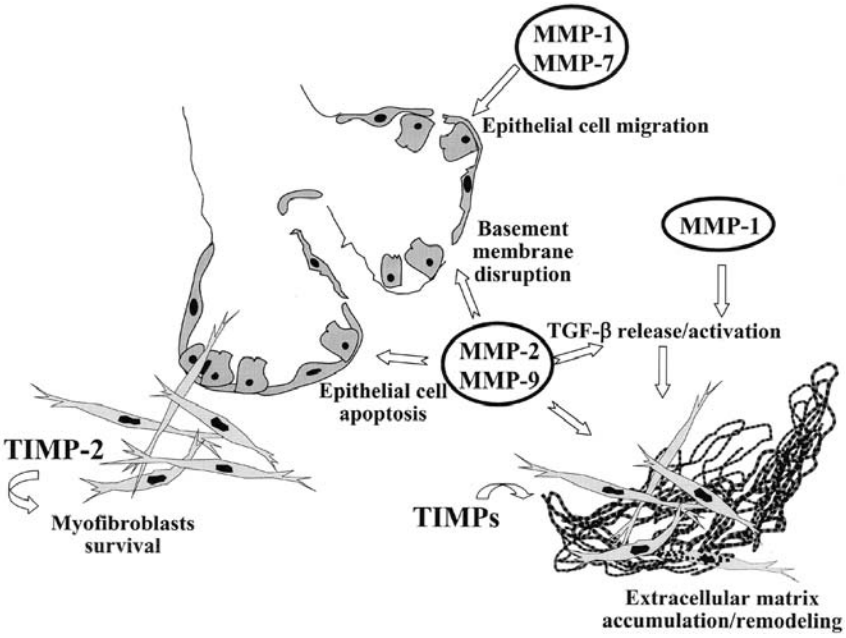

Figure 3 Hypothetical scheme summarizing the possible roles of MMPs and TIMPs in the pathogenesis of pulmonary fibrosis. In the injured alveoli, MMP-1 and MMP-7 are strongly upregulated in the alveolar epithelium and may participate in cell migration. Increased production of MMP-2 and MMP-9 may contribute to fibrogenesis through at least three mechanisms: basement membrane disruption, epithelial cell apoptosis, and the release of different growth factors, the latter also with the participation of MMP-1. Increased levels of growth factors in the lung milieu enhance fibroblast migration/proliferation and the accumulation of extracellular matrix; this process is additionally facilitated by the tissue inhibitor of metalloproteinases. TIMP-2 may have an additional role of enhancing fibroblast survival.

V. Conclusions

Increased deposition of ECM, including fibrillar collagens, fibronectin, elastic fibers, and proteoglycans, is the hallmark of the aberrant tissue remodeling that characterizes pulmonary fibrosis. In this chaotic reaction, the expression, location, and relationships of MMPs and TIMPs in the lung microenvironment appear to play a critical role in enhancing ECM accumulation, basement membrane disruption, and epithelial cell apoptosis among other effects (Fig. 3). A better understanding of the multiple roles that these two unique families of proteins play in the disease process may open new avenues for therapeutic approaches.

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19

Extracellular Matrix

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I. Introduction

Extracellular matrix is a general term that refers to the structural, macromolecular components of a tissue (1). In the lung, it includes interstitial connective tissues as well as basement membranes below airway and alveolar epithelial cells and surrounding vascular endothelial cells. Lung extracellular matrix plays an important role in growth and development, as well as in tissue homeostasis and repair. The principal extracellular matrix molecules that make up the pulmonary interstitium are fibrillar collagens and elastic fibers. In addition to their structural properties, these extracellular matrix molecules function as regulators of biological processes by interacting with growth factors, cytokines, and cell surface receptors (2,3). The complete molecular structure and function of the extracellular matrix, however, have not yet been elucidated.

This chapter focuses on the principal constituents of the extracellular matrix that comprise the interstitial connective tissue of the lung in idiopathic pulmonary fibrosis (IPF); namely, type I collagen and elastin. In addition, this chapter reviews the role of matrix metalloproteinases (MMPs) in extracellular matrix remodeling. The interactions between extracellular matrix molecules

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and growth factors are also examined. Finally, a group of secreted regulatory macromolecules known as matricellular proteins is reviewed.

II. Extracellular Matrix Molecules

A. Type I Collagen

Type I collagen is the most abundant extracellular matrix protein in humans and it is produced in a variety of tissues in a tightly regulated manner. Type I collagen is a heterotrimeric molecule consisting of two α1 chains and one α2 chain. In humans, the α1 chain is encoded on chromosome 17 and the α2 chain on chromosome 7. Following posttranslational modifications, the chains are assembled into a triple helical structure and secreted (4–6). In the extracellular space, the N and C propeptides are cleaved from procollagen molecules that are subsequently assembled into fibrils and then into fibers. Although type I collagen is necessary for the structural integrity of tissues, its excess accumulation results in fibrosis and organ dysfunction. The regulation of type I collagen production is complex and incompletely understood. It includes processes that control gene transcription and posttranscriptional mechanisms such as mRNA stability (7–9).

Transcriptional Regulation

Transcriptional regulation of type I collagen genes is mediated by interactions between *cis*-acting DNA sequences and *trans*-acting protein factors (10). Regulatory sequences within the α1(I) and α2(I) collagen gene promoters have been identified using transfection studies of different promoter segments controlling a reporter gene. In studies using transgenic mice and different segments of the α1(I) and α2(I) collagen gene promoters, cell-specific expression of these genes appears to be controlled by different *cis*-acting elements (11,12). For example, an 800-bp element of the α1(I) collagen gene promoter induces the expression of a reporter gene in dermal fibroblasts, whereas another element located approximately 1.7 kb 5′ of the transcription start site induces expression of a reporter gene in osteoblasts and odontoblasts, but not in other cell types. The *trans*-acting factors that bind to these *cis*-acting elements have not been identified. Similar results demonstrating the cell specific expression of α1(I) collagen have been reported using α1(I) collagen minigenes in transgenic mice (13). A more recent report described interactions among several *cis*-acting elements, including two short elements termed TSE1 and TSE2, that appear to regulate α1(I) collagen gene expression in tendon fibroblasts (14).

A variety of effector molecules increase type 1 collagen expression. These include, but are not limited to, transforming growth factor-β (TGF-β), insulin-like growth factor-1, interleukin-4 (IL-4), and endothelin. Individually

or in combination, these effector molecules stimulate increases in type I collagen formation by acting at different sites in the collagen biosynthetic pathway (15). Other factors such as prostaglandin E_2, interferon γ, tumor necrosis factor-α, and corticosteroids inhibit type I collagen production by fibroblasts.

TGF-β is a potent stimulus of fibrotic reactions in tissues. It increases fibroblast proliferation, induces collagen production, and inhibits collagen degradation. The rate of transcription of type I collagen genes and the stability of the resultant mRNA transcripts are increased by TGF-β (8,16). TGF-β signal transduction is mediated by the Smad family of proteins (17–19). Following TGF-β stimulation, Smad2 and Smad3 are phosphorylated and form multimeric complexes with Smad4. These complexes translocate to the nucleus and regulate gene transcription by binding DNA directly or in association with other transcription factors.

Studies of the α2(I) collagen promoter identified a putative TGF-β response element that appears to mediate the transcriptional effects of TGF-β (20,21). TGF-β may modify the binding affinity of a multimeric protein complex containing Sp1 to the TGF-β response element (21). However, Smad proteins have not been identified within this complex. Adaptor protein (AP-1) may mediate some of the TGF-β effects on α2(I) collagen gene transcription (22).

Intratracheal instillation of bleomycin in rodents results in pulmonary fibrosis (23). In our studies of C57BL/6 mice, intratracheal administration of bleomycin results in dramatic increases in lung type I collagen as assessed by Western blot analysis using an antibody to the C-terminal propeptide (Fig. 1). In this established animal model of human IPF, the mechanisms underlying fibrogenesis are gradually being elucidated. TGF-β appears to play an important role in the pathogenesis of the fibrotic process (24,25). Transcriptional activation of the rat α1(I) collagen gene by TGF-β may be

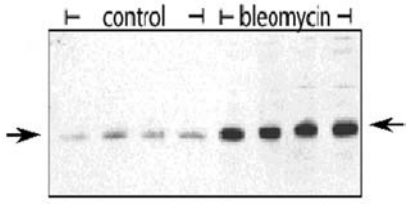

Figure 1 The effect of bleomycin on lung collagen levels in mice. C57BL/6 mice were treated with intratracheal saline (control) or bleomycin, as indicated. After 21 days, the lungs were harvested. Total protein was isolated from the lungs and 100 μg per lane was resolved by SDS-PAGE. Western blot analysis was performed using an antibody to the type I collagen C-terminal propeptide.

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mediated by a *cis*-acting element within the promoter (26,27). This putative TGF-β activation element was studied in transgenic mice expressing a 3.6-kb rat α1(I) collagen promoter linked to a chloramphenicol acetyl transferase reporter gene (28). Interestingly, following bleomycin treatment, transgenic mice with a mutation in the TGF-β activation element display a similar level of reporter gene activity to transgenic mice with a wild-type α1(I) collagen promoter. A more recent report of mice deficient in Smad3 demonstrates suppressed type I collagen levels in the lungs following bleomycin treatment when compared with wild-type mice treated with bleomycin (29). This study presents further evidence that TGF-β signaling is important during bleomycin-induced fibrogenesis.

Posttranscriptional Regulation

The rate of transcription of the α1(I) collagen gene and the stability of the mRNA transcript are low in quiescent fibroblasts within tissues. In activated hepatic stellate cells, the transcription rate of the α1(I) collagen gene is only 2-fold greater than in quiescent cells; however, the stability of the mRNA transcript is increased 16-fold (9). Activated fibroblasts derived from the skin of patients with systemic sclerosis are characterized by increases in steady-state levels of α1(I) collagen mRNA that are in large part due to increases in mRNA stability (30). Therefore, in activated fibroblasts, the increase in steady-state levels of α1(I) collagen mRNA may be significantly influenced by the increase in stability of the mRNA transcript.

In human lung fibroblasts, phosphatidylinositol-3-kinase (PI3K) activation increases the stability of the α1(I) collagen mRNA transcript without altering the rate of transcription of the α1(I) collagen gene (31). Inhibition of PI3K by LY-294002 or wortmannin decreases basal and TGF-β–induced increases in α1(I) collagen mRNA in these fibroblasts. In addition, LY-294002 decreases the expression of a truncated α1(I) collagen minigene driven by a cytomegalovirus promoter in mouse lung fibroblasts. These data indicate that PI3K activation increases α1(I) collagen mRNA stability and that, in vivo, PI3K activity may regulate basal levels of α1(I) collagen mRNA expression in lung fibroblasts.

The posttranscriptional regulation of mRNA is determined in part by specific nucleotide sequences located in the 5′ untranslated region (UTR) and the 3′ UTR of the mRNA transcript (32,33). Oncogenic ras regulates type I collagen genes at both transcriptional and posttranscriptional levels (34). Overexpression of mutated ras decreases the stability of the α1(I) collagen mRNA transcript;. however, the *cis*-acting elements mediating this change were not identified. In other studies, a regulatory element was identified in the 3′ UTR of α1(I) collagen mRNA from hepatic stellate cells (9). Mutation of

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this element in an α1(I) collagen minigene decreases the stability of the mRNA transcript. A protein complex containing an αCP subunit binds to this region of the 3′ UTR. Moreover, the subunit αCP is also found in a protein complex involved in the stabilization of α-globin mRNA (35).

A stem-loop structure found in the 3′ UTR of the granulocyte colony stimulating factor regulates mRNA stability (36). A stem-loop structure is also found in the 5′ UTR of α1(I), α2(I), and α1(III) collagen mRNA (37–39). In experiments examining the potential regulatory role of the 5′ stem-loop in the expression of reporter genes in hepatic stellate cells, the stem-loop decreased the expression of the reporter genes in quiescent cells but not in activated cells (38). The inhibitory effect of the stem-loop was in part mediated by decreases in the stability of the mRNA transcripts. These studies demonstrate the potential role of the UTR in regulating the stability of α1(I) collagen mRNA.

Interactions between fibroblasts and extracellular matrix elements may also influence α1(I) collagen gene transcription and mRNA stability. For example, lung fibroblasts cultured on flexible-bottom surfaces coated with laminin or elastin and exposed to mechanical strain increase steady-state levels of α1(I) collagen mRNA, whereas fibroblasts cultured on fibronectin do not (40). The stability of the α1(I) collagen mRNA transcript was not evaluated in these studies; however, fibroblast–extracellular matrix interactions increase the stability of other mRNA transcripts, such as integrin subunits α3 and α5 (41). These data suggest that cell–extracellular matrix interactions influence mRNA stability.

Multiple factors influence the accumulation of the extracellular matrix in the lung following bleomycin treatment of rodents. As noted, TGF-β plays an important role during this fibrotic response. TGF-β gene expression increases in hamster lungs treated with bleomycin (25). In addition, the administration of a TGF-β antibody to mice reduces the accumulation of lung collagen induced by bleomycin (24). Bleomycin hydrolase deficiency in the lung may also contribute to fibrogenesis following the administration of bleomycin (42). In a mouse model of pulmonary fibrosis induced by the intraperitoneal injection of bleomycin, the intranasal administration by adenovirus-mediated gene transfer of the bleomycin resistance gene (*streptoalloteichus hindustanus* (Sh) ble) prevented collagen deposition in the lungs as assessed by hydroxyproline content analysis (43).

Recent studies suggest the connective tissue growth factor (CTGF) may mediate some of the effects of TGF-β (44). CTGF belongs to the CCN family of conserved, modular proteins with diverse biological functions. Most CCN family members are secreted proteins that contain 343–381 amino acid residues including 38 cysteine residues. CCN proteins have four distinct structural domains; however, the contribution of these domains to their biological function is not known. The N-terminal domain of CTGF shares sequence

homology with insulin like growth factor binding protein-1 (45). The two internal domains are the von Willebrand type C domain, whose function has not been described, and the thrombospondin type 1 domain, which may mediate cell attachment (46–48). The C terminal domain mediates dimerization and contains a cysteine knot motif that is found in other growth factors including TGF-β. Since some of the receptor-binding properties of these growth factors reside in variable regions within the cystine knot, the C-terminal domain may also mediate receptor binding (49). CTGF binds to integrins in platelets and endothelial cells, suggesting its involvement in cell adhesion signaling (50,51).

Upon synthesis, CTGF is secreted and becomes associated with the cell surface and the extracellular matrix (52,53). As an extracellular matrix molecule, CTGF may serve as an adhesion molecule to regulate cell function. In addition, CTGF may regulate cell function indirectly through cooperative interactions with other growth factors (54,55). Since TGF-β induces CTGF expression and CTGF induces collagen production in fibroblasts, it has been suggested that CTGF may mediate both the mitogenic and fibrogenic activities of TGF-β (44). However, CTGF-independent pathways of TGF-β action have been identified. For example, IL-4 increases α1(I) collagen mRNA levels in lung fibroblasts, whereas IL-4 attenuates the TGF-β–stimulated increase in CTGF mRNA expression (56). Furthermore, discordance between CTGF and type I collagen expression is demonstrated in lung fibroblasts maintained in amino acid-deficient media (57). TGF-β activity and CTGF expression are similarly dissociated in studies of mink lung epithelial cell proliferation (55) and myofibroblast differentiation (58).

B. Elastin

Elastin is one of the principal extracellular matrix molecules that make up the pulmonary interstitium. It is important to both lung morphology and function (59,60). During lung development, elastin regulates cellular growth, migration, and differentiation. Under normal conditions, mature elastin is a stable protein with a turnover rate approaching the life span of the animal (61). However, elastin production can be rapidly activated following lung injury (62,63). Although the pathogenesis of IPF has been studied primarily in the context of collagen regulation, the aberrant regulation of elastin contributes to distorted pulmonary architecture, impaired gas exchange, and fibrosis. In a murine model of chemical and hyperoxic lung injury, histopathological changes of fibrosis are associated with marked increases in elastin mRNA expression (63).

In the lung, elastin is produced by several cell types including fibroblasts and vascular smooth muscle cells. In extravascular lung tissue, the interstitial

fibroblast is the primary source of elastin (64). In studies of cultured interstitial lung fibroblasts and vascular smooth muscle cells, various effector molecules including TGF-β (65), insulinlike growth factor-1 (66), and retinoic acid (67) increase elastin mRNA expression. Other effector molecules such as IL-1β decrease elastin mRNA levels (64). Both the stability of the mRNA transcript and the rate of gene transcription contribute to changes in the steady-state level of elastin mRNA.

In studies of rat lung fibroblasts, hypoxia decreases the steady-state level of elastin mRNA by a process that is primarily due to decreases in mRNA stability and to a lesser extent due to decreases in the rate of gene transcription (68). Whereas many of the effects of TGF-β are associated with transcriptional regulation, the TGF-β induction of steady-state levels of elastin mRNA occurs by stabilizing the mRNA transcript (69,70). A recent study reported that TGF-β stabilizes elastin mRNA by activating Smads, protein kinase C-δ, and the stress-activated protein kinase, p. 38 (71).

Following mRNA translation, tropoelastin, a soluble form of elastin, is secreted by vesicles to the plasma membrane. During this process, a specific elastin-binding protein appears to protect tropoelastin from intracellular proteolysis (72). The elastin-binding protein also constitutes part of a heterotrimeric receptor complex that links the extracellular matrix to the intracellular compartment (73).

Multiple isoforms of tropoelastin exist as a result of alternative splicing of a single gene (74). Although the functional differences among individual tropoelastin isoforms are unknown, several tropoelastin mRNA transcripts and their encoded isoforms appear to be tissue specific and developmentally regulated (74,75). These differences may be important for the interaction of tropoelastin with microfibril proteins such as TGF-β–binding proteins (75).

Tropoelastin undergoes few posttranslational modifications; however, the formation of lysine-derived cross links is important in maintaining the properties of the mature elastin protein (59,60). As tropoelastin is secreted from the cell, it interacts with specific glycoprotein microfibrils that are necessary for alignment and subsequent fiber formation (76). Incomplete cross linking or abnormal assembly of elastic fibers can result in pathological conditions such as pulmonary emphysema and fibrosis (77,78). Degradation of extracellular matrix components including elastin is a feature of these disorders. The extent of elastin breakdown may be assessed by measuring the urinary levels of desmosine and isodesmosine, cross-linked amino acids derived from elastin (79). Although damaged elastic fibers can be repaired, the recovery of physiological function of the tissue may not occur (80).

Recently, the integrin ligand fibulin-5, also known as DANCE, was found to be essential for elastic fiber organization (81,82). In mice rendered deficient in fibulin-5, pulmonary emphysema, vascular abnormalities, and loose skin develop. Multiple tissues in these mice exhibit disorganized elastic fiber

formation with fragmented elastin, but without any associated increase in elastase activity.

During normal lung development, elastin synthesis is regulated by a variety of growth factors including retinoic acid (83). The administration of retinoic acid to fetal rats induces airway branching (84). On postnatal day 10 in rats, cellular retinoic acid–binding protein expression increases and coincides with increases in elastin expression (83). Massaro examined the role of retinoic acid on rat postnatal lung development in two separate studies (85,86). The first study demonstrated that the daily intraperitoneal injection of retinoic acid increases the number of alveoli (85). The second study examined a rat model of elastase-induced emphysema. The administration of retinoic acid to rats following the initial lung injury resulted in improved lung morphology and function (86). The precise mechanisms regulating elastin fiber assembly and alveolar formation and the role of extracellular matrix remodeling in these processes are unknown.

III. Extracellular Matrix Remodeling

Extracellular matrix remodeling is an important feature of fibrogenesis. The abnormal deposition of extracellular matrix or its disruption by proteolytic action involves alterations in the structural integrity of tissues. Changes in cell shape and in stress-strain relationships between cells residing within alveolar structures may then ensue. Cellular behavior is modified by alterations in extracellular matrix–cell interactions and by release of bioactive proteolytic fragments as well as connective tissue–bound growth factors that are sequestered in the extracellular matrix. During fibrotic reactions in the lung, the infiltration of inflammatory cells, the activation fibroblasts, and the migration of fibroblasts to fibroblastic foci likely require matrix degradation. We have found evidence of increased levels of breakdown products of collagen and elastin in the urine of patients with IPF (unpublished observations).

Remodeling of the extracellular matrix involves the activation of matrix metalloproteinases (MMPs) and perhaps other enzymes. The MMPs comprise a multigene family of secreted or cell surface enzymes with overlapping substrate specificity that participate in a wide variety of physiological functions. Members of this family include collagenases, gelatinases, stromelysins, matrilysin, and membrane-bound metalloproteinases (MT-MMPs). Unraveling the role of the MMP system in the pathogenesis of fibrogenic reactions is a formidable task because of the multiple levels of regulation that exist for each MMP, the variations in expression and abundance for individual MMPs, the differences in their substrate specificity, and the differences in localization for each MMP (87). The complexity of these effects has thus far precluded a clear understanding of the role they play in fibrogenic reactions. Only limited

information is currently available related to MMPs in IPF. Most of the available information regarding MMPs and fibrosis has been obtained from the study of other tissues and pathological states.

The production of MMP proenzymes depends in part on mRNA levels that are regulated by both transcriptional and posttranscriptional mechanisms. These processes vary among the individual MMPs. Many of the genes are inducible by exposure to growth factors, cytokines, or physical stress (88). MT-MMP and MMP-11 (stromelysin-3) are secreted in active form, whereas activation of other MMPs occurs by cleavage of the proenzyme by an enzymatic process that is itself regulated. Inactivation of MMPs occurs by degradation or by binding to endogenous tissue inhibitors of metalloproteinases (TIMPs) or other inhibitors such as α_2-macroglobulin (89). In addition to inhibiting the action of specific MMPs, TIMPs have other biological activities identified in vitro that involve regulation of cell growth (90,91).

Examination of normal human lungs reveals low-level expression of MMP-1 and MMP-2 and TIMP-2 in alveolar type II cells and macrophages. MMP-9 is expressed primarily in neutrophils. Studies of fibrotic human lungs using immunohistochemical techniques found alterations in expression of MMPs and TIMPs in fibrotic areas. Because extracellular matrix accumulates in IPF, a relative deficiency of protease activity may exist in these areas. The action of MMPs may also enhance fibrogenesis, as demonstrated by reduced bleomycin toxicity with the use of batimastat, a MMP inhibitor (92,93). MMPs are also required for normal wound healing as evidenced by mice deficient in MMP-3 that display delayed healing of cutaneous wounds (94). In vitro, type I collagen stabilizes MMP-2 by preventing its degradation (95,96). Increases in MMP-1 and MMP-2 and TIMP-1 and TIMP-2 are found in hyperplastic epithelial cells. In human IPF, levels of MMP-2 and MMP-9 are increased in myofibroblasts (97). Similar results were obtained by other investigators (98–100). Selman reported higher expression of TIMPs compared with MMPs in patients with IPF, suggesting a bias toward connective tissue accumulation. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are detected in myofibroblast subpopulations. TIMP-1 is expressed in interstitial macrophages, whereas TIMP-2 is found in fibroblast foci, TIMP-3 in the lamina of vessels, and TIMP-4 in epithelial and plasma cells (99).

Some information is available related to the expression of certain MMPs and TIMPs in the course of bleomycin-induced fibrosis in rodents. Importantly, however, species differences in the expression and regulation of these enzymes may preclude extrapolation to studies of human IPF (87,101,102). Bleomycin treatment in mice induces MMP-2 within 24 h and remains elevated for 21 days. Kunugi found that MMP-2 is strongly induced in reactive alveolar epithelial cells following bleomycin treatment (100). Interestingly, MMP-2 is activated by an MT-MMP that is also induced. The activity of MT-MMP is regulated by integrin activation (96) and inhibited by TIMP-2 and TIMP-3

(95). Mice deficient in MT-MMP have significant phenotypic abnormalities including soft tissue fibrosis (103,104).

MMP-12 (macrophage metalloelastase) gene expression is slightly increased following bleomycin treatment at 24 h, but it is markedly increased at 8 and 21 days. MMP-12 is localized to fibrotic lesions in the lung. TIMP-1 is upregulated at 1 day and further elevated at 21 days with persistence at 60 days (105). The upregulation of TIMP-1 mRNA and protein following bleomycin treatment was confirmed by Madtes (106). Of note, TIMP-2 immunoreactive protein is also increased in this model but mRNA levels are unchanged, indicating differential regulation (105).

Failure to adequately upregulate MMPs or inhibit MMP activity by TIMPs or other antiproteases could result in connective tissue accumulation, suggesting a potential pathogenic mechanism for IPF. Myofibroblasts derived from the skin of patients with scleroderma demonstrate both increased collagen synthesis and TIMP-1 levels (107). Regulation of proteinase activity by TIMPs is clearly important in maintaining normal alveolar architecture. Mice deficient in TIMP-3 but not TIMP-1 or TIMP-2 develop airspace enlargement and accumulate collagen (108). TIMP-3 inhibits MMP-1, MMP-2, MMP-3, and MMP-9 as well as several ADAM (a disintegrin and a metalloproteinase domain)–type proteins (109–111). Unlike TIMP-1 and TIMP-2, TIMP-3 induces apoptosis in several cell types (87,112). These data do not distinguish between the concept that connective tissue is remodeled by proteases, albeit at low levels, in the normal lung or that TIMP-3 exerts its own independent effects on lung remodeling.

Studies of TIMP-deficient mice have not yet provided direct support for the role of these inhibitors in controlling the degree of tissue fibrosis in general and in IPF in particular. For example, in hepatic fibrosis associated with schistosomiasis, TIMP-1 and TIMP-2 are increased and TIMP-3 is unchanged. However, no changes are found in the development of fibrosis following infection with *Schistosoma mansoni* in TIMP-1–deficient and TIMP-2–deficient mice (113). Matrix formation after experimental myocardial infarction is associated with increased MMP activity. However, MMP-9–deficient mice display decreased collagen levels in infracted areas with increased expression of MMP-2, MMP-13, and TIMP-1 (114). TIMP-1 deficiency does not attenuate renal interstitial fibrosis caused by unilateral ureteral obstruction (115). These data suggest that the hypothesis implicating an MMP/TIMP imbalance as a cause for fibrogenesis is oversimplified, and that there exists insufficient data related to the development of lung fibrosis in TIMP-deficient mice. Moreover, the complexities of the interactions among MMPs as well as the overlapping specificities of these enzymes and their inhibitors preclude definitive conclusions. Other factors that are still poorly defined may regulate this system and determine the extent of connective tissue deposition. For example, proteolytic processing of procollagen releases a MMP inhibitor (116).

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IV. Extracellular Matrix and Growth Factors

Certain extracellular matrix components can serve as a reservoir for growth factors. These growth factors bind the extracellular matrix and can be released following tissue injury. Heparan sulfate is found on the cell surface and in the extracellular matrix. It binds several growth factors including members of the fibroblast growth factor (FGF) family, vascular endothelial growth factor, and heparin-binding epidermal growth factor, as well as MT-MMPs. The binding of FGF to heparan sulfate allows them to accumulate in the extracellular matrix and protects them from degradation (117). Binding of FGF to heparan sulfate likely requires 6-O-sulfation and facilitates binding of FGF to its high-affinity receptor and activation of tyrosine kinase (118). Other growth factors that activate collagen are sequestered in the matrix including TGF-β and insulinlike growth factor-1.

TGF-β binds to decorin, a small leucine-rich proteoglycan. Decorin itself binds to type I collagen, type II collagen, and type VI collagen, promotes fiber stability, and may affect the differentiation of certain cell types (119). Decorin-deficient mice show increased skin fragility and abnormal collagen fibers (120). Decorin colocalizes with type I collagen during the development of renal fibrosis (121). Decorin is considered to be a potential antifibrotic agent, because decorin binds TGF-β and potentially sequesters the growth factor. Overexpression of TGF-β in the rodent lung induces fibrosis that can be ameliorated by coexpression of decorin but not biglycan, another TGF-β–binding proteoglycan (122,123). Overexpression or administration of decorin attenuates bleomycin-induced fibrosis (122–124). These data suggest that pharmacological administration of decorin has significant antifibrotic activities that are likely different from the physiological role of this extracellular matrix component.

V. Matricellular Proteins

Mesenchymal cells bind to specific extracellular matrix components via integrins that in turn regulate cellular behavior. Matricellular proteins are a group of matrix glycoproteins that were initially reported to influence cell adhesion in culture. They include SPARC (secreted protein acidic and rich in cysteine), thrombospondin-1 (TSP-1), and tenascin-C (TN-C). These matrix proteins do not contribute directly to the length-tension properties of the alveolar wall; however, they may affect the deposition of other matrix components, for example, by influencing type I collagen fibril assembly. Matricellular proteins may also affect cellular behavior such as migration or growth factor activation.

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Matricellular proteins are expressed during development and during wound healing (125). They bind extracellular matrix proteins including collagen and vitronectin, disrupt cell-matrix interactions, and may be important in promoting cell migration following lung injury. In normal adult lungs, minimal expression of matricellular proteins is detected by immunohistochemistry, whereas in the lungs of patients with IPF, increased expression of SPARC, TN-C, and TSP-1 is found with differential quantitative and spatial distribution. However, the precise role of these matrix components during fibrosis remains uncertain. Preliminary examination of mice deficient in SPARC and TN-C suggest that they may modulate, but not determine, collagen deposition in IPF.

A. SPARC

SPARC is synthesized by endothelial cells and other cell types (125–127). In vitro, addition of SPARC to bovine aortic endothelial cells inhibits cell proliferation and disrupts focal adhesions (128). SPARC also impairs myocyte differentiation, suggesting it may play a role in myofibroblast differentiation (129). The protein contains a follistatinlike domain, an angiogenic domain, and an extracellular calcium-binding module (127,130). Proteolytic cleavage of SPARC produces bioactive peptides, suggesting the potential for a variety of other physiological functions (131). TGF-β activates the production of SPARC by endothelial cells, and SPARC may regulate endothelial barrier function through changes in cell shape (132,133).

During tissue remodeling, SPARC is upregulated in the lung and in other tissues (134). SPARC colocalizes histologically with α-smooth muscle actin, suggesting that it appears in myofibroblasts, which are a key cell type in fibrogenesis (135). Histological examination of skin in SPARC-deficient mice suggests a possible role in collagen fibril assembly (125). Although the impairment of fibrillagenesis would be expected to interfere with wound healing, the rate of wound healing appears to be accelerated in SPARC-deficient mice. Moreover, collagen and TGF-β production is decreased in SPARC-deficient primary mesangial cells as compared with wild-type cells (136). In a rat model of glomerulonephritis, SPARC stimulates the expression of TGF-β (137). These results demonstrate the complex role of SPARC in fibrotic reactions. SPARC may have effects that both enhance and inhibit different components of the fibrotic process.

In lung biopsies from patients with IPF, SPARC is found in the intracellular compartment of fibroblast foci (138). Following bleomycin treatment of the rodent lung, levels of SPARC mRNA and protein increase. Histological studies suggest that the protein is more abundantly expressed following bleomycin treatment in mice than during interstitial fibrosis in humans. The discrepancy could be related to the particular antibody used or to

the dose of bleomycin given. Alternatively, it could suggest a fundamental difference between the human disease and the animal model of fibrosis.

Similar to many other matrix components, SPARC is induced in cultured fibroblasts by treatment with TGF-β. An early report suggested that treatment of SPARC-deficient mice with bleomycin resulted in less fibrosis than in wild-type controls (139). A subsequent report found more fibrosis following intratracheal bleomycin administration in SPARC-deficient animals (140). In general, the interpretation of bleomycin experiments in mice is difficult, because minor genetic differences in mice strains may lead to large differences in bleomycin sensitivity. In these studies, however, the divergent results suggest that the differences in genetic background are unlikely to account for the differences in fibrosis. Interestingly, the amount of fibrosis is similar in SPARC-deficient and wild-type mice after intraperitoneal bleomycin administration. This route of administration likely causes a more homogeneous pattern of fibrosis with less variability within treatment groups. These apparently conflicting results further demonstrate the unclear role of SPARC in regulating the fibrotic response.

B. Tenascin

The tenascin (TN) family of glycoproteins (TN-C, TN-R, TN-W, TN-X, and TN-Y) comprises closely related molecules with restricted patterns of expression (141–143). TN-C was the first isoform described and is present in the lung during morphogenesis and following injury (143). In vitro studies suggest that TN-C influences adhesive and signaling functions of various cell types. TN-C forms a hexabrachion structure with arms that attach to a central core (143,144). These arms contain epidermal growth factor–like repeats, fibronectin type III domains, and a terminal globular domain that resembles the β and γ chains of fibrinogen (143).

In the pulmonary vasculature, TN-C expression is associated with the progression of pulmonary hypertension (145). Several studies indicate that TN expression is upregulated in human interstitial fibrosis, particularly in areas of active disease and hyperplastic type II cells (138,146). Kuhn reported that TN-C is found in abundance in fibroblast foci and in the basement membrane below the epithelium lining honeycomb cysts. Wallace noted that TN is not identified in normal lung; however, in patients with IPF, TN is expressed in areas of active disease. Immunoelectron microscopy of usual interstitial pneumonitis (UIP) demonstrates labeling in association with collagen fibers and within type II pneumocytes. In culture, type II pneumocytes produce TN (146). Increased TN expression under metaplastic bronchiolar type epithelium is associated with early mortality in patients with IPF (147). Following bleomycin-induced lung injury in rats, TN mRNA and protein are rapidly upregulated in areas of inflammation with maximal expression at

3 days (148). In this model, type I collagen and type III collagen mRNA expression persist during the recovery phase, whereas TN mRNA expression decreases (148). TN protein is also found in alveolar septal walls in areas of inflammation.

The exact function of TN-C during the development of pulmonary fibrosis is unclear. The lungs of animals deficient in TN-C are grossly normal. The mice display behavioral abnormalities and have subtle abnormalities in wound healing (149). The absence of gross phenotypic alterations suggests that the role of TN-C overlaps with other TN isoforms or with other matrix elements. The effect of TN deficiency on bleomycin-induced fibrosis is unknown. TN, fibronectin, and vitronectin bind the $\alpha 8\beta 1$ integrin. This integrin is expressed in normal lung and is upregulated in myofibroblasts following bleomycin treatment (150). However, the early appearance of TN following bleomycin injury suggests that it is not directly involved in the accumulation of type I collagen in this model, as the deposition of collagen occurs later during the recovery phase (148). It may be that TN-C is involved in the activation of myofibroblasts that subsequently produce connective tissue matrix. Studies of vascular smooth muscle cells indicate that activation of $\beta 3$ integrins by treatment with matrix metalloproteinases results in production of TN-C. TN-C production then leads to alterations in smooth muscle cell shape and increases in epidermal growth factor–dependent growth (151). In other tissues, TN-C expression is upregulated following injury and is found in areas of epithelial proliferation in close proximity to sites of matrix deposition (152,153).

C. Thrombospondin

Thrombospondin-1 (TSP-1) is a trimeric protein present in large quantities in platelet alpha granules. It is also produced by endothelial cells, smooth muscle cells, and fibroblasts in culture (154). The protein contains a heparin-binding domain, an $\alpha 1$(I) collagen–like region, three type I repeats, epidermal growth factor–like repeats, and calcium-binding repeats (155,156). Early studies indicated that it played a role in hemostasis, but TSP-1–deficient mice do not have a coagulation defect (157). TSP-1 is chemotactic for fibroblasts and is a regulator of wound repair. In addition, TSP-1 disrupts focal adhesion and has antiangiogenic properties (154,158). Thrombospondin-2 (TSP-2) is a closely related trimeric protein; however, TSP-2 is encoded by a different gene and has a different physiological action than TSP-1 (159,160). During development, TSP-1 is expressed at day 14 in the columnar epithelium. In contrast, TSP-2 expression is observed after 16 days and is localized beneath the epithelium of the bronchi and bronchioles (161).

There is currently little available information concerning the potential role for either TSP-1 or TSP-2 in the normal adult lung or in IPF. TSP-1 is

detected in low abundance in patients with IPF in the extracellular matrix beneath reparative fibrosis (138). In patients with pulmonary hypertension, TSP is detected within the thickened intimal layer of hypertensive vessels (162). Early in life, TSP-1–deficient mice develop inflammatory cellular infiltrates in the lung, suggesting that TSP-1 may limit inflammation following lung injury. TSP-1–deficient mice are not suitable for challenge with bleomycin because of the ongoing inflammatory response in the lung.

TSP-1 can activate latent TGF-β in vitro (163), and TGF-β–deficient animals develop lung inflammation. These data suggest that TSP-1 may contribute to fibrosis by activating TGF-β. TSP-1 interacts with its receptor CD36 via type I (properdin) repeats. This interaction may have a role in activating latent TGF-β (164). Alveolar macrophages derived from bleomycin-treated lungs produce increased amounts of active TGF-β1 as well as TSP-1/CD36 receptor complexes. Treatment of bleomycin-treated animals with a CD36 synthetic peptide decreases fibrosis (165). Activation of latent TGF-β could account for this result; however, alternative mechanisms could exist. In other tissues, correlative data suggest a mediating role for TSP during the early events in the evolution of fibrosis. The expression of TSP precedes the development of tubulointerstitial fibrosis in the kidney and is associated with the extent of subsequent fibrosis (166).

TSP-2 is abundantly expressed by fibroblasts in experimental wounds (154). The phenotype of TSP-2–deficient mice is characterized by abnormal collagen fibril assembly, accelerated wound healing, reduced scarring, and increased angiogenesis. As noted, SPARC-deficient mice display enhanced wound healing. Dermal fibroblasts derived from TSP-2–deficient mice show increased production of MMP-2 that induce an adhesive defect (167,168). Indeed, SPARC increases metalloproteinase-2 production is some cell lines (169,170). To date, there is no available information describing alterations in fibrogenesis in the lungs of TSP-2–deficient animals.

Acknowledgments

This work was supported by the National Institutes of Health grants HL04232, ES11443, and HL66547 and the Veterans Administration REAP Program.

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Role of Fibroblasts and Myofibroblasts in Idiopathic Pulmonary Fibrosis

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I. Introduction

Pulmonary fibrosis is characterized by lung inflammation and abnormal tissue repair resulting in the replacement of normal functional tissue with an abnormal accumulation of fibroblasts and deposition of extracellular matrix in the interstitium and alveolar spaces. The process involves cellular interactions via a complex cytokine-signaling mechanism and heightened collagen gene expression, ultimately resulting in their abnormal deposition in the lung. Current understanding of the pathogenesis of pulmonary fibrosis suggests that in addition to inflammatory cells, the fibroblast and fibroblastlike cells (e.g., myofibroblasts) play important roles in the diverse processes that constitute fibrosis. Indeed, there is now mounting evidence to support the concept that the fibroblasts can be potent effector cells, releasing many of the same cytokines, as do the various inflammatory cells. Furthermore, in conjunction with its ability to elaborate extracellular matrix, the myriad activities of the cytokines produced by these cells would argue for active roles for this cell type in the response to tissue injury, inflammation, repair, and fibrosis.

Adding to the complexity in analyzing the role of the fibroblast is the heterogeneity of fibroblasts, which has been documented in cells isolated from different tissues, as well as from within a single tissue. With respect to the pulmonary fibroblast, for example, phenotypic differences exist between primary fibroblast lines from patients with pulmonary fibrosis and primary lines from control adult lung tissue. Heterogeneity in fibroblast populations has been documented in lesions in other fibrotic tissues, and in chronic diseases such as scleroderma, keloidosis, gingivitis, nasal polyposis, and wound healing.

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A key common feature found in fibrotic lesions including pulmonary fibrosis and wound healing is the de novo emergence of fibroblastlike cells, termed myofibroblasts, owing to their expression of significant amounts of α-smooth muscle actin and contractile properties. This myofibroblast is found in areas undergoing active fibrosis where there is extensive remodeling with active synthesis of extracellular matrix components, such as collagen. It appears that this cell is the primary cellular source of collagen as well as a major source of fibrogenic cytokines and chemokines in pulmonary fibrosis. Although platelet-derived growth factor (PDGF) and stem cell factor (SCF) are responsible for differentiating myofibroblasts from embryological stem cells in fetal development, the genesis of the myofibroblasts in adult wound healing and fibrosis appears to be regulated by a different group of cytokines.

This chapter will review the significance of fibroblast activation in fibrosis. The first part summarizes the known classes of cytokines that are either produced by fibroblasts or have an effect on fibroblast function. The second part will review some important issues in fibroblast heterogeneity and the role of myofibroblasts in the pathogenesis of pulmonary fibrosis.

II. Overview of Fibroblasts as Effector Cells in Pulmonary Fibrosis

The fibroblasts of the lung along with the myofibroblasts are derived from mesenchymal tissues and localized primarily in the interstitial tissue space (1). These cells can be distinguished from each other based on differences in morphology, contractile protein content composition, and extracellular matrix production. Myofibroblasts express α-smooth muscle actin and have a phenotype intermediate between fibroblasts and smooth muscle cells (2–6). However, these cells are heterogeneous with respect to their cytoskeletal phenotype, especially those found in various fibrotic diseases and chronic lesions (3–7). Variability in many properties of fibroblasts, including those derived from lung tissue, has been reported (4). Structural and functional differences have been reported in fibroblasts from different species, from different tissues within a species, and even from the same tissue taken from donors of different ages (8–10).

The past decade has seen a considerable change in how the fibroblast is viewed in terms of its in vivo role under normal and disease conditions. Early studies focus on the ability of fibroblasts to elaborate interstitial collagens and other extracellular matrix components. Abnormal deposition of these connective tissue components has long been known to be a stereotypical feature of the fibroblast in fibrotic lesions. Pulmonary fibrosis is a stereotypical response to a variety of insults to the lung and is characterized by a persistent alveolitis, derangement of normal alveolar architecture, and abnormal

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accumulation of fibroblast and extracellular matrix in the interstitium and alveolar spaces (11–12). Experimental studies have attempted to reproduce these components of pulmonary fibrosis in humans, and one that fit some of these criteria is the model using the antitumor antibiotic bleomycin. Above certain cumulative doses, this agent causes pulmonary fibrosis in humans and experimental animals (13–20). It is known to induce increased lung fibroblast net collagen synthesis in vitro and in vivo (15,18,20,21). The use of specific antibodies to the various collagen types shows selective increases in types I, III, and V (22–24). More recent studies have also shown increases in type VI collagen in pulmonary fibrosis, with no evidence for differential regulation of gene expression for α1(VI) and α3(VI) subunits (25). Collagen VI mRNA is expressed by fibroblasts, mostly with myofibroblast characteristics, and coexpressed with collagen type III rather than type I in pulmonary fibrosis.

As part of the remodeling process in fibrosis, the increased and abnormal deposition of collagen is likely a result of alterations in both synthesis and degradation (26–28). Indeed, there is higher expression of tissue inhibitor of metalloproteinase (TIMP) types $1, 2, 3, 4$ compared to collagenases in lung tissue of patients with pulmonary fibrosis, supporting the hypothesis that a nondegrading fibrillar collagen microenvironment is prevalent in this disease (29). Because the fibroblast/myofibroblast is the primary cellular source of interstitial collagens, the increased collagen biosynthesis by these cells is considered a key factor in the formation of the fibrotic lesion and its propagation (6,18,30).

In addition to being the primary source of the extracellular matrix in fibrotic lesions, the fibroblast expresses and responds to certain classes of cytokines relevant to both inflammation and fibrosis. Many of these cytokines may be responsible for the altered phenotypic characteristics of fibroblasts from fibrotic lungs, such as its profibrotic secretory phenotype, with a lower growth rate plus increased spontaneous apoptosis and the expression of collagen, transforming growth factor-β (TGF-β), gelatinase B, and all TIMPs (31). Thus, cytokine production by fibroblasts should be considered to be as a potentially equally important factor as extracellular matrix production in the pathogenesis of pulmonary fibrosis. It is the production of such cytokines that characterizes the fibroblast as more of an effector cell than perhaps its role as a source of matrix components. It is indisputable, however, that secretion of both cytokines and matrix would have important functional consequences on neighboring cells of import to both inflammation and fibrosis.

III. Cytokines in Pulmonary Fibrosis

Cytokines are critical to a myriad of fundamental homeostatic and pathophysiological processes such as fever, wound healing, inflammation, tissue

repair, and fibrosis (11,16–18,30–38). They play important roles in regulating cell function such as proliferation, migration, and matrix synthesis. It is the balance or the net effect of the complex interplay between these mediators which appears to play a major role in regulating the initiation, progression, and resolution of tissue repair processes. Failure to resolve or abnormal repair results in fibrosis. Various cytokines, both promoting and inhibiting inflammation and/or fibrogenesis, have been implicated in the pathogenesis of fibrosis. Since the other specialized chapters on cytokines have reviewed this topic in detail, this chapter will focus primarily on the role of cytokines as it relates to the fibroblast/myofibroblast.

The potentially relevant roles of cytokines and fibroblast source in pulmonary fibrosis are summarized in Table 1. Pulmonary fibrosis and animal models of lung injury and fibrosis commonly exhibit evidence of alveolar epithelial injury, which occur early in the acute inflammatory phase and are subsequently followed by recruitment of monocytes/macrophages, lymphocytes, and eosinophils into the lung (Fig. 1). During the inflammatory phase, important roles for macrophages and monocytes have been proposed on the basis that they represent important sources of fibrogenic cytokines and growth factors such as tumor necrosis factor-α (TNF-α), TGF-β, PDGF, and endothelin-1 (ET-1) (32–39). In addition, chemokines including the factor regulated upon activation in normal T-cell expressed and secreted (RANTES), and macrophage inflammatory protein (MIP-1α) also play important roles in the pathogenesis of pulmonary fibrosis. As in wound healing and tissue repair, these cytokines stimulate and recruit other inflammatory cells (e.g., lymphocytes, monocytes, and polymorphonuclear leukocytes) into the lung. These effector cells could in turn release the same and additional cytokines, which stimulate fibroblasts to replicate, differentiate to myofibroblasts, release cytokines and synthesize collagen and other extracellular matrix components, and reduce breakdown of collagen (Fig. 2).

IV. Fibroblasts and Cytokines

Fibroblast migration, proliferation, extracellular matrix synthesis and degradation, all of which play important roles in chronic inflammation and fibrosis, are regulated by various growth factors and cytokines. Among them are specific fibrogenic cytokines and growth factors that have been proved to be important in the pathogenesis of pulmonary fibrosis in animal models. Some of these are of potential autocrine/paracrine regulatory significance, since they are synthesized by the fibroblast itself. For the purposes of this chapter, the polypeptide growth factors (such as TGF-β, PDGF), cytokines (such as TNF-α and interleukins), and chemokines (such as MCP-1 and MIP-1α) will be generically referred to as cytokines. Also in such a limited

Table 1 Key Cytokines Expressed by Fibroblasts or with Effects on Fibroblasts[a]

		Major biological activities						
Cytokines	A	Induced by	Induces	Proliferation	Chemotaxis	Collagen synthesis	Fibrogenic in vivo	Refs.
$TGF\beta_1$	Y	MCP-1	FGF, TGFβ, IL-1	±	+	+ +	+	33–75
PDGF	Y	FGF, TGFβ, IL-1	U	+	+	+	+	95–126
FGF2	Y	U	U	+	+	+	+	127–135
EGF/TGFα	Y	U	PDGF	+	0	+	+	149–166
TNFα	Y	U	IL-1, -6, -8, MCP-1	+	0	+	+	77–94
IL-1	Y	TNFα, IFNγ	IL-6, IL-8	+	0	+	+	180–190
IL-4	U	U	GM-CSF, G-CSF	+	+	+	+	191–211
IL-6	Y	IL-1β, TNFα	MIP-1α, IL-8	+	+	+	+	219–229
IL-10	N	U	Il-1, 6,8, TNFα,	−	+	−	±	245–252
IL-12	N	U	Il-10, IFNγ		−	−	±	253–257
IFNγ	N	U	IL-1	−	−	−	−	267–276
IL-8	Y	TNFα, FASL	U	+	+	+	+	230–237
MCP-1	Y	IL-1β, TNFα	TGFβ	−	+	+	+	282–290
M1P-1α	Y	U	U	−	+	+	+	291–292

[a]A (Made by fibroblasts?); Y (Yes); N (No); U (Undetermined); ++ (Major role or strongly stimulatory); + (stimulatory); − (inhibitory); ± (variable - dose-dependent); 0 (no effect).

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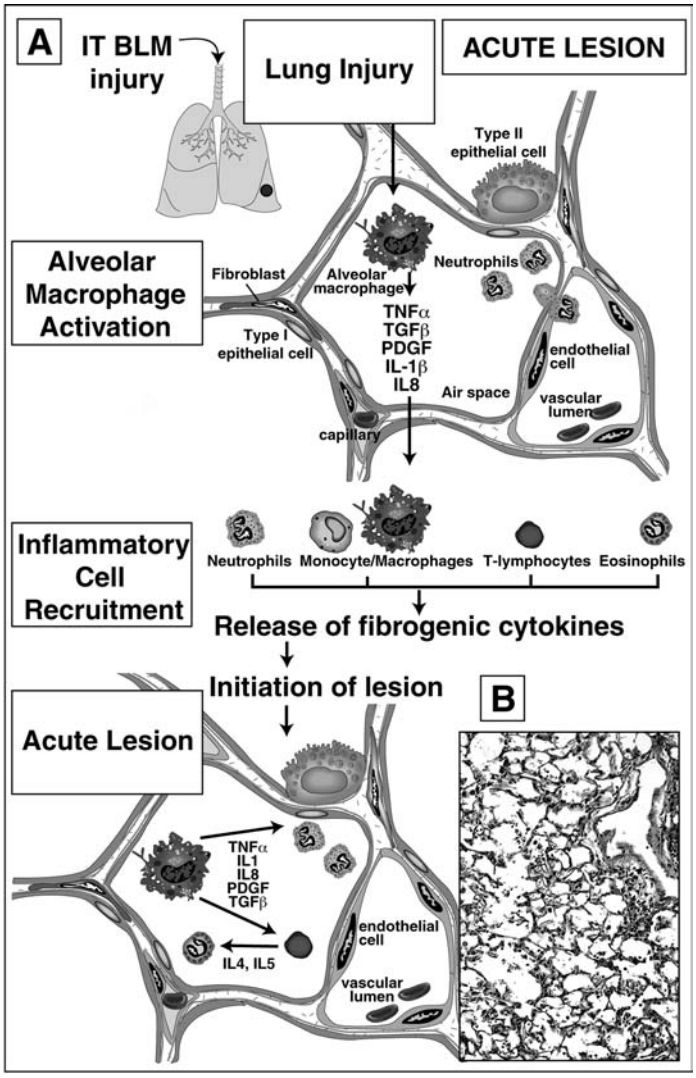

Figure 1 Summary of cellular networks in acute lung injury and inflammation. Depending on etiological and other factors, many forms of pulmonary fibrosis are associated with some degree of lung injury, which may represent an initiating or enhancing factor in the fibrotic process. The events summarized by this schematic drawing are deduced from many human as well as experimental animal model studies. (A). Acute lung injury, such as by an endotracheal administration of bleomycin (BLM), elicits an inflammatory reaction and rapid recruitment of neutrophils to the lung followed by activation and recruitment of mononuclear cells. Activated alveolar macrophages release chemokines and cytokines, leading to recruitment and activation

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review, only several of the more important cytokines can be briefly discussed vis-à-vis their potential importance in fibroblast/myofibroblast pathobiology within the context of pulmonary fibrosis (summarized in Table 1).

A. TGF- β

Transforming growth factor-β is a family of multifunctional cytokines that includes at least five known isoforms, three of which are expressed by mammalian cells (TGF-β1−3) (40,41). These isoforms arise from different genes, and the promoter structures and regulatory elements for them are different (42). Most of the transforming growth activity is accounted for by TGF-β1, and TGF-β3. TGF-β1 is a multifunctional polypeptide produced by a wide variety of cells and capable of regulating cell proliferation and differentiation as well as synthesis of many components of the extracellular matrix (43–44). With respect to pulmonary inflammation and fibrosis, TGF-β1 has a broad spectrum of activities, including being a chemoattractant for fibroblasts and monocytes/macrophages (45–47) and an activator of these cells to synthesize a number of cytokines such as TNF-α, PDGF, IL-1β, and TGF-β1 itself (48). The production of these cytokines in turn contributes to the fibrotic processes by their effects on both fibroblast and inflammatory cell function (see Fig. 2). TGF-β is one of the most potent inducers of extracellular matrix production, including collagens I, III, V, fibronectin, proteoglycans, and other components (49), whereas at the same time reducing the breakdown of collagen and other matrix proteins by stimulating protease inhibitor expression (29). TGF-β1 and TGF-β3 appear to be equally potent in increasing extracellular production in lung fibroblasts (50). This TGF-β–induced activation of fibroblasts parallels their differentiation into myofibroblasts (3,51), which have been shown to be a key source of collagen and certain cytokines in fibrotic lung tissue (see Fig. 2). In these studies, myofibroblasts identified by their expression of α-smooth muscle actin are shown to be key sources of procollagen I as well as TGF-β1 itself and the chemokine MCP-1 (51–59). Interestingly, these cells have been found to be susceptible to Interleukin-1β (IL-1β)–induced apoptosis in vitro (51). On the basis of these functional properties and morphological features, these cells appear to play

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of lymphocytes and monocytes. Activated T cells and infiltrating monocytes/macrophages release additional fibrogenic cytokines and chemokines, leading to recruitment of additional leukocytes, including in certain situations, eosinophils, to the site of injury. (B) Corresponding light micrograph of a lung section from a day 7 post–bleomycin-treated animal illustrating the early inflammatory cell infiltration into the interstitial and alveolar space, with evidence of interstitial edema and injury to the alveolar wall. Hematoxylin and eosin (H&E) stained section photographed at 400× magnification.

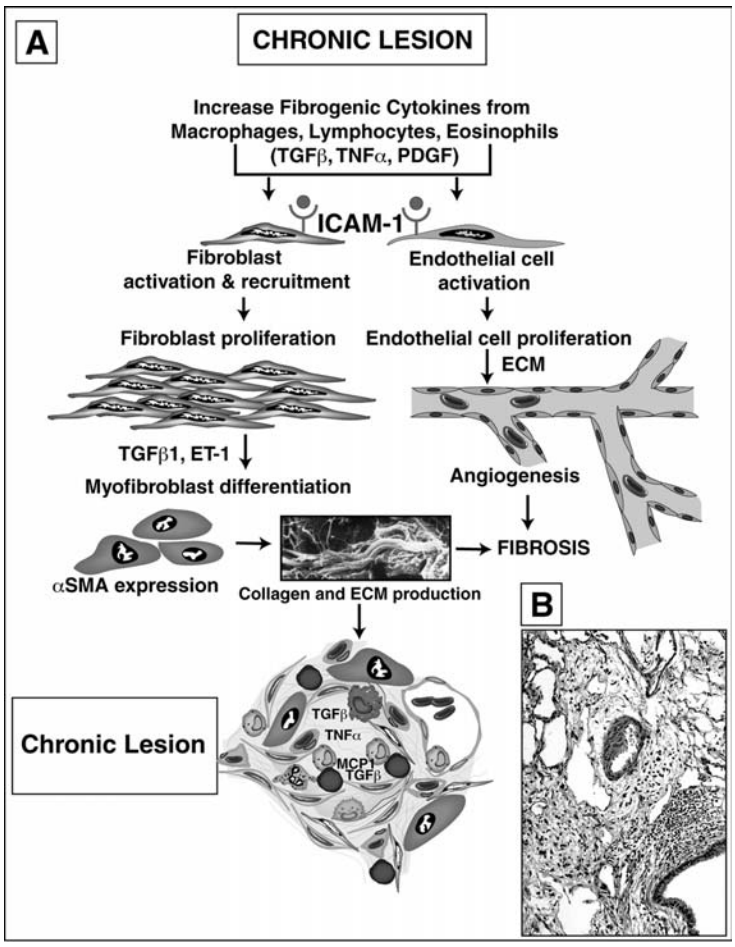

Figure 2 Summary of cellular and cytokine networks in chromic stages of lung injury and fibrosis. Progression from the acute phase of lung injury is accompanied by attempts at tissue repair and remodeling, which may result in chronic progressive fibrosis. (A) Increased fibrogenic cytokine expression by macrophages, lymphocytes, and eosinophils (when present), results in activation and recruitment of fibroblasts and endothelial cells. This leads to the expansion of lesion via activation and proliferation of fibroblasts and endothelial cells, inducing extracellular matrix synthesis and perhaps angiogenesis. Fibroblast activation, differentiation to myofibroblast, and enhanced matrix synthesis are key feature of this period of active fibrosis. (B) Light micrograph of lung section from a day 14 post–bleomycin-treated animal shows the characteristic features of increased mesenchymal cell infiltration, distortion of alveolar architecture due to intra-alveolar and interstitial fibrosis, and abnormal increased deposition of matrix. At this stage, chronic inflammation remains evident. H&E stained section photographed at 400× magnification.

critical roles in the increase in extracellular matrix deposition and increased contractibility of lung parenchyma, which are associated with pulmonary fibrosis (52–57). Thus, this property of TGF-β vis-à-vis myofibroblast differentiation represents a key profibrogenic activity of this cytokine. This is further discussed in the section on myofibroblasts below. Another important property of TGF-β is its ability to stimulate or inhibit proliferation of a variety of cell types including mesenchymal cells. The effects are dependent on its concentration, its cell density, and the presence of other growth factors (60,61). The importance of these cell growth effects in the context of pulmonary fibrosis has not been directly established, partly because of the myriad of other activities exhibited by this cytokine.

In the lung, TGF-β is produced by numerous cell types, including fibroblasts, alveolar macrophages, eosinophils, and endothelial and epithelial cells (33,61–62). It appears that a number of cytokines may mediate its cellular effects indirectly via endogenous TGF-β in autocrine and/or paracrine fashion. For example, certain agents such as MCP-1 stimulate collagen synthesis by inducing endogenous TGF-β1 expression, which could be inhibited by suppression of endogenous TGF-β1 gene expression using antisense oligonucleotides (63,64). In vivo, this potent role of TGF-β as a direct inducer of extracellular matrix synthesis is supported by evidence of its elevated expression in lungs undergoing pulmonary fibrosis, which precedes the noted increase in lung collagen expression (33). Studies of lung tissue from patients with pulmonary fibrosis show that TGF-β is increased in alveolar walls at the sites of active fibrosis and matrix deposition. In addition, overexpression of active TGF-β1 by transfection with an adenoviral vector in the lungs of the fibrosis-resistant TNF-α receptor knockout mice induces lung fibrosis (65). Moreover, administration of TGF-β antibodies decreases lung collagen content in a murine model of pulmonary fibrosis. Increased production of TGF-β1 has been associated with fibrotic processes in many organs as well (33–37,66–72). Additionally, administration of soluble TGF-β receptor type II to the injured carotid artery reduces neointimal lesion formation (73). In addition to regulating TGF-β by neutralizing with antibodies or soluble receptors, recent attempts have focused on modulation of intracellular TGF-β signaling. For example, TGF-β signaling via the Jun D isoform of activator protein-1 (AP-1) is found to be important in mediating its effects on collagen synthesis in lung fibroblasts (74). Both TGF-β induced signaling and collagen deposition are inhibited by cyclosporine A and interferon-γ treatments (74). Finally, an additional fibrogenic property of TGF-β may be related to its differential effects on fibroblast eicosanoid production and proliferation. Prostaglandin E_2 (PGE_2) is a potent inhibitor of fibroblast proliferation and collagen gene expression. However, this property seems to be lost in lung fibroblasts from patients with pulmonary fibrosis, which is associated with diminished induction of cyclooxygenase-2 by TGF-β (75). Thus, TGF-β may

stimulate collagen synthesis without having an inhibitory effect on fibroblast proliferation, making it a much more potent fibrogenic factor in this condition.

Activin is a member of the TGF-β superfamily with diverse biological activity. Human fetal lung fibroblasts (HLF-1) express activin type I and II receptors on the surface. Activin A exerts its biological activity on this cell through binding to its specific receptors. Activin A stimulated collagen gel contraction and structural remodeling similar to that in pulmonary fibrosis (76).

B. TNF-α

TNF-α and TNF-β are produced primarily by monocytes and lymphocytes, respectively (77,78). TNF-α is known to stimulate fibroblast proliferation, and is chemotactic for and activates macrophages and neutrophils. TNF-α has potential roles in tissue remodeling by modulating fibroblast extracellular matrix degradation (79) and migration into a collagen gel (80). TGF-β, a cytokine known strongly to stimulate the production of collagen by fibroblast, is induced in endothelial cells and bronchiolar-alveolar epithelial and mesenchymal cells by TNF-α (81–83). Other cytokines induced by TNF-α include IL-1, IL-6, and MCP-1. This spectrum of biological activities suggests important direct and indirect roles for TNF-α in wound healing and tissue remodeling. With respect to potential direct roles in fibrogenesis, both TNF-α and TNF-β have potent mitogenic activity for fibroblasts, which is downregulated by IFN-γ. TNF-α–treated fibroblasts exhibit phosphorylation of a number of cytosolic proteins at tyrosine, threonine, and/or serine residues, but the mitogenic response correlates well with the increased stimulation of tyrosine phosphorylation (84). In addition to proliferative effects on fibroblasts, TNF-α induces IL-8 and MCP-1 expression in these cells (85). TNF-α induces NO release from mononuclear cells, which may mediate its proinflammatory effects, and thus secondarily its profibrotic activity (86).

There is abundant evidence to implicate TNF-α in the pathogenesis of pulmonary fibrosis. First, increased expression in the lungs of patients with pulmonary fibrosis and in animal models of pulmonary fibrosis is well documented (35). Given its proinflammatory activity in the lung (87,88), it is not surprising that TNF-α receptor knockout mice fail to develop lung injury associated fibrosis (89,90). Administration of neutralizing anti–TNF-α antibodies or soluble TNF-α receptor constructs attenuates pulmonary fibrosis in animal models (35,91,92). Interestingly, inhibition of pulmonary fibrosis by anti–TNF-α antibodies is accompanied by suppression of TGF-β1 and IL-5 expression, as well as reduced eosinophilia (35), suggesting the importance of TNF-α as a key upstream mediator of both eosinophilic inflammation and fibrosis. This role may be mediated by its previously noted ability to induce

cytokine expression in a variety of cell types. TNF-α is important in fibrotic disease in other organs, but paradoxically (vis-à-vis fibrosis), it also seems to play a role in pulmonary emphysema through lung inflammation and activation of the elastolytic enzymes (93). Finally, a basis for heightened TNF-α expression in pulmonary fibrosis may be due to dysregulated gene expression (94).

C. PDGF

The PDGF family consists of dimers of two polypeptide (A and B) chains encoded by separate genes, which in the lung are produced by virtually all cell types, including fibroblasts, macrophages, and endothelial, smooth muscle, and epithelial cells (95). Of significance to the pathogenesis of fibrosis, PDGF is a major mitogen and chemoattractant for cells of mesenchymal origin, including fibroblasts and smooth muscle cells, as well as inflammatory cells, including neutrophils and macrophages (96–111). It has been reported to increase procollagen synthesis, collagenase activation, and fibronectin gene expression (111,112). Of importance to tissue remodeling, PDGF can also induce interstitial matrix metalloproteinases (MMPs) including MMP-1, -3, and -9 (113). Additionally, heparin, TIMP-1, and TIMP-2 can inhibit PDGF-stimulated lung fibroblast chemotaxis (114). Heparin inhibits PDGF-induced lung fibroblast proliferation, whereas angiotensin II induces proliferation (115). The ability of heparin and related glycosaminoglycans to inhibit fibroblast chemotaxis and proliferation may be due to an inhibitory effect on MMP activity, suggesting a potential role in extracellular matrix remodeling in inflammatory lung disease. A potential complication in analyzing the role of PDGF in pulmonary fibrosis is the observation that PDGF isoforms differ in their functional effects on fibroblasts (97,98) and bind to different receptor subtypes (96,98–100). Their differential in vivo importance is highlighted by studies using isoform-specific knockout mice. Studies using PDGF-A knockout mice reveal that PDGF-AA and its receptor are essential for the development of myofibroblasts in the lung, and surviving offspring develop fetal emphysema (101). PDGF-Rα is also essential for maximal mitogenic and chemotactic responses to PDGF (102–104).

There is ample evidence to support the importance of PDGF in fibroproliferative diseases such as pulmonary fibrosis, obliterative, bronchiolitis, and atherosclerosis (116–119). Increased lung expression of both isoforms in pulmonary fibrosis has been well documented (120–123). The pathogenic significance of this elevated expression is suggested by studies showing that inhibition of fibrosis by certain agents correlates with inhibition of PDGF expression (124,125). Upregulation of PDGF-Rα expression has been reported in a model of pulmonary fibrosis, and may be a key mechanism for lung myofibroblast hyperplasia and development of fibrosis (119).

The importance of signaling via PDGF receptors is underlined by inhibition of fibrosis by treatment with a PDGF receptor–selective tyrosine kinase inhibitor (126).

D. Fibroblast Growth Factors

Originally, the fibroblast growth factors (FGFs) consisted of two isoforms, one with an acidic isoelectric point (aFGF) and the other with basic isoelectric point (bFGF). Recently, with identification of seven additional FGFs, the family has gained a new nomenclature, acidic FGF is now FGF-1, basic FGF is FGF-2 and new forms are FGF-3 to FGF-9 (127,128). FGFs are key growth factors involved in angiogenesis, directing endothedial cell migration, proliferation, and plasminogen activator synthesis (129–131). Stimulation of virtually all cells involved in wound repair has been demonstrated, and play important roles in the genesis of granulation tissue (132). bFGF is chemotactic for a number of cells involved in granulation tissue formation, and induces collagenase production by fibroblasts (133). Recently, FGF-2 has been found to induce telomerase expression in rat lung fibroblasts, especially those isolated from injured and inflamed lungs (134,135). The significance of telomerase expression is uncertain, but may be related to the increased proliferative capacity of fibroblasts and the expanded fibroblast population in fibrotic lungs.

E. Insulinlike Growth Factor

The insulinlike growth factors (IGFs) consist of two key members, a basic IGF-I and a neutral peptide IGF-II (136). IGFs and their receptors are expressed by most tissues, especially fetal and postnatal lung fibroblasts as well as macrophages (137). IGF-I is highly mitogenic, and it may act as an autocrine or paracrine growth factor involving cellular proliferation and differentiation, these properties often being exerted in concert with other cytokines. IGF-I may play a role in pulmonary fibrosis, since its autocrine production is partly responsible for fibroblast proliferation after exposure to PDGF (138). In the dual control model cycle, fibroblast proliferation needs the additive effects of two signals: a competence and progression signal (139). PDGF is a competence factor and IGF-1 is a progression factor (139,140). Increased expression of IGF-I and PDGF in patients with pulmonary fibrosis has been shown (141–144). IGF-I expression in the lung localizes to macrophages, alveolar epithelial cells, and ciliated columnar epithelial cells (145). In addition, the level of IGF-binding proteins 3 and 9, major serum binding proteins that enhance the action of IGF-I, is significantly increased in bronchoalveolar lavage fluid from patients with pulmonary fibrosis as compared to healthy controls (146). Increased lung IGF-I and PDGF expression is found also in pulmonary sarcoidosis and silicosis, as well

as in animal models of fibrosis (144,147). An additional role for IGF-I in pulmonary fibrosis may be mediated by its ability to stimulate fibroblast collagen production (148). In summary, IGF-I is likely to play an important role in the pathogenesis of pulmonary fibrosis by its ability to stimulate fibroblast proliferation as well as collagen and laminin gene expression, thus contributing to the overall deposition of extracellular matrix and connective tissue formation.

F. Epidermal Growth Factor/TGF-α

TGF-α, a member of the epidermal growth factor (EGF) family that includes TGF-α, EGF, and heparin-binding EGF (HB-EGF) could play an important role in modulating the proliferative and fibrotic responses of the injured lung, with well-known effects on fibroblast proliferation. Human TGF-α shares some homology with human EGF and HB-EGF (149) and binds to the EGF receptor (150). TGF-α is chemotactic for fibroblast and stimulates cellular proliferation, including fibroblasts and epithelial cells, and endothelial cells (151–153). Activation of the EGF receptor stimulates collagen synthesis (154,155). Additionally, TGF-α induces the expression the MMP-1, -2, -3, -9, and TIMP-1 and TIMP-3 by fibroblasts and epithelial cells (156–159). TGF-α mRNA is present throughout prenatal lung development (160). In human fetal and postnatal lung, TGF-α immunolocalizes to airway and alveolar epithelial cells and to vascular smooth muscle (161). Although it appears to be active in lung development, there is evidence that TGF-α also participates in diseases where there is significant lung remodeling. Recent studies show elevated TGF-α expression in the vascular endothelium, type II cells, and fibroblasts of lungs from pulmonary fibrosis patients (162). It appears that TGF-α expression may be upregulated early in response to acute lung injury (163), which may be important in driving subsequent fibrosis. Animal model studies provide some support for such a possibility. Thus, early upregulation of lung TGF-α expression upon bleomycin-induced lung injury precedes increased lung collagen synthesis characteristic of the active fibrosis phase (164). Its importance in driving fibrogenesis is supported by the observation that transgenic mice expressing human TGF-α under control of regulatory regions of the human surfactant protein-C gene develop pulmonary fibrosis. Further support is provided by studies showing reduction of bleomycin-induced pulmonary fibrosis in TGF-α–deficient animals despite normal levels of expression of other EGF family members (165). Finally, studies using tyrphostins, AG1296, or AG1478 that specifically block autophosphorylation of PDGF or EGF receptors, respectively, show inhibition of the proliferative response of epithelial and mesenchymal cells in fibrotic lesions, and is accompanied by reduced lung collagen synthesis (166). Taken together the evidence suggests that TGF-α may play a role in fibrogenesis by recruiting and

stimulating fibroblasts, promoting their proliferation, and subsequent increased extracellular matrix production.

G. Granulocyte-Macrophage Colony-Stimulating Factor

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a 23-kD glycosylated protein member of the hemopoietic cytokine family that is expressed by a number of lung cell types, especially lung fibroblasts following stimulation by TNF-α or IL-1 (167,168). It is chemotactic for granulocytes and monocytes, induces activation and proliferation of these cells, and stimulates myeloid stem cell differentiation to granulocytes, monocytes, and macrophages (169). Additional diverse activities include stimulation of macrophage IL-1RA production (170) and keratinocyte proliferation (171–172). Clues of its potential importance in fibrosis are suggested by studies of transgenic mice overexpressing GM-CSF in the lung, which show increases in lung growth and exhibit type II cell hyperplasia (173). However, administration of neutralizing antibodies to GM-CSF results in increased lung collagen synthesis in bleomycin-induced pulmonary fibrosis, which is consistent with the observation of early reduced lung GM-CSF expression in this model (174,175). Similarly, GM-CSF knockout mice exhibit an enhanced bleomycin-induced pulmonary fibrosis without affecting lung inflammation, although lung levels of the antifibrotic lipid prostaglandin E_2 (PGE_2) are diminished in knockout mice (176). These findings seem to suggest an antifibrotic role for GM-CSF, possibly mediated by PGE_2. However, other studies appear to conflict with such a conclusion. Thus, subcutaneous administration of GM-CSF to rats induces fibroblast proliferation and α-smooth muscle actin expression indicative of myofibroblast differentiation (177). Such an effect would be consistent with a profibrogenic role, since this myofibroblast phenotype is characteristic of lung fibrosis as well (178). More direct evidence of such a profibrogenic activity is provided by studies in which lung-localized overexpression of GM-CSF by a recombinant replication-deficient adenoviral vector elicits a significant eosinophilic and mononuclear cell response and fibrosis (179). Thus, the actual role of GM-CSF in pulmonary fibrosis remains controversial, and its effect on the fibroblast appears to be indirect, possibly mediated by TGF-β (176).

H. Interleukins

The interleukin (IL) family consists of several members, originally known for their regulatory effects on leukocytes. The limited review here will focus on selected interleukins and their known effects on fibroblasts/myofibroblasts or their expression by these cells, which may be of potential relevance to the pathogenesis of pulmonary fibrosis.

IL-1

The three known constituents of the IL-1 gene family are IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1RA), which all binds to the same receptor (180). They are synthesized as inactive precursor molecules, and are expressed predominantly by mononuclear cells (181). Of direct relevance to fibrosis are the known direct effects of IL-1 on fibroblast proliferation and collagen gene expression, which are both complicated by simultaneous endogenous stimulation of PGE_2, an inhibitor of both fibroblast proliferation and on collagen synthesis (182). Additionally, IL-1 is chemotactic for fibroblasts, and potently regulates their production of other cytokines, including IL-6, IL-8, TNF-α, MCP-1, and GM-CSF (182–185). Paradoxically, IL-1β selectively induces NO-dependent apoptosis in myofibroblasts (52). In vivo, IL-1 can act synergistically with TNF-α to mediate sepsis and inflammation, as well as a number of other biological effects (186), including fever, and serves as a chemoattractant for monocytes, macrophages, neutrophils, and leukocytes (187). It is this complex spectrum of IL-1 activities in conjunction with other cytokines that could result in enhanced fibroblast proliferation and myofibroblast differentiation leading to the development of progressive fibrosis (46–48, 188,189). This conclusion is supported by the recent observation that transient lung overexpression of IL-1β by adenoviral gene transfer induces acute inflammation and lung fibrosis that is associated with upregulation of lung PDGF and TGF-β expression (190). Nevertheless, owing to the complex biological effects of this cytokine, its actual role in pulmonary fibrosis remains uncertain.

IL-4

IL-4 is a proinflammatory cytokine with stimulatory activities on fibroblasts as well as mononuclear cells (191). Its regulatory effects on B-cell function and the promotion of proliferation of IL-2–dependent T-Cell lines are well known (192), whereas its receptor, IL-4R, is expressed by a variety of hematopoietic cells and fibroblasts (34,38,193–195). It is a key member of the Th2 family of cytokines (193,194), and regulates B-cell IgE production (196,197) and the switching of naïve cells to the Th^2 phenotype (198–201). Of relevance to pulmonary fibrosis is the observation of elevated IL-4 and IL-5 expression by infiltrating inflammatory lung cells in lungs of patients with pulmonary fibrosis (202–205). Expression of both these cytokines is also increased in scleroderma-associated pulmonary fibrosis (206,207). Potential roles for IL-4 in fibrosis are suggested by its ability specifically to increase lung fibroblast adhesion molecule and inflammatory cytokine expression, as well as to stimulate expression of extracellular matrix, including collagen and promote chemotaxis and myofibroblast differentiation (191,208–211). Bleomycin-induced lung injury induces significant early elevation of lung IL-4 expression, primarily

by macrophages and T lymphocytes is areas of active fibrosis (38). Thus, such upregulated expression by these cells may cause direct effects on fibroblasts, inducing them to proliferate and differentiate to myofibroblasts (see below) and upregulate TGF-β expression and extracellular matrix expression, thus promoting fibrosis.

IL-5

IL-5 is another member of the Th2 family of cytokines and has a number of important regulatory effects on eosinophils, T cells, and B cells (212–215). It is important in regulating eosinophil differentiation, migration, activation, and chemotaxis (212,216). It plays a role in Th2-dependent disease processes and immune responses, such as in lung eosinophilia associated with allergic inflammation (217). The potential importance of eosinophils vis-à-vis pulmonary fibrosis is suggested by its presence in lungs with certain types of fibrosis, and rests on its ability to express fibrogenic cytokines. Eosinophilia is prominent in a rodent model of bleomycin-induced pulmonary fibrosis, coinciding with the period of active fibrosis and heightened cytokine gene expression. Using dual staining for eosinophil marker and cytokine expression, expression of TGF-β1, MCP-1, and IL-5 by eosinophils can be demonstrated directly, and further shows that this cell type represents a key cellular source of these cytokines at the peak of eosinophilia (11,17,33,34,36). Studies with the same animal model show that IL-5 is essential for lung eosinophilia, and full expression of the fibrogenic response, as assessed by fibrogenic cytokine expression, myofibroblast differentiation, and heightened collagen gene expression (36). However, IL-5 appears to be unnecessary for rapid scarring due to massive lung injury with high acute mortality (218). In summary, IL-5 may play a role by virtue of its ability to recruit and activate eosinophils to secrete a number of proinflammatory and fibrogenic cytokines, as well as promote tissue injury via release of oxidants and harmful proteins.

IL-6

IL-6 is a multifunctional cytokine with important roles in the acute phase response and the regulation, activation, and differentiation of T cells and B cells (219,220). It is produced by most nucleated cells, including fibroblasts, upon stimulation by IL-1, TNF-α, PDGF, and TGF-β, alone or in combination (219–221). It can activate endothelial cells to recruit leukocytes, and stimulate the production of collagen and glycosaminoglycan in cultured human dermal fibroblasts (222,223). In view of these activities, it is logical that its role in the development of inflammatory tissue damage and fibrotic tissue remodeling has been suggested (224). High levels of IL-6 are present in the serum and synovial fluids of patients and animals with rheumatoid arthritis (RA) and in the serum of patients with progressive systemic sclerosis

(225–227). IL-6 expression is elevated in cultured fibroblasts from patients with progressive systemic sclerosis; potentially due to pathogenic B-cell stimulation (226,228). An additional potential role for IL-6 in pulmonary fibrosis is its ability to upregulate MIP-1α expression, a cytokine known to be important in an animal model of fibrosis (229).

IL-8

IL-8 is a member of the CXC chemokine family and a potent chemoattractant and activator of neutrophils and T lymphocytes, and thus serves to amplify the inflammatory cascade (230,231). It is produced by a wide variety of cell types including mononuclear cells, endothelial cells, epithelial cells, and fibroblasts in response to stimulation by certain cytokines, such as TNF-α (232–234). Of special interest is its increased expression by alveolar macrophages and elevated concentration in bronchoalveolar lavage fluid from patients with pulmonary fibrosis (235). Interestingly, increased expression of IL-8 in the serum of such patients correlates significantly with impairment of lung function (236). In addition to promoting inflammation, an additional potential role for IL-8 in fibrosis may lie in its angiogenic activity (237).

Other Interleukins

IL-9 is a T-cell–derived cytokine of the Th2 family originally discovered as a growth factor for activated T cells and mast cells (238,239). It binds to a receptor of the cytokine kinase receptor superfamily that is present on subsets of T and B lymphocytes, mast cells, and macrophages (240). Naïve T cells do not respond to IL-9, but upon activation they express IL-9 receptor and proliferate in response to IL-9 (241). It is involved in the development of T-cell lymphomas, and stimulates proliferation of hematopoietic progenitor cells (242). Increased IL-9 production in the lungs results in bronchial hyperresponsiveness and airway inflammation (243). Despite being a Th2 cytokine, which tend to be profibrogenic (243), IL-9 overexpressing transgenic mice exhibit reduced silica-induced pulmonary fibrosis relative to wild-type controls (244). Thus, IL-9 paradoxically seems to be playing an antifibrogenic role; at least in this animal model.

IL-10 is another Th2 cytokine that is known to suppress inflammation, possibly by downregulating expression of a number of proinflammatory cytokines and inducing activated neutrophil apoptosis (245–249). With regard of fibroblast effects, IL-10 suppresses collagen production and proliferation in these cells. Consistent with such a potential antifibrotic role, lower levels of IL-10 protein are found in bronchoalveolar lavage fluid from pulmonary fibrosis patients compared with those from healthy control subjects, which may explain the enhanced pulmonary inflammation and fibrosis in affected patients (250). Its antifibrotic activity is confirmed in an animal model study showing

reduction in bleomycin-induced lung injury by introduction of the IL-10 gene (251). It appears that IL-10 also has a direct inhibitory effect on constitutive and TGF-β–stimulated collagen expression by lung fibroblasts (251). In contrast to the findings in this animal model, however, silica-induced pulmonary fibrosis in IL-10 knockout mice is impaired relative to wild-type mice, suggesting a profibrotic role for IL-10, at least in this animal model (252).

IL-12 is a key regulatory cytokine driving Th1 cell development, promotes growth and activation of T lymphocytes and natural killer (NK) cells (253). It is a heterodimeric protein (p70) composed of a 40-kD (p40) and a 35-kD subunit (p35) (254). Normal lung fibroblasts can inhibit IL-12 production by expression of IL-10, which is impaired in fibrotic lung fibroblasts resulting in a significantly reduced capacity to downregulate the expression of CD40 on monocytes as compared to normal fibroblasts (255). However, the in vivo role of IL-12 in pulmonary fibrosis appears to be controversial. In one study, administration of IL-12 attenuates bleomycin-induced pulmonary fibrosis, possibly mediated via induction of IFN-γ, an inhibitor of fibroblast proliferation and collagen production (256). However, another study using the same animal model shows inhibition of fibrosis by neutralizing anti–IL-12 antibody treatment (257). There is currently no ready explanation to account for these discrepant results.

IL-13 is a cytokine produced by activated Th2 cells, mast cell, and human alveolar macrophages (258,259) with well-known effects on primary immune cell immunoglobulin production, proliferation of B cells, and the differentiation of cells of the monocytic lineage (260). It appears to be an important mediator in the pathogenesis of asthma, hepatic fibrosis, progressive systemic sclerosis, and pulmonary fibrosis (261–264). Similar to IL-4, IL-13 is a potent stimulator of fibroblast proliferation, myofibroblast differentiation, and collagen deposition in vitro (263,264), and may mediate its in vivo lung fibrogenic effects via selective stimulation and activation of TGF-β1 (265). Its profibrogenic role is also apparent in airway wall fibrosis (266).

Interferons are cytokines produced by cells in response to stimulation by certain viruses, bacteria, antigens, and mitogens. IFN-α and IFN-β are produced by leukocytes and fibroblasts in response to viral infection (type I), whereas a third isoform, IFN-γ, is secreted by T cells, NK cells, and type II alveolar epithelial cells in response to specific antigens or mitogens (type II) (267,268). IFN-γ inhibits fibroblast chemotaxis, proliferation, and extracellular matrix production, suggesting a direct antifibrogenic property (268–271). Consistent with this property is the ability of this cytokine to inhibit an experimental model of bleomycin-induced pulmonary fibrosis (272). Thus, IFN-γ appears to have an antifibrotic therapeutic potential for controlling pulmonary fibrosis (273). A recent clinical study reports improvement in lung function by treatment with INF-γlb in combination with prednisolone in patients with idiopathic pulmonary fibrosis (274). However, other studies

suggest that the effects of IFN-γ are more complex than merely inhibiting fibrosis via its fibroblast/myofibroblast inhibitory activities. Thus, lung IFN-γ expression is actually elevated in pulmonary fibrosis, as well as in an experimental animal model of pulmonary fibrosis (275,276). Furthermore, in response to bleomycin-induced lung injury, IFN-γ knockout mice exhibited significantly less lung parenchymal inflammation, weight loss, mortality, and pulmonary fibrosis relative to wild-type control mice. These results are consistent with a proinflammatory role for IFN-γ, with perhaps secondary profibrotic roles due to inflammatory cell–derived profibrogenic cytokine expression. Thus, the effectiveness of IFN-γ as an antifibrotic therapeutic agent may be limited by this proinflammatory property, which may overcome its direct antifibrotic activity on fibroblasts.

I. Chemotactic Cytokines

Chemotactic cytokines or chemokines belonging to a supergene family that is now known to have diverse biological activities beyond chemotaxis, including regulation of complex processes, such as angiogenesis, hematopoiesis, and organogenesis (277–281). Two major groups in this family are designated as the C-C (e.g., MCP-1) and the C-X-C chemokines (e.g., IL-8) based on the location of the cysteine residues. MCP-1 and M1P-1α expression increases during wound healing and may regulate the recruitment of leukocytes and promote production of the extracellular matrix; thus potentially integrating inflammatory and reparative processes during wound healing. These chemokines are produced by a variety of cell types, including macrophages, fibroblasts, endothelial cells, and keratinocytes. With regard to pulmonary fibrosis, there is evidence of a significant role for both chemokines as well. MCP-1, known as the JE gene product in the mouse, is first identified as a growth factor that is inducible in murine 3T3 fibroblasts (282). A number of cell types including fibroblasts, macrophages, and lymphocytes have been shown to both produce and/or be modulated by MCP-1 or MIP-1α (229). MCP-1 being a potent chemoattractant for monocytes and an activator of T cells, NK cells, and immature dendritic cells (283–286), would be expected to play important roles in the chronic inflammation in a wide variety of inflammatory disease, including pulmonary fibrosis and granulomatous lung disease (17,287). MCP-1 is known also to be critical for the development of characteristic glomerular crescents and the deposition of type I collagen in crescentic nephritis (288). Additionally, there is evidence to support a direct fibrotic role for this chemokine in terms of its ability to stimulate lung fibroblast collagen gene expression upon binding to specific receptors and mediated by endogenous upregulation of TGF-β1 expression (63,289). Upregulation of lung MCP-1 expression has been documented in pulmonary fibrosis, and found to localize to mononuclear cells and eosinophils (17,290).

Treatment with anti–MCP-1 antibodies significantly reduces lung inflammation, and mice deficient in CCR2, the receptor for MCP-1, exhibit reduced pulmonary fibrosis in animal models (291). In addition, analysis of lung tissue and bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis has demonstrated elevated levels of MCP-1 and MIP-1α compared with normal individuals. MIP-1α has been implicated in the pathogenesis of pulmonary fibrosis, and mediates mononuclear phagocyte recruitment during the response to bleomycin (292). Both MCP-1 and MIP-1α are expressed in a time-dependent manner after bleomycin administration (17,292), and passive immunization of these animals with anti–MIP-1α antibodies attenuated leukocyte accumulation and attenuated fibrosis (293). It seems that MIP-1α plays an indirect role in pulmonary fibrosis via upregulation of chronic inflammation, whereas MCP-1 can potentially play both an indirect role as well as via a direct effect on fibroblast activation. Mice treated with anti-CCR1 (MIP-1α receptor) antibodies or mice deficient in CCR2 exhibit reduced lung fibrosis or atherosclerosis lesion formation respectively (294–297).

V. Myofibroblasts and Pulmonary Fibrosis

A growing body of literature over the past decade has made it evident that there is phenotypic heterogeneity among fibroblasts in fibrotic lesions (298). An activated fibroblast phenotype of relevance to tissue repair and fibrosis is characterized by smooth muscle–like features, including the expression of α-smooth muscle actin, and hence their identification as myofibroblasts (6,21,37,299). However, these cells are themselves heterogeneous with respect to their cytoskeletal phenotype and function. Their genetic program ultimately determines the response of the fibroblast to stimuli. Various factors, such as cytokines, complement proteins, extracellular matrix, and others, interact with this program to elicit specific phenotypic responses and differentiation to myofibroblasts. In general, there are several common normal activities of myofibroblasts. They represent a key component of tissue/organ growth and differentiation by secretion of cytokines, growth factors, and through secretion and formation of interstitial matrix and expression of receptors for these factors and matrix components. However, in addition to these normal developmental roles, myofibroblasts play important roles in many diseases, including developmental abnormalities in their absence (6,21,37,300–302). First studied for their role in wound healing, myofibroblasts have since been found in pathological conditions in the lung, including asthma and pulmonary fibrosis. One of the key events in wound healing is the infiltration of fibroblasts to synthesize extracellular matrix critical for tissue repair and proper healing to occur. This process involves fibroblast proliferation and differentiation into the myofibroblast. Myofibroblasts play a central role in extracellular matrix

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deposition and wound contraction, but gradually disappear as the healing process diminishes and successful tissue repair is achieved. A similar sequence of events occurs in injury and/or inflammation in other tissues undergoing fibrosis or abnormal repair. In the lung, a common feature of pulmonary fibrosis in human and animal model studies is the de novo appearance of myofibroblasts that express α-smooth muscle actin, inflammatory mediators, fibrogenic cytokines, chemokines, and growth factors as well as extracellular matrix components (2,6,21,37,51,52,54,299). It appears that the origin of these myofibroblasts is not exclusively the fibroblast, but may also potentially be derived from other types of interstitial cells as well (303–306). Furthermore, myofibroblasts are not homogeneous with respect to expression of intermediate filaments such as vimentin, desmin, or α-smooth muscle actin itself (303–306), which may perhaps account partly for their diverse origins. The focus of this chapter will be on recent information about the interactions between myofibroblasts with parenchymal cells and their role in pulmonary fibrosis. The major cytokines, growth factors, and soluble factors secreted by these cells are also discussed in the context of myofibroblast differentiation, survival, or apoptosis, and its potential importance in the progressive nature of human pulmonary fibrosis.

A. Origin of Myofibroblasts

It is not clear whether myofibroblasts originate from progenitor stem cells or simply transdifferentiate from resident tissue fibroblasts or other cells, such as vascular, intestinal, or smooth muscle cells (307–309). The close anatomical relationship of pericytes to vascular smooth muscle and intestinal myofibroblasts to intestinal smooth muscle suggests a potential bidirectional route of transdifferentiation. In the kidney, the renal tubular cells may differentiate to myofibroblasts under certain conditions (310). Although PDGF is important for myofibroblast differentiation in lung development (101), other cytokines appear to be responsible for myofibroblast differentiation in the adult lung during lung injury and fibrosis. The emergence of these cells in pulmonary fibrosis is not dissimilar to the recruitment of inflammatory cells to sites of tissue injury. The recruited inflammatory cells undergo certain phenotypic alterations when recruited to sites of injury (311). Pulmonary fibrosis in humans and in animal models is accompanied by the appearance of a new population of α-smooth muscle actin and vimentin-positive cells in the interstitium and in the intra-alveolar fibrotic foci (19,37,56,178,312). Similar cells with this myofibroblast phenotype have also been isolated from the bronchoalveolar lavage fluid of patients with scleroderma, which is absent in samples from healthy individuals (313). The cellular origin of such myofibroblasts in fibrotic tissues still is unclear. The possible candidates would include adventitial fibroblasts, smooth muscle cells of adjacent blood vessels

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or airways, or pericytes. Kinetic studies in an animal model suggest that myofibroblasts likely emerge from fibroblasts located in the adventitial areas surrounding the airways and adjacent blood vessels, perhaps under the influence of TGF-β upregulation (18,35,37,314,315). This conclusion is supported by in vitro studies showing that activation of fibroblasts by fibrogenic cytokines such as TGF-β can induce their differentiation to myofibroblasts (19,51,52,58,314,315). Additional known stimuli for differentiation of fibroblasts to myofibroblasts include endothelin, IL-4, and IL-13 (316–318). As discussed above, expression of these stimuli are upregulated in lungs undergoing active fibrosis; thus such a role for these cytokines in vivo represents additional potential mechanisms for emergence of the myofibroblast in pulmonary fibrosis.

B. Myofibroblast Heterogeneity

Lung myofibroblasts in pulmonary fibrosis are heterogeneous with respect to cytoskeletal protein expression. Thus, attempts have been made to distinguish between them based on their morphology and cytoskeletal protein expression, although the functional significance of these phenotypic differences remains unclear. Vimentin, desmin, and α-smooth muscle actin are the three components that have been used to identify myofibroblast populations (3,5,6,37,53,56,178,302,319). Based on immunohistochemical staining for these proteins, myofibroblasts express both vimentin (V) and α-smooth muscle actin (A), and thus are classified as VA. However, they are heterogeneous with respect to desmin expression (D), indicating the presence of at least two subpopulations, VA and VAD, in an experimental model of pulmonary fibrosis (37). These two subpopulations show differences with respect to tissue localization, with the VA-type myofibroblast being found predominantly in a subpleural location, whereas the VAD type tends to localize to active fibrotic foci in more central areas (37). Both subtypes, however, do express high levels of collagen; thus making it unclear if they differ significantly in terms of their overall role in pulmonary fibrosis. The basis for this heterogeneity is unknown, and may be due to a different cell of origin and/or differential regulation of desmin expression by as yet undetermined stimuli.

C. Regulation of Myofibroblast Differentiation

A hallmark of the myofibroblast phenotype is the expression of α-smooth muscle actin. The factors that regulate the emergence and fate of lung myofibroblasts in pulmonary fibrosis are not completely understood, but associated alterations in the expression of a variety of cytokines are likely to be involved. Many fibrogenic cytokines, such as TGF-β, PDGF, GM-CSF, FGF, TNF-α, and IL-4, are known to upregulate α-smooth muscle actin expression, whereas IFN-γ and IL-1β have been reported to downregulate such expression

(19,51,52,58,314,316–318,320,321). An additional factor in the activation of the myofibroblast includes the requirement for matrix molecules; namely, the ED-A (EIIIA) domain of fibronectin (322). During tissue injury, expression of this ED-A domain splice variant of fibronectin is increased, and serves as the binding site for cell membranes and other matrix molecules. It is necessary for TGF-β stimulation of α-smooth muscle actin expression and collagen secretion by myofibroblasts. Interactions between TGF-β and PDGF have also been reported with respect to generation of CTGF and expression of PDGF receptors (323). Recent studies identifying IL-1β as a major inducer of the PDGFR-α in rat myofibroblasts found that staurosporine upregulates PDGFR-α gene expression (324). The requirement for kinase signaling is also shown for TGF-β as well as IL-4 and IL-13 induction of the myofibroblastic phenotype in the human lung fibroblast (317,325). These studies show induced increases in C-Jun-NH2 terminal kinase (JNK), P38 MAPK, and extracellular signal-regulated kinase (ErK) phosphorylation, which are associated with myofibroblast differentiation that could be inhibited by certain specific kinase inhibitors. Thus, TGF-β signaling via this kinase pathway is essential for subsequent α-smooth muscle actin expression, which is indicative of myofibroblast differentiation. Downstream to this signaling mechanism, regulation of the α-smooth muscle actin gene promoter mediates ultimate gene expression. This appears to require a TGF-β control element in the promoter (2) similar to that described in smooth muscle cells (326). Subsequently, a gut-enriched Krüppel-like factor is found to bind to this element and mediate TGF-β–induced SM22 gene expression in smooth muscle cells (327). However, whether the same transcription factor is involved in α-smooth muscle actin expression in myofibroblast differentiation remains to be determined. Other known promoters of myofibroblast differentiation include endothelin, and angiotensin II (316,328). In addition to these mediators, myofibroblasts are subject to regulation by extracellular matrix components (329). This cell-matrix interaction is mediated by the usual specific cell surface receptors or integrins, heterodimeric transmembrane glycoproteins consisting of α and β chains, and with well-known important roles in wound healing and tissue repair. A potentially significant protein of interest here is vitronectin, a glycoprotein found in serum and the extracellular matrix; and of added significance is its increased expression in interstitial lung disease (330). Paradoxically, however, vitronectin is known to downregulate α-smooth muscle actin expression in human lung fibroblasts, whereas neutralizing antivitronectin antibodies stimulate α-smooth muscle actin expression (329). Other matrix proteins such as fibronectin, collagens, and laminin do not significantly affect α-smooth muscle actin expression in the same cells (329). Mechanical factors also have been reported to influence myofibroblast differentiation and function (328). Thus, a variety of stimuli and mechanical factors have regulatory effects on myofibroblast differentiation as viewed from the perspective of regulation of

α-smooth muscle actin gene expression. Since many of these factors are present in pulmonary fibrosis, it is likely that some (or possibly all) of them could play some role in myofibroblast emergence in vivo in the pathogenesis of pulmonary fibrosis

D. Myofibroblasts and Inflammation

Myofibroblasts can play a major role in the inflammatory response by virtue of its ability to elaborate inflammatory mediators (54). For example, they produce chemokines, cytokines, and growth factors (Fig. 3) with the capability to enhance or diminish the inflammatory and immune responses (2,6,19,21,52); and as mentioned above, some of these appear to be involved as well in regulating the emergence of the myofibroblast phenotype itself (6,19, 21,52,329). In addition to secretion of such soluble regulators of inflammation, the myofibroblast can directly promote inflammation by the expression of selective adhesion molecules with proinflammatory and profibrogenic activity such as intercellular adhesion molecule-1 (ICAM-1) (331). Thus, inflammatory

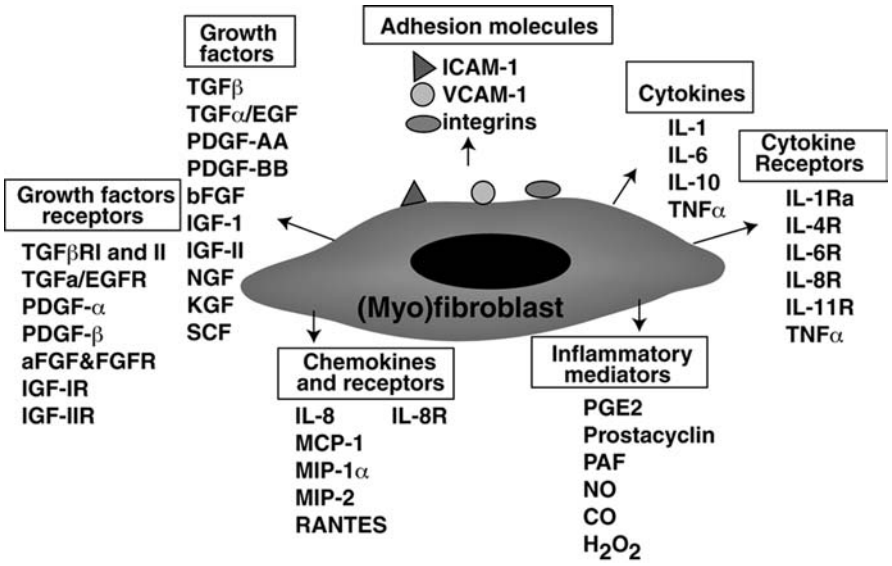

Figure 3 Fibroblast/myofibroblast expression of cytokines and cognate receptors, other mediators, and adhesion molecules. Activated (myo)fibroblasts express a number of cytokines, chemokines, growth factors and their receptors, and inflammatory mediators, as well as adhesion molecules. These products can contribute to tissue injury, activation and proliferation of myofibroblasts, and increased extracellular matrix deposition and promote fibrosis.

cells may dock on such ICAM-1–expressing myofibroblasts and contribute to inflammatory cell activation with consequent secretion of cytokines and other mediators that can enhance inflammation and tissue injury (Fig. 3).

Myofibroblasts also express α and β integrins that are part of their matrix-binding mechanism. Since these integrins influence many cellular activities, including adhesion, migration, proliferation, apoptosis, and cell survival, their expression by myofibroblasts may impact on these diverse processes of relevance to pulmonary fibrosis. Thus treatment of mice with antibodies to leukocyte β_2 integrins significantly inhibits lung injury and fibrosis in an animal model (332). However, since ICAM-1 and other adhesion molecules/integrins are expressed by many other cell types, the results of this study cannot provide direct proof that such potential leukocyte-myofibroblast interactions play critical roles in fibrosis in this animal model. Recently, however, there is evidence to suggest that α8β1 integrin may be a marker for activated myofibroblasts, and that proliferation of α8-expressing cells appears to be a common feature of fibrosis (333). Although the relevance of expression of this particular integrin to inflammation is uncertain, it is clear that the myofibroblast has the wherewithal to regulate inflammation both via soluble and insoluble signals.

E. Myofibroblast and Wound Healing

In the previous section, myofibroblasts were shown to have the capacity to regulate inflammation, a process involved in the pathogenesis of a multitude of diseases. Here the focus will be on the more obvious roles that they can play as a mesenchymal cell in processes with a significant connective tissue component. Historically, their identification at wound healing sites suggests a fundamental role in tissue repair. However, this complex process characterized by inflammation, cell migration, proliferation, extracellular matrix synthesis, angiogenesis, and remodeling has many analogous elements in diverse fibrotic diseases in many organs, and is subject to regulation by many of the same myofibroblast-derived mediators and products as discussed above (32). Myofibroblasts appear de novo presumably in response to TGF-β signaling, and are responsible for synthesis of the extracellular matrix and cytokines as well as wound contraction. Biosynthesis of the extracellular matrix is a key factor in the restoration of cutaneous tissue defects due to injury or wounding (334). Hence, as healing progresses, the emergent myofibroblasts progressively assume a synthetic role contributing to the increased deposition of matrix components. The direct role of myofibroblasts in regulation of matrix degradation is unclear, but both matrix synthesis and degradation are intimately involved with the remodeling phase. However, the degradation of matrix components is controlled by a variety of collagenase enzymes, MMPs

and their inhibitors, TIMPs, whose synthesis and secretion are subject to regulation by the previously discussed myofibroblast-derived growth factors and other mediators, as noted above in the discussion on cytokines. The repair process is ultimately completed by the terminal differentiation of epithelial and parenchymal cells, and notably by disappearance of myofibroblasts, presumably by undergoing apoptosis (335). The factors that terminate the repair process are incompletely understood, but the decline during this period in the expression of inflammatory and fibrogenic cytokines (e.g., TGF-β) and mediators may be an important factor. Additionally, certain cytokines having anti-inflammatory or antifibrogenic activities may also play an active role in downregulating the repair process. Thus, IL-10 is known to be anti-inflammatory (246,248,249,251), whereas IFN-γ is antifibrogenic and inhibits α-smooth muscle actin expression (269–274,336,337). Despite these well-documented in vitro activities, their potential in vivo roles as signals for downregulating the repair process remain undefined. Nevertheless, a proper termination of this process is necessary in successful wound healing, and a failure to do so may result in hypertrophic scarring and other fibrotic diseases.

F. Myofibroblasts in Pulmonary Fibrosis

The presence of myofibroblasts in lung fibroproliferative lesions is well documented and shown to localize to areas of active fibrosis characterized by evidence of increased extracellular matrix deposition (6,19,31,33,37,53,56,178, 312,313,315,338–340). Hence, early studies have postulated their importance as a source of the increased extracellular matrix synthesis observed in such lesions (312). Indeed, a subsequent study in an animal model provides direct evidence that the myofibroblast is the key cellular source of type I collagen gene expression in fibrosis (37). Although the elaboration of matrix is a key factor in fibrosis, given the complex functional capabilities of the myofibroblast as reviewed above, its role likely extends beyond the mere production of the extracellular matrix. It appears to have the potential to affect many of the key processes that comprise pulmonary fibrosis, including inflammation, tissue remodeling, and genesis of connective tissue. The potential role of the myofibroblast in inflammation has been discussed previously on the basis of the observation that it is an important source of chemokines and cytokines with the capability of recruiting and activating inflammatory cells (17,33,54). The role of these cytokines in pulmonary fibrosis is well documented (6,21,37,51,52,299), and as discussed in preceding sections, some of these cytokines appear to be involved in the regulation of myofibroblast differentiation and function as well (2,51,104,314,317,325). By virtue of its ability to express TGF-β (19,33), the myofibroblast can directly promote its own differentiation from fibroblasts (19,51,52,314), which in combination with the

enhanced matrix production results in the increased net deposition of connective tissue that is a key hallmark of pulmonary fibrosis (Fig. 4). An additional potential role of the myofibroblast is suggested by its α-smooth muscle actin content, its ability to contract collagen gels that is enhanced by TGF-β, and its postulated role in wound contraction (19,52,304). Thus, the association between increased contractility of fibrotic lung tissue with the presence of myofibroblasts may suggest their contribution to the overall altered mechanical properties of the lung in pulmonary fibrosis (55,56). Despite this extensive evidence for the capabilities and profibrogenic phenotypic features of the myofibroblast, direct evidence for its specific importance in pulmonary fibrosis is lacking. In the previously cited studies, there is evidence for an association between the genesis of fibrosis and the presence of myofibroblasts, and conversely, downregulation of fibrosis is accompanied by reduction in myofibroblast numbers. Despite this correlation, however, in view of the current lack of a specific method for elimination of myofibroblasts, direct proof has not been forthcoming. Also, the existence and role of other (nonmyofibroblast) activated fibroblast phenotype(s) have not been ruled out. Nevertheless, the totality of the current evidence suggests that the myofibroblast may be one of the key participants in the upregulation, maintenance, and perhaps even propagation of the fibrotic process; and as such, its persistence may represent a primary cause for progressive fibrosis leading to end-stage disease. Hence, development of the ability to control the emergence and/or survival of the myofibroblast may be helpful in developing new therapeutic approaches. Such a goal, however, requires a fuller understanding of the mechanisms underlying myofibroblast emergence and disappearance in the specific context of pulmonary fibrosis.

In the normal lung, the interstitium contains a population of mesenchymal cells expressing myogenic intermediate filaments, including desmin and vimentin but not α-smooth muscle actin (37,178,333). In studies possible only with an animal model, kinetic analysis of the emergence of α-smooth muscle actin–expressing myofibroblasts after lung injury show that these cells do not appear until day 3 after bleomycin-induced lung injury (37). The first few cells with this phenotype are found initially in the adventitia of bronchioles and adjacent blood vessels, wherein no α-smooth muscle actin–expressing cells exist in control uninjured lungs. Since these sites are clearly spatially separated and demarcated from the smooth muscle layer of the airway wall, the vascular media, or the pericytes of the vascular channels, it is unlikely that these cells are the source of myofibroblasts. Furthermore, the pericytes are already expressing α-smooth muscle actin in the normal lung, whereas the newly emerging myofibroblasts arise from areas with cells that are previously negative for this actin isoform. Over the next few weeks after induction of injury, the number of myofibroblasts increase, peaking at about the second week after injury, and then gradually beginning to decline until only a few

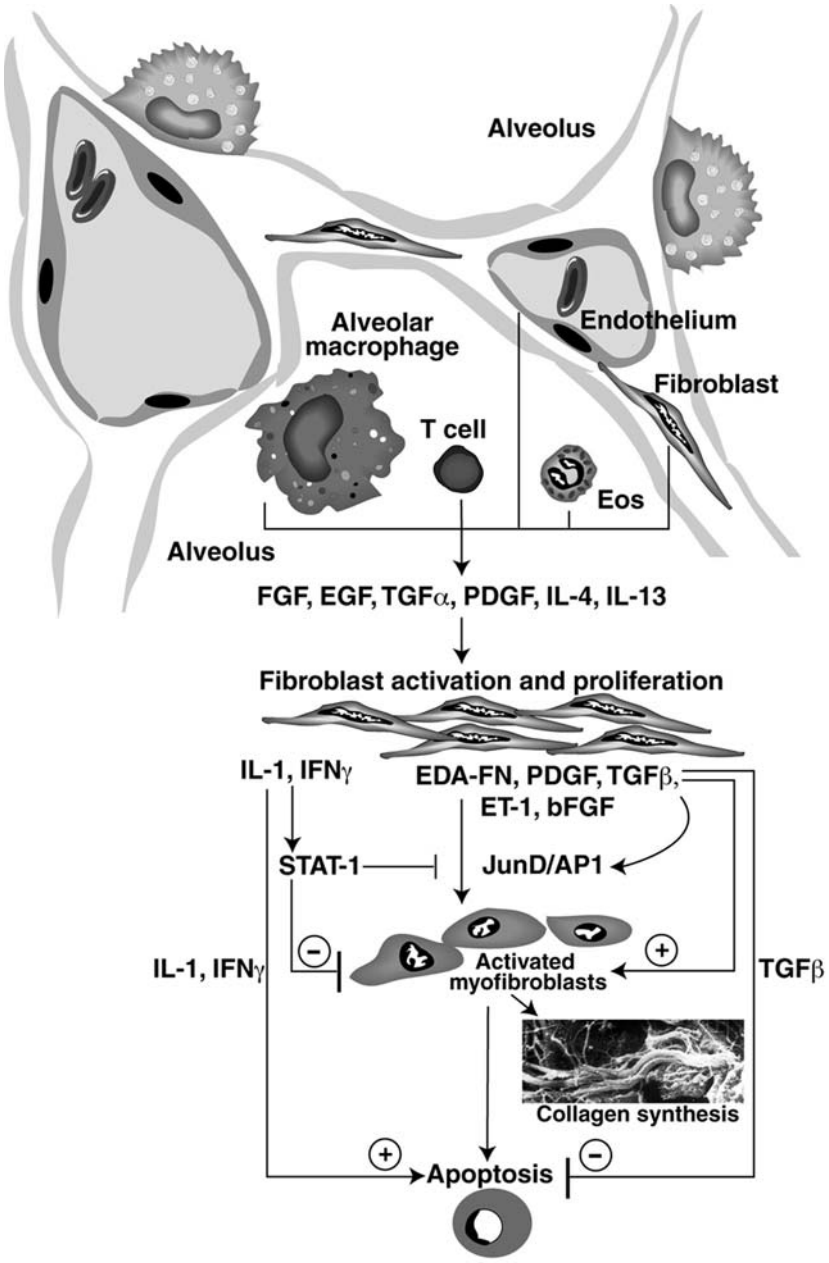

Figure 4 Proposed scheme depicting the genesis and role of myofibroblasts. During pulmonary fibrosis, activated fibroblasts proliferate and undergo phenotypic modulation to myofibroblasts expressing α-smooth muscle actin under the influence of TGF-β,

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occasional ones are identifiable toward a month after injury. The peak of increase in myofibroblast numbers corresponds to a period of peak increases in a number of cytokines, including TGF-β, while the period of decline is associated with declining cytokine expression. Thus in this self-limiting model of lung injury and fibrosis, the adventitial fibroblast appears to be the source of myofibroblasts, whose differentiation may be induced by the high levels of TGF-β expressed by inflammatory and other cells at the same time period. This possibility is supported by in vitro studies showing the ability of TGF-β and several other cytokines directly to induce myofibroblast differentiation in fibroblast cultures. The mechanism of how such cytokines regulate differentiation is not completely understood, but may be mediated via pathways similar to regulation of a variety of genes by these cytokines. TGF-β along with IL-4 and IL-13 are known inducers of lung myofibroblast differentiation as defined by α-smooth muscle actin expression, which appears to require JNK, but not p38 MAP kinase or Erk, to mediate this effect (317,325). Downstream mechanisms that lead to induction of α-smooth muscle actin gene expression are unclear. However, there is evidence for the importance of at least a TGF-β–controlling element in the promoter of this actin gene in lung fibroblasts (2), which may be similarly controlled by GKLF as previously identified for an analogous element found in the promoter of the SM22 gene in smooth muscle cells (326,327). The Smad pathway (341) may also be involved in this TGF-β–induced myofibroblast differentiation in the lung, as suggested by evidence of nuclear translocation of Smad2 and changes in the level of Smad7 expression in TGF-β–induced myofibroblast differentiation in other tissue sites (342,343). Thus, the available evidence suggests complex signaling and promoter-regulatory mechanisms are involved in cytokine-induced myofibroblast differentiation, a key step in the emergence of this cell in pulmonary fibrosis.

Expansion of the myofibroblast population is likely sustained by the high levels of TGF-β expression, some of which is derived from the emerging myofibroblasts themselves (Fig. 4). Moreover, TGF-β is found to prevent

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IL-4, IL-13, EDA-FN, and/or endothelin. JunD/AP1 appears to be important in mediating signaling by TGF-β and IL-4/IL-13 during myofibroblast differentiation. Activated myofibroblasts exhibit high levels of extracellular matrix production and directly promote fibrosis. TGF-β^1 derived from myofibroblasts and other cells promote myofibroblast differentiation and survival from apoptotic stimuli. In contrast, IL-1β and IFN-γ (via STAT-1) downregulate α-smooth muscle actin expression and myofibroblast apoptosis. Thus the persistence or survival of the myofibroblast may be key in the persistence of the cycle of myofibroblast differentiation and increased deposition of extracellular matrix that is characteristic of progressive pulmonary fibrosis leading to end stage lung disease.

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myofibroblasts from undergoing apoptosis, partly by preventing a reduction in the expression of the antiapoptotic protein bcl-2 (51,344). Thus, under the conditions of elevated TGF-β (and other cytokines) expression found in lung fibrotic lesions, fibrosis can potentially be sustained by the induction of myofibroblast differentiation and promotion of its survival, that is, persistence. Furthermore, if sufficient numbers of myofibroblasts were to express adequate levels of TGF-β, they in turn could sustain a steady-state level of myofibroblast numbers to ensure maintenance of fibrosis independent perhaps of other sources of this cytokine (i.e., leukocytes). This could account for the observation of active fibrotic lesions in the absence of "inflammation" (338), although as noted previously, the myofibroblast could perhaps be viewed as an inflammatory cell in its own right (54).

Resolution of tissue repair or downregulation of active fibrosis in self-limiting animal models of pulmonary fibrosis is accompanied by the gradual loss or disappearance of myofibroblasts. This clearly does not happen in progressive pulmonary fibrosis such as that seen in human interstitial lung diseases. Hence, understanding the mechanism that governs this down-regulation may be beneficial in terms of uncovering possible defects in this mechanism that may lead to persistence of fibrosis, as well as identification of new therapeutic strategies based on activating this turn-off signal or process. The mechanism underlying this disappearance of the myofibroblast is uncertain, but there is evidence that this reduction in myofibroblast numbers may be mediated, at least in part, by an apoptotic process (335,345). The signal for induction of apoptosis in these cells is unknown, although serum (which contained TGF-β) deprivation in vitro is known to induce apoptosis (52,344), suggesting that loss of TGF-β signaling may be one trigger for apoptosis in myofibroblasts. Conversely, TGF-β is known to protect them from apoptosis (51,344). Interestingly, lung myofibroblasts are selectively (relative to fibroblasts) susceptible to apoptosis by NO exposure, although they themselves do not express iNOS in response to IL-1β (51,52). An additional potential mechanism is the expected reduction in the rate of myofibroblast differentiation as TGF-β expression declines in the terminal stages of tissue repair or diminution of active fibrosis in experimental animal models. Finally, IFN-γ is also known to inhibit α-smooth muscle actin expression (see Fig. 4), thus providing an additional pathway for reducing myofibroblasts (336,337,346), which may account in part for the potential therapeutic value of this cytokine in idiopathic pulmonary fibrosis (274).

In summary, complex regulatory mechanisms are involved in the emergence of the myofibroblast, its survival, and its disappearance. Although some of these may not be unique to the myofibroblast, the applicability of findings from studies in other cell types under different conditions requires confirmation. Additional potentially novel mechanisms may eventually be discovered as well. Despite the lack of understanding, the role of TGF-β

prominently stands out. Its potent profibrogenic role is underlined by its multiple roles in the induction of myofibroblast differentiation and in its preservation (from apoptotic signals), the combination of which results in survival or persistence of the myofibroblast. It is therefore not surprising that neutralizing TGF-β activity or signaling is effective in the amelioration of fibrosis in lung and other tissues. Despite the ability of NO or IFN-γ to downregulate myofibroblast numbers, their in vivo roles appear to be more complicated than that for TGF-β; partly due to the fact that both NO and IFN-γ have additional activities that promote the inflammatory response and possibly consequent tissue injury. Additional studies are necessary to evaluate more completely their in vivo roles vis-à-vis inflammation versus fibrosis, as represented by myofibroblast persistence.

VI. Conclusions

The presence of an activated fibroblast phenotype in fibrotic lesions is well documented. The importance of such a phenotype in the fibrotic process presumably rests on its multiple functional capabilities. The heterogeneity of fibroblast populations is well known, and extends to activated cells present at sites of tissue repair and fibrosis. However, amid this complexity in terms of fibroblast populations, a unique phenotype characterized by the expression of α-smooth muscle actin and other features could be discerned to arise de novo in many fibrotic lesions, including in pulmonary fibrosis. In view of the distinct and stable phenotypic differences from fibroblasts, the cell with this phenotype, the myofibroblast, is considered to have arisen from a process of transdifferentiation or differentiation. Although other activated fibroblast phenotypes may be present and play a role in pulmonary fibrosis, the myofibroblast, by virtue of its easily identifiable and distinct marker, namely, α-smooth muscle actin expression, has been the most intensely studied. In the lung, myofibroblasts play important roles in normal growth and development as well, although their origin may not necessarily be identical to those seen arising de novo in pulmonary fibrosis. The emergence of the myofibroblast in tissue repair is associated with the period of active extracellular matrix elaboration, which if allowed to persist beyond the period necessary for proper repair, may lead to progressive fibrosis. Additional key characteristics of myofibroblasts germane to the pathophysiology of pulmonary fibrosis include their contractile properties and expression of inflammatory and fibrogenic cytokines. Thus, they are well equipped to drive, maintain, and propagate the fibrotic process. Also, in view of these capabilities, it is tempting to ascribe a decisive role for the myofibroblast as the cellular instigator for persistence and progression of fibrosis, whose demise in accordance with this view would be key to termination of this process. Correlational evidence supports such a view,

since downregulation of fibrosis is characterized by loss of myofibroblasts. Although such a focus on the myofibroblast may seem unjustified, the lack of easily distinguishable markers for other potential activated fibroblast phenotypes makes it more difficult to study the potential role(s) of such cells. Nevertheless, more research is necessary to prove such a decisive role for the myofibroblast and to rule out (or rule in) other fibroblast phenotypes. Although an effective cell-specific method for elimination of the myofibroblast in vivo is currently unavailable, recent studies have provided important and intriguing clues with respect to myofibroblast differentiation and apoptosis. Future research in these and related areas should uncover more details that may be useful for testing this hypothetical role of the myofibroblast, and evaluating the feasibility of targeting this cell in novel therapeutic approaches.

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Peptide and Provisional Matrix Signals in Idiopathic Pulmonary Fibrosis

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I. Introduction

Idiopathic pulmonary fibrosis (IPF) is a deadly disease presenting patients with few therapeutic options other than transplantation. Fibrotic lesions are found scattered throughout the lung parenchyma at different stages of progression. Some alveolar units are inflamed, others manifest epithelial denudation with fibroblastic foci, and still others are scarred shut with a mature collagenous matrix. For reasons that remain to be elucidated, fibrosis begins at the lung bases and periphery working its way upward and inward. Attempts to treat inflammation with the goal of interdicting fibrosis have been disappointing. The scope of research has therefore expanded, addressing signals that regulate lung fibroblast fate and alveolar reepithelialization. Such efforts may help us uncover new therapeutic targets.

IPF evolves as if it resulted from a cryptic alveolar injury, although the evidence to support injury as the first step in the process has not been forthcoming. As in acute lung injury, the innate immune system is activated and there is damage to both the alveolar epithelium and the endothelium. The injured alveoli become filled with a provisional matrix composed of fibronectin, polymerized fibrin, and other serum proteins. This matrix provides a scaffold for subsequent fibroblast ingress and proliferation. In response to external signals from peptide growth and differentiation factors as well as matrix derivatives, sessile interstitial fibroblasts differentiate into motile myofibroblasts and migrate into the alveoli from the interstitium where they proliferate and deposit collagen. The affected alveoli contract, losing surface area and functional capillaries and the ability to participate in normal gas exchange.

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In order for the fibrosing lung to regain reasonable function, the alveolar epithelial barrier must be preserved in intact alveoli and reconstituted in those that are injured by the disease process. Healing requires that surviving alveolar epithelial progenitor cells (generally regarded to be the type II cell, but stem cells and circulating progenitors may also have a role) proliferate, migrate on the provisional matrix, and differentiate into type I cells, thus reconstituting the normal air-lung interface—a process that must be coordinated with fibroblast apoptosis and degradation of the provisional matrix. This sequence is observed in acute lung injury but not in IPF for reasons that remain undefined. In this chapter, we will discuss peptide and provisional matrix signals that illustrate some of the general concepts regarding the evolution and, in principle, the regression of pulmonary fibrosis. The reader is referred to comprehensive reviews of peptide growth factors, integrins, and provisional matrix for more details.

II. Peptide Signals in Pulmonary Fibrosis

Several peptide growth factors appear to be important in the pathogenesis of pulmonary fibrosis. We have selected a few examples to illustrate some general principles. These include transforming growth factor-β1 (TGF-β1), connective tissue growth factor (CTGF), the fibroblast growth factor (FGF) family, and the platelet-derived growth factor family (PDGF).

A. TGF-β1

Platelets, activated macrophages, and lung parenchymal cells all release the peptide cytokine TGF-β1. TGF-β is a member of a superfamily that includes more than 40 members and has been assigned an important role in fibroproliferative diseases. There are three mammalian TGF-β isoforms: TGF-β1, -2, and -3. Each isoform can bind to two distinct TGF-β receptors. These receptors are serine/threonine kinases and are designated TGF-β type I receptor (Alk5) and TGF-β type II receptor. TGF-β isoforms are secreted as large complexes that are sequestered in the extracellular matrix in a latent form associated with matrix proteins. TGF-β action is controlled not only by its production and release, but also by the regulation of latent complex activity. For example, some TGF-β1 latent complexes bind to the $\alpha v\beta 6$ integrin. Ligation of the $\alpha v\beta 6$ integrin results in activation of TGF-β1, a mechanism that appears to be plausible in the pathobiology of pulmonary fibrosis (1).

TGF-β1 is a powerful morphogen that induces interstitial mesenchymal cells to differentiate into myofibroblasts and signals them to synthesize collagen by activating the cytoplasmic transcription factors Smad2 or 3. Smad2 or 3 in turn binds to Smad4 and translocates to the nucleus where it associates with TGF-β response elements in target genes. In vitro, TGF-β1 stimulates type II

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cells to produce FGF-2 (2) and increases expression of FGF receptors 1 (FGFR-1 or Flg) and 2 (FGFR-2 or Bek) on human lung fibroblasts (3). Both of these effects may set the stage for other profibrotic signals to act, and thus can be viewed as central steps in the genesis of fibrosis.

TGF-β1 is a potential target for antifibrotic therapy. Preclinical studies in a hamster bleomycin model of pulmonary fibrosis (4) showed promise. Instillation of soluble TGF-β receptor into the trachea 2 days after intratracheal instillation of bleomycin attenuated pulmonary fibrosis. Administration of TGF-β antibodies intravenously 5 min and 5 days after intratracheal bleomycin reduced lung collagen accumulation in mice (5). These preclinical studies suggest TGF-β interdiction, at the level of receptor-ligand interactions or downstream, deserves serious consideration as the basis for new antifibrotic compounds.

B. CTGF

CTGF is a 38-kD peptide expressed by fibroblasts but not other cell types such as epithelial cells and leukocytes. The CTGF promoter contains a TGF-β1 response element, suggesting it may function as a downstream effector in TGF-β1–induced fibroproliferation. Recent evidence indicates that CTGF is important in the genesis of fibrosis. CTGF mRNA expression is upregulated in a murine model of bleomycin-induced pulmonary fibrosis (6) and is increased in the bronchoalveolar lavage of patients with IPF (7). CTGF induces fibroblasts to synthesize collagen (8), further strengthening the case for its role in the perpetuation of fibroproliferation.

C. FGFs

The FGF family includes over 20 members that are structurally similar and bind heparin and heparan sulfate (9,10). The FGFs bind to specific cell surface tyrosine kinase receptors (FGFRs), of which four have been described. Upon ligand binding, the FGFRs autophosphorylate a cytoplasmic tyrosine residue that in turn activates cytoplasmic SH2 domain–containing signaling molecules. FGFs mediate the growth, differentiation, migration, and survival of many different cell types. In this chapter, we will highlight the role of a prototype FGF, FGF-2 (basic FGF), in pulmonary fibrosis.

FGF-2 was initially purified from bovine pituitary extracts (11). It can bind to any of the high-affinity receptors FGFR-1 to -4, but FGF-2 specifically activates a splice variant of FGFR-2 known as FGFR-2-IIIc (11). FGF-2 has been identified in the lungs of patients who died following acute lung injury where it was immunolocalized to macrophages in those airspaces containing fibroblastic proliferation (12). Mast cell FGF-2 is increased in the lungs of patients with silica-induced pulmonary fibrosis (13) and IPF (14). Repressors and counter regulators of FGF-2 have been sought. In this regard, there is

evidence that FGF-1 may be antifibrogenic. FGF-1 induces human lung fibroblasts to express collagenase mRNA and protein and decreases both type I collagen mRNA and protein (15). Other negative regulators of the profibrotic activity of FGF-2 have not yet been identified.

D. PDGFs

PDGF is a dimer encoded by two genes, A and B. It is a potent chemoattractant, proliferative signal, and survival factor for fibroblasts. Binding of PDGF to its receptor on target cells stimulates cell migration and cell cycle entry. It also induces autocrine insulin-like growth factor-1(IGF-1) release, which may be important for its antiapoptotic function. It is expressed at low levels in normal tissues, but expression is increased following tissue injury. PDGF probably is produced by platelets early in the fibroproliferative process. Later on, macrophages and parenchymal cells, including epithelial cells, endothelial cells, and fibroblasts produce it. A large body of experimental and clinical evidence supports the pathogenetic role played by PDGF in the development of pulmonary fibrosis in humans. This includes studies in vascular fibrosis, fibrosis of the airways and lung parenchyma, kidneys, and liver.

III. Provisional Matrix Signals in Pulmonary Fibrosis

The alveolar provisional matrix, composed of fibronectin and fibrin as well as other serum proteins, forms when plasma proteins leak into the injured alveoli from the capillaries and the coagulation cascade is activated. By giving cells a nurturing substratum for migration and proliferation, it provides a scaffold for fibroproliferation as well as for alveolar reepithelialization.

Interactions of the cell with matrix proteins are governed by specific surface receptors. These include integrins and proteoglycans. Integrins are heterodimeric proteins composed of an α and β subunit. The major integrin that regulates cell interactions with the matrix protein fibronectin is $\alpha 5\beta 1$. The $\alpha v\beta 3$ integrin mediates cell interactions with fibrinogen. Once a matrix ligand binds to an integrin on the target cell surface, a sequence of intracellular signal transduction events occurs that results in a change in cell function, such as cell adhesion and migration.

Proteoglycans are cell surface glycoprotein matrix receptors capable of mediating the adhesion and migration of both transformed and nontransformed cells (16,17). To date, no one has shown that proteoglycans mediate type II cell adhesion or migration. However, proteoglycans such as CD44 are located on type II cell surfaces (18). Based on the above considerations, it is likely that a detailed understanding of how fibroblasts and type II cells interact with provisional matrix proteins will lead to important insights regarding disease progression.

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A. Fibroblast Interactions with the Provisional Matrix

The early fibrotic lesions in IPF contain myofibroblasts, proteoglycans, and collagen. By analogy with acute lung injury, available data suggest that myofibroblasts migrate from the interstitium through gaps in the epithelial basement membrane on to the alveolar provisional matrix (19). One of the proteins present in the provisional matrix is fibronectin. Myofibroblasts found in areas of alveolar fibrosis display integrin $\alpha5\beta1$ on their surface, which is consistent with a functional role in mediating adherence to fibronectin in the provisional matrix (20). The stimuli responsible for the movement of myofibroblasts into the alveolar provisional matrix are not fully defined. Candidates known to be present in the alveolar microenvironment include PDGF and fibronectin. Unfortunately, to date, a suitable experimental model of slowly progressive, durable fibrosis is lacking. This has precluded definitive gain and loss of function studies to define the critical macromolecular signals.

B. Type II Alveolar Epithelial Cell Interactions with the Provisional Matrix

As in acute lung injury, the alveoli in IPF are damaged. One hallmark of the evolving IPF lesion is alveolar epithelial cell death. The precise details of this process and the mode of cell death are not fully understood. In this connection, fibrotic lung fibroblasts, in contrast to their normal counterparts, are capable of inducing epithelial cell apoptosis (21). Surviving type II epithelial cells are thought to be the major progenitor cells that must repopulate the alveolar epithelium. In contrast to what happens when the lung heals after acute lung injury, in IPF, the repopulation process goes awry. Although there is a robust proliferation of hyperplastic type II cells, it occurs on top of intra-alveolar accumulations of fibroblasts incorporating them into a thickened, expanded interstitium where capillaries are entrapped and lose contact with the air-lung interface. It is as if the normal repair process is subverted by a delayed type II cell response allowing the intra-alveolar fibroblasts to get a foothold in the airspace—or a failure of timely fibroblast apoptosis—or both. If lung healing is to occur, efforts to accelerate type II cell migration and proliferation on the provisional matrix may be a logical approach.

Type II cells have been found on the alveolar provisional matrix following lung injury in humans (22). Type II cells adhere and migrate on the provisional matrix proteins fibronectin and fibrinogen, which are functions that are regulated by the $\alpha5\beta1$ and $\alpha v\beta3$ integrins, respectively (23,24). In IPF, alveolar epithelial cells found in areas of intra-alveolar fibrosis display $\alpha5\beta1$ on their surface (20). Learning more about how to regulate $\alpha5\beta1$ or $\alpha v\beta3$ integrin on alveolar epithelial cells and how to enhance its function could lead to new therapies focused on stimulating reepithelialization of damaged alveoli.

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Integrin signaling is a complex process that involves different intracellular signaling molecules depending on the function that is being regulated. For example, β1 integrin regulation of cell migration on fibronectin leads to tyrosine phosphorylation of intracellular phosphatidylinositol 3 kinase (PI3 kinase) (25). One downstream effector of PI3 kinase is protein kinase B (PKB)/Akt. In response to β1 integrin engagement, PI3 kinase activates AKT by phosphorylation of a serine residue, and this pathway is important in adhesion and fibroblast survival (26). It is possible that these molecules could be targets for manipulation in the treatment of IPF.

Another matrix protein that is important in cell migration is hyaluronan. Cells bind to hyaluronan with high affinity using RHAMM and CD44 cell surface receptors. Both receptors provide a link between hyaluronan and the cytoskeleton and intracellular signaling cascades (27). Of interest, there is evidence that integrins and proteoglycans may function cooperatively in signaling information from outside to inside the cell (28).

C. Regulation of Epithelial and Fibroblast Apoptosis by Matrix and Growth Factors

The cell death process is an important component of the genesis and progression of pulmonary fibrosis. In chronic fibroproliferative lung disease, it is inferred, but not proved, that the predominant mode of epithelial cell death is apoptosis. Apoptosis can be triggered in mature adult tissues through at least two well-defined pathways, which have the capacity for cross talk. The type 1 pathway, leading to activation of caspase-8, is triggered by death ligands such as tumor necrosis factor (TNF-α) and Fas, both of which have been found in the lungs of patients with IPF. In addition, their cognate cell surface death receptors, Fas/CD95 and TNF-α receptor (29), have been found on the lung epithelium and can function to cause epithelial cell death with resultant fibrosis. The second pathway, initiated by pathological stress such as ischemia or toxin exposure, is transduced through a series of steps into mitochondrial release of cytochrome *c*. Subsequent formation of the apoptosome, a complex containing cytochrome *c*, adapter protein Apaf-1, and procaspase-9, leads to activation of caspase-9 (30). When activated, caspases-8 and -9 convert the inactive proform of effector caspases-3, -6, and/or -7 into their active forms, which in turn cleave critical cellular targets resulting in death (31). Proteins in the Bcl-2 family tightly regulate mitochondrial release of cytochrome *c*. The proapoptotic Bcl-2 family proteins, such as Bax and Bad, form pores in the outer mitochondrial membrane. In contrast, the antiapoptotic family members, Bcl-2 and Bcl-X_L, inhibit pore formation (29). In some cells, these two pathways converge, and receptor-induced activation of caspase-8 also results in mitochondrial release of death promoters with subsequent activation of the apoptosome-dependent caspase cascade (32).

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Growth factors and integrins provide critical survival signals to epithelial cells holding these death pathways in abeyance in part through activation of the Akt kinase–dependent survival pathway. For example, interleukin-1 has been found to interdict Fas ligand–mediated death by activating Akt, as well as by repressing activation of caspase-8. In addition, ligation of the $\alpha 5\beta 1$ integrin by fibronectin triggers activation of the PI3/Akt kinase cascade which is instrumental in preserving cell viability. Growth factors and integrins also maintain cell viability by modulating the activity of the cellular protein synthesis machinery. In this regard, cell viability is regulated by the activation state of the cap-dependent translation initiation apparatus, eIF4F. In contrast to their normal counterparts, epithelial and mesenchymal cell malignancies as well as fibroblasts from fibrotic lesions require a high level of cap-dependent translation to suppress apoptosis. In pulmonary fibrosis, unremitting signals emanating from aberrant accumulations of certain extracellular matrix components and peptide growth factors may contribute to the abnormal persistence of fibroblasts in the alveolar airspace as fibrosis eventuates.

D. Therapeutic Implications for Epithelial Cytoprotection and Elimination of Fibroblasts

Manipulation of death pathways with the goal of cytoprotecting epithelial cells and triggering apoptosis of fibroblasts comprising fibrotic lesions can now, in principle, be conceptualized. Convincing data have been already reported in model systems, indicating that epithelial cell apoptosis can be inhibited by trophic growth factors such as keratinocyte growth factor (33). The key missing pieces of information at present relate to the lack of evidence that the primary mode of epithelial death in IPF is apoptosis—and if death is indeed apoptotic, which death pathway is triggered. In contrast, the importance of fibroblast apoptosis for the regression of fibrosis after tissue injury is established. However, the identity of the signals mediating fibroblast apoptosis, whether ligand initiated, withdrawal of trophic matrix or peptide signals, or intrinsically programmed, remain undefined. This critical information may emerge from systematic studies of patients combining data on clinical characteristics and course with genomic and proteomic analyses.

IV. Conclusions

Treatment of IPF continues to be a major problem for patients and clinicians. Understanding the mechanisms that regulate the development of pulmonary fibrosis will provide new avenues for creating novel therapies. Peptide signals appear to be important in the pathogenesis of IPF. In addition, cell interactions with the provisional matrix are essential both for the promulgation of the fibrotic process and its resolution. Attempts to target specific peptides by

inhibitory molecules could provide rewarding results. Blocking myofibroblast adhesion and migration on provisional matrix proteins could be another track for investigation. Augmenting alveolar epithelial cell migration on provisional matrix proteins is still another possible target for repairing the lung in IPF.

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Role of Alveolar Type II Epithelial Cells in Pulmonary Fibrosis

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I. Introduction

The lung's response to injury is limited to a narrow range of histological reactions irrespective of etiology. Inflammatory tissue damage and repair pathways compete as they attempt to destroy or restore preexisting lung epithelium, microvasculature, and scaffolding. Normal architecture is restored when the connective tissue scaffolding is intact and the epithelial cells proliferate and reform on the underlying basement membrane (1). In cases of severe or prolonged inflammatory insult, repair by epithelial cells is not possible. Normal architecture is lost and replaced with disorganized connective tissue along distorted scaffolding resulting in pulmonary fibrosis and loss of gas exchange units.

The traditional concept of lung repair has focused on diseases in which an inflammatory reaction is a prominent histological feature. This concept formed the basis of the "inflammatory fibrosis hypothesis" which asserts that chronic inflammation, that is, alveolitis, is the initial event that triggers fibrotic lung disease (2,3). Diffuse alveolar damage (DAD), the pathological correlate of acute respiratory distress syndrome (ARDS), is a classic model typifying this hypothesis. DAD is distinguished by two phases: (1) an exudative phase and (2) a proliferative or organizing phase. The exudative phase is characterized by alveolar epithelial cell injury and inflammation resulting in damage to the epithelium and endothelium. Histologically, this phase is distinguished by a fibrinous intra-alveolar exudate and hyaline membranes representing sloughed alveolar lining cells and insoluble plasma proteins. Repair is initiated by type II

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cell proliferation heralding the proliferative or organizing phase of DAD. In this phase, cuboidal cells extend along the alveolar wall in an attempt to cover the previously denuded basal lamina. Concurrently, fibroblasts and myofibroblasts migrate through the damaged alveolar basal lamina into the intra-alveolar exudate. Fibroblasts then convert the exudate to cellular granulation tissue and ultimately with the deposition of collagen into dense fibrous tissue (4).

Recent literature (5) has contrasted this traditional model of lung injury with idiopathic pulmonary fibrosis (IPF), a disease in which inflammation is not a prominent histological feature. The evolving hypothesis of IPF pathogenesis suggests that epithelial injury activates an abnormal fibrogenic response independent of any inflammatory response (3,5). Recently, a point mutation in the surfactant protein C gene (SP-C) was discovered in a kindred with familial pulmonary fibrosis (6). The mutation (128 T→A in exon 5) substitutes a glutamine for a conserved leucine residue which presumably results in abnormal folding and processing of SP-C precursor protein. Electron microscopy of affected lung revealed alveolar type II cell atypia with abnormal lamellar bodies. This phenotype was replicated in mouse lung epithelial cells transfected with the SP-C mutation. One adult affected with this mutation demonstrated usual interstitial pneumonia (UIP) pathology. This study suggests that the familial UIP phenotype associated with this mutation results from type II cell injury, supporting Selman's hypothesis. Interestingly, children in the kindred with the SP-C mutation demonstrated nonspecific interstitial pneumonia (NSIP) pathology, indicating that both NSIP and UIP pathology can result from the same mutation.

A considerable body of data has emerged on the role of the alveolar type II cell in lung repair and pulmonary fibrosis. Type II cell hyperplasia (Fig. 1) is a prominent histological feature seen in various injury and fibrosing patterns including DAD and UIP, the pathological correlate of IPF. Conflicting data exist as to the consequence of these proliferating and differentiated type II cells. There is good evidence that alveolar type II cells function to limit fibrosis and restore structural integrity of the lung parenchyma following injury. Mechanisms potentially include (1) proliferation and repopulation of the alveolar surface post injury, (2) surfactant synthesis and secretion with resulting decrease in surface tension, (3) interaction with other repair cells by limiting fibroblast proliferation and clearance of the inflammatory exudate, and (4) restoration of the lung architecture by synthesis of matrix components and matrix degrading proteins. Other data, however, suggest that the alveolar type II cell may in fact contribute to pulmonary fibrosis.

The critical issue to keep in mind is that the progression of IPF is very slow and the pathogenesis and involvement of type II cells may be different from the rapid wound healing hypothesis of DAD and evolution of pulmonary fibrosis in animal models. A critical histopathological feature of UIP is the fibroblastic focus which is shown in Fig. 2 and is not seen in DAD.

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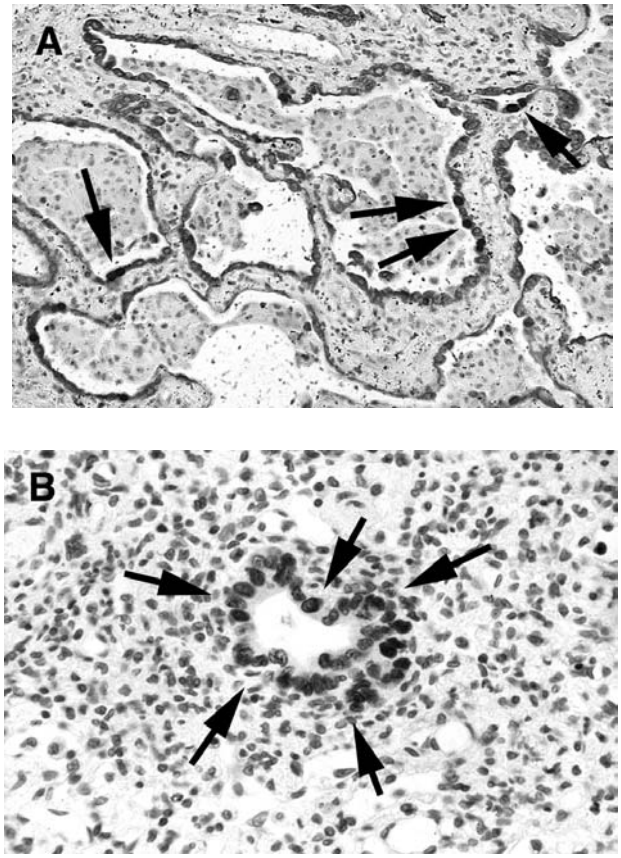

Figure 1 Proliferating epithelial cells in interstitial lung disease. (A) Panel shows the lung of a patient with a desquamative interstitial pneumonitis (DIP)-like reaction. The epithelium is stained brown with cytokeratin and the proliferating cells are stained black with an antibody to proliferating cell nuclear antigen (PCNA). The arrows point to proliferating epithelial cells. (B) Panel B shows PCNA staining of proliferating epithelial cells 1 week after installation of 2 units bleomycin into the left lung of a rat. There are islands of proliferating epithelial cells in the midst of an inflammatory exudate and connective tissue that is relatively free of proliferating activity as measured by PCNA staining. It should be noted in these examples that most of the proliferating cells in these histopathological sections are epithelial cells.

Nevertheless, this chapter will consider the role of the type II cells in the traditional wound healing paradigm, on which there is abundant information, and the new concept of indolent IPF, on which there is little information. We will provide a focused review on the role of the alveolar type II cell in either promoting or inhibiting pulmonary fibrosis.

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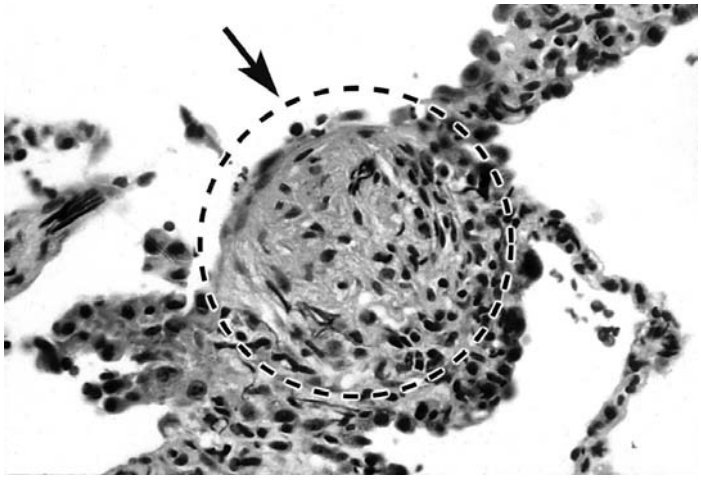

Figure 2 Fibroblastic focus. The encircled lesion is a characteristic feature of UIP and is thought to be the origin of new fibrosis that occurs in IPF. Interesting from the perspective of this review is that these lesions rarely have a well-defined hyperplastic epithelium covering their alveolar surface. The luminal surface has a more attenuated epithelium.

II. Regulation of Alveolar Type II Cell Proliferation and Differentiation

A. Alveolar Type II Cells

Alveolar epithelium is comprised of two cell types—type I and type II cells. Type I cells are flat, relatively metabolically inactive cells that cover over 95% of the surface area of the lung. Their thin structure facilitates the exchange of oxygen and carbon dioxide between air and blood. They are distinguished by their squamous shape, very large surface area, and lack of lamellar inclusions. They do not express the surfactant proteins but do express specific markers such as T1α, aquaporin 5, and carboxypeptidase M. In contrast, type II cells are cuboidal, metabolically active cells designed to be synthetic, secretory, and reparative. They possess lamellar bodies, which are membrane bound inclusions containing lamellated, phospholipid bilayers that are the storage granules for pulmonary surfactant.

The relationship between type I and II cells is complex. Alveolar type II cells serve as stem cells capable of repopulating the alveolar epithelium following injury (7). Maintenance of the differentiated state requires continuous regulation (8) with active, continuous suppression of type I–specific genes that are not normally expressed in the fully differentiated cell. The

differentiated phenotype of type II cells can be maintained in vitro by culture on specific matrices (9–11) and is enhanced by various soluble factors (12,13). In vivo and in vitro alveolar type II cells lose their differentiated phenotype and acquire the differentiated characteristics of alveolar type I cells in a process termed "transdifferentiation" (14–16). There is an element of "cell plasticity" exhibited by these epithelial cells in which this process is reversible in vitro (14). It is unknown whether this cell plasticity and reversibility of transdifferentiation occurs in vivo. Traditionally, type I cells are thought to be terminally differentiated.

B. Regulation of Type II Cell Hyperplasia

As the role of epithelial cells in lung fibrosis has been elucidated, it has become apparent that alveolar epithelial cells are both producers and effectors of growth factors that modulate lung repair and fibrosis.

A number of growth factors have been shown to be capable of stimulating type II cell DNA synthesis and growth including acidic fibroblast growth factor (aFGF, FGF-1) (17), basic fibroblast growth factor (bFGF, FGF-2) (17), keratinocyte growth factor (KGF or FGF-7 (18), FGF-10, hepatocyte growth factor (HGF) (18,19), transforming growth factor = α (TGF-α) (20), heparin-binding EGF (HB-EGF) (21), and epidermal growth factor (EGF) (22). These studies are usually performed with thymidine incorporation and confirmed by autoradiography and labeling indexes. Proliferation of alveolar type II cells occurs in vitro when plated at low density or in the presence of rat serum and KGF (20,23).

The source of these mitogens in vivo is likely pulmonary fibroblasts, macrophages, and type II cells themselves. To support this hypothesis, Brandes et al. (24) demonstrated that conditioned media from stimulated macrophage cultures doubled basal type II cell proliferation. Similarly, Everett et al. (25) showed that hyperoxic conditioned media derived from hamster fibroblasts exposed for 4 days to 100% oxygen induced a two- to three fold increase in type II cell proliferation. Panos et al. isolated KGF and HGF from fibroblast-conditioned medium (18). In addition, several of these growth factors are bound to extracellular matrix and could be an additional source of these growth factors.

The rate of cell proliferation in vitro is influenced markedly by other modulating factors including serum, insulin, and substrate upon which the cells are grown. Serum (either fetal bovine serum or rat serum) is necessary for proliferation of alveolar type II cells, as demonstrated by thymidine incorporation (26). The presence of insulin and/or alveolar- macrophage-conditioned media was insufficient to maintain growth of these cells in the absence of serum but had synergistic effects in the presence of serum. Insulin receptors have been found in isolated type II cells (27), and insulin has been shown to increase DNA synthesis in human alveolar type II cells (28).

Sugahara et al. (29) have demonstrated the role of insulin in regulating the metabolism of alveolar type II cells and stimulation of amino acid transport in primary culture. The importance of these growth factors and hormones on type II cells in vivo are poorly defined. Rat bronchoalveolar lavage fluid has been shown to stimulate DNA synthesis in alveolar type II cells (30). HGF was identified in this biological fluid and was found to be responsible for most of the stimulatory effect on proliferation (31).

The proliferation of cultured alveolar type II cells can be greatly enhanced by plating these cells on matrix rather than tissue culture plastic. Type II cells plated on an extracellular matrix prepared from bovine corneal epithelial cells resulted in a sixfold increase in thymidine incorporation compared to cells cultured on plastic in 10% fetal bovine serum (FBS) (22). This effect was further enhanced by replacing the FBS with rat serum. More recently, substrates composed of a mixture of rat tail collagen and basement membrane proteins (Matrigel) have been used (23).

Of all growth factors found to increase alveolar type II cell proliferation, most attention in recent years has focused on KGF. KGF, also known as FGF-7, is a member of the FGF family. KGF is produced by various mesenchymal cells and acts primarily on epithelial cells. Recombinant KGF or an adenovirus that expresses KGF greatly stimulate type II cell proliferation in vivo (32–34). The effects of KGF on type II cell proliferation have also been demonstrated in vitro (13,18). As discussed below, although many growth factors have been shown to promote type II cell proliferation, KGF is special in its ability to induce both proliferation and differentiation of type II cells in vitro.

C. Regulation of Type II Cell Differentiation

As noted above, cellular differentiation represents the acquisition of a specialized phenotype by a cell within a tissue (16). In lung development, these processes culminate in the formation of two distinct alveolar epithelial cells. Type II cells are distinguished by cuboidal morphology and the presence of lamellar bodies, the storage granules for pulmonary surfactant. These differentiated type II cells express distinct cell surface markers on the apical membrane including lectin A maclura pomifera agglutinin (MPA)–binding glycoprotein (35), alkaline phosphatase (36), neural endopeptidase (37), and glycoprotein 330. The differentiated type II cell serves a variety of functions including (1) synthesis and secretion of surfactant, (2) progenitor cell responsible for maintaining the alveolar epithelium, (3) transepithelial transport of sodium from the apical to basolateral surface to minimize alveolar fluid, and (4) effector and modulator of lung inflammation through secretion of growth factors, cytokines, and chemokines.

Cellular differentiation of the alveolar type II cell is a dynamic process regulated by local growth factors, cell shape, cell-cell interaction, cell-surface adhesion molecules, and cell-matrix interaction. The substratum upon which the type II cells are cultured is the most critical factor in maintaining a differentiated phenotype. Alveolar type II cells cultured on tissue culture plastic flatten out and rapidly lose their differentiated morphological and biochemical characteristics (38). Substrata rich in laminin allow alveolar type II cells to maintain a cuboidal morphology and a differentiated phenotype. In contrast, substrata rich in fibronectin result in transdifferentiation into alveolar type I cell phenotypes as the cells flatten out and lose lamellar bodies. The three considerations that are important for maintaining a differentiated phenotype are (1) cell-cell interactions, (2) cell-extracellular matrix interactions, and (3) maintaining cuboidal morphology (9).

D. Matrix Effects on Differentiation

EHS (Engelbreth-Holm-Swarm) matrix has been extensively studied as a substrate capable of maintaining functional differentiation of the alveolar type II cell. EHS matrix, comprised of laminin, type IV collagen, heparin sulfate proteoglycan, and entactin (39,40) reproduces in vivo conditions essentially mimicking the alveolar basal lamina on which alveolar type II cells grow. Shannon et al. (41) demonstrated that alveolar type II cells cultured on EHS matrix retained a cuboidal morphology. This permissive cytoarchitecture appeared to be a prerequisite responsible for maintaining differentiated function. This hypothesis was supported by culturing alveolar type II cells on collagen gels whose surface area could be altered by detaching them from the culture vessel. Cuboidal type II cells expressed differentiation markers, whereas more flattened, spread out type II cells did not (9). This effect was reversible by detaching the gels from tissue culture plastic allowing the flattened cells to resume their native cuboidal cell shape (10). One mechanism proposed to account for this observation is that the extracellular matrix (ECM) modulates its effects by organizing the cytoskeleton of the cells with which it interacts (10,42). Many ECM components are connected to interacting cell cytoskeleton by transmembrane receptors including the integrin superfamily and syndecan allowing for cell-matrix signal transduction. Disruption of the type II cell microfilaments or microtubules by various drugs significantly reduced the half-lives of surfactant protein gene expression, providing further evidence for the role of the cytoskeleton in maintaining type II cell differentiation (43).

E. Effect of Soluble Factors on Differentiation

Various soluble factors modulate differentiation of alveolar type II cells including KGF, FGF-1 (acidic FGF), rat serum, and dexamethasone. KGF

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and FGF-1 increase expression of surfactant proteins A, B, and D (44,45). In addition, intratracheal instillation of KGF increases SP-A and SP-D in bronchoalveolar lavage (BAL) fluid of rats (33). The effects of corticosteroids on alveolar type II cells is complex. Dexamethasone stimulates some but not all markers of differentiation (23,46). Dexamethasone added to cell cultures increased SP-B and improved the ultrastructural appearance of lamellar bodies such that they more closely resembled the appearance in vivo (23). Rat serum increases incorporation of acetate into phosphatidylcholine (47). However, when rat serum is used alone, it does not increase the mRNA levels for the surfactant proteins (48).

In summary, although several growth factors stimulate type II cell proliferation, very few modulate type II cell differentiation. In vivo, differentiated type II cells play an important role in lung repair and identifying the factors responsible for differentiation would define potential therapeutic targets in lung injury. The differentiated phenotype of the alveolar type II cell in vitro is modulated by both the matrix on which the cells are cultured and various soluble factors. Matrices that allow the cells to adopt a cuboidal morphology (mimicking the in vivo state) appear to be critical element in this process. Soluble factors including FGF-1, KGF, and dexamethasone are important in modulating this process.

III. Evidence that Alveolar Type II Cells Limit Fibrosis

A. Reepithelialization and Prevention of Appositional Atelectasis

1. Type II Cell Hyperplasia

In 1954, Macklin (49) identified several important functions of alveolar type II cells and speculated on their role in modulating lung injury. He suggested that these cells are important in reducing surface tension, enhancing clearance of inhaled particles, and diminishing the transudation of interstitial fluid into the alveolus. A cartoon summarizing some of these features is shown in Fig. 3. Subsequent investigators noted that type II cell hyperplasia is a prominent feature of the histological response to lung injury (50,51). Proliferating type II cells are seen in lung tissue of virtually all patients with various patterns of lung injury including NSIP, UIP, bronchiolitis obliterases–organizing pneumonia (BOOP), or DAD. Additionally, this feature has been identified in various animal models of lung injury (52,53). Traditionally, type II cell hyperplasia has been conceptually associated with limiting inflammation and promote lung repair. Animal studies have demonstrated that impairing epithelial cell regeneration resulted in more extensive alveolar fibrosis (54–56).

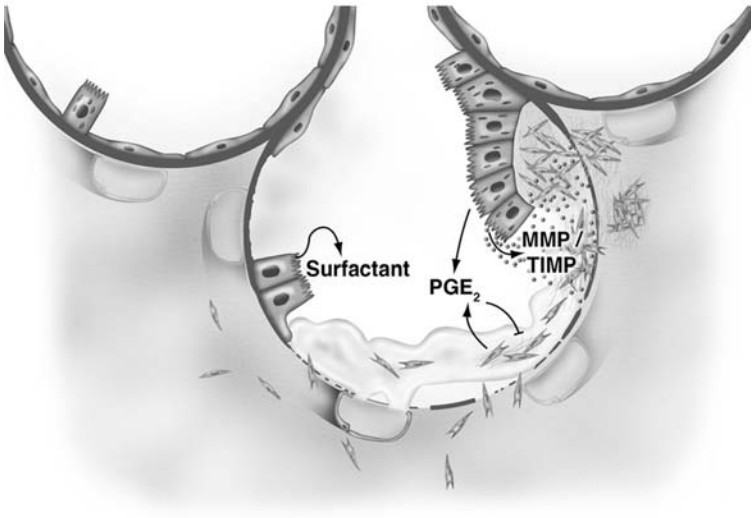

Figure 3 Hypothesis that alveolar type II cells inhibit fibrosis. This diagram depicts proliferating type II cells restoring the integrity of the alveolar epithelium along a denuded basement membrane. This is the classic "wound healing" hypothesis. Several secreted products of alveolar type II cells that act to inhibit fibrogenesis are depicted. PGE_2, prostaglandin E_2; MMP, metalloproteinase; TIMP, tissue inhibitor of metalloproteinase.

2. Alveolar Progenitor Cell

Kapanci (7) popularized the concept that alveolar type II cells serve as stem cells responsible for repopulating the alveolar epithelial surface area. This hypothesis was based on hyperoxic lung injury studies in monkeys. In these studies, sloughing of alveolar type I cells was followed by type II cell hyperplasia. These type II cells spread out along the denuded surface, and as healing occurred, type I cells replaced the type II cells. These observations led these investigators to conclude that the type II cell serves as the progenitor cell of the alveolar epithelium. Autoradiographic studies in rats exposed to nitrogen dioxide (57), oxygen (58), or ozone (59) demonstrated that alveolar type II cells have the capacity to replicate, whereas type I cells do not. Evans demonstrated the transition of alveolar type II cells into type I cells occurred within 48 h following exposure to nitrogen dioxide (60). Katzenstein (61) confirmed these animal experiments using tissue derived from lung biopsies of patients with acute interstitial pneumonia.

Uhal (62) has proposed the following model of alveolar epithelial kinetics in the adult lung based on current data. Alveolar type II cells in the lung are

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quiescent (G_0 phase) and must traverse G_1 phase before beginning DNA synthesis. Cell division results in two relatively undifferentiated daughter cells, both of which have the potential to revert to the type II cell phenotype or to transdifferentiate into a type I cell phenotype. However, the factors which determine the fate of these two independent daughter cells are not known.

Alveolar type II cells are capable of both self-maintenance and terminal differentiation, and thereby serve as the progenitor cell of the alveolar epithelium. These cells, however, have restricted lineage and are therefore not "true" stem cells. Recent work has demonstrated that stem cells derived from bone marrow engrafted into lung parenchyma develop a morphological and molecular phenotype of alveolar type I (63) and type II (64) cells.

These studies indicate a potential therapeutic role for bone marrow–derived stem cells in the treatment of diffuse alveolar damage.

3. Type II Cell Migration

Equally important in the remodeling process are signaling pathways that mediate type II cell migration along the regenerating basement membrane. In vivo studies of 3-methylindol–induced lung injury have been performed to delineate the effects of proliferation from migration on pulmonary fibrosis. Monotacraline was used selectively to inhibit type II cell proliferation without inhibiting migration (65) and pulmonary fibrosis did not occur. Woods et al. inferred that type II cell migration is an essential element of lung repair, and coverage of denuded basal lamina by any method (e.g., migration independent of proliferation) is sufficient to prevent pulmonary fibrosis.

In addition to its effects on proliferation and differentiation, KGF enhances the spreading and motility of alveolar epithelial cells. Mechanisms through which enhanced migration occurs have not yet been defined in alveolar epithelial cells. If KGF signaling pathways are similar to those of human keratinocytes (66), matrix metalloproteinases (MMPs) (67), and urokinase plasminogen activator (UPA) (68,69) are likely regulators.

TGF-α has been shown to enhance alveolar epithelial repair in an in vitro model of wound repair (70). Alveolar type II cells were plated on polystyrene tissue culture chamber slides and grown to confluence. A wound was made and time-lapse microscopy was used quantitatively to measure healing time. TGF-α significantly enhanced wound closure, and antibody to TGF-α significantly inhibited wound healing. Similar studies identified beneficial effects of interleukin-lβ on alveolar epithelial wound repair (71).

4. Alveolar Collapse

Alveolar collapse and fusion of adjacent alveolar walls has been observed in experimental models of pulmonary fibrosis. Alveolar cells are believed to act as an antiadhesive surface that prevents opposing alveolar walls from sticking

together (72,73) ''Collapse induration'' or ''atelectatic induration'' refers to the inability of alveoli to reexpand once the denuded alveolar walls coalesce (78), resulting in permanent apposition and loss of alveolar surface area. These collapsed areas appear as thickened or fibrotic alveolar septa when they are observed under light microscopy (74). As remodeling occurs, further thickening of the alveolar wall results from mural incorporation of organized airspace exudate and interstitial fibrosis as collagen bands replace the alveolar walls. The proliferation of type II cells is thought to prevent this atelectatic induration and fusion of two adjacent alveolar walls.

B. Barrier and Transepithelial Transport

The alveolar epithelial cell plays a dual role in protecting the alveolus from fluid accumulation. The alveolar epithelium acts as a relatively impermeable barrier protecting against fluid and solutes from leaking across the lung interstitium and vasculature into the alveolar spaces. In addition to its barrier function, the alveolar epithelial cell actively transports sodium ions from the alveolar lumen to the interstitium. This property is critical in promoting clearance of edema fluid from the alveolar space and restoration of alveolar gas exchange. A synergistic effect of KGF and beta-adrenergic therapy in upregulating alveolar epithelial fluid transport has been demonstrated in vivo (75). In vitro studies suggest that KGF modulates transepithelial ion transport by upregulating $Na/K = ATPase$ pumps in lung epithelial cells (76). Alveolar type II cells contain greater numbers of Na pumps when compared to alveolar type I cells (77). As suggested by Borok et al. (76), upregulation of Na pumps in cells treated with KGF may represent a marker of type II cell differentiation. Restoration of the alveolar epithelium is critical in preventing alveolar flooding and clearance of alveolar fluid necessary for effective gas exchange.

C. Role of the Epithelial-Alveolar Basal Lamina

The importance of the basal lamina in allowing for ordered lung repair was elegantly demonstrated by electron microscopy in an oleic acid model of lung injury (1). In this study, alveolar epithelial cells proliferated along denuded basal lamina scaffolding, reestablishing lung structure and function within 3 weeks of injury. In contrast to repair of other organs, the lung did not produce a new layer of basal lamina but rather guided alveolar cell attachment and proliferation along the original denuded basal lamina. The investigator concluded that intact scaffolding is an essential requirement for the orderly regeneration of the alveolar capillary barrier, and suggested that cell-specific receptors and signals guide this complicated process. In a bleomycin model of lung injury, Vaccaro et al. (78) confirmed that basal lamina thickening does not occur in the early phase of lung injury (1 week). Basal lamina thickening and duplication, however, was noted as a late (30–60 days) feature of the repair and

remodeling process. Katzenstein (74) has demonstrated in electron microscopic studies the extensive epithelial type I cell necrosis and denudation of alveolar basal lamina that occurs in inflammatory lung injury. Folding of the basal lamina in these areas seemed to interfere with normal reepithelialization. These studies collectively suggest that successful repair seems to depend on an intact epithelial basal lamina that defines the topography for guiding epithelial cell migration and regeneration.

Remodeling of the ECM is a complex event governed at least in part by matrix governed at least in part by matrix metalloproteinases (MMPs). MMPs are zinc endopeptidases that degrade ECM, promote neovascularization, and facilitate cell migration during reepithelialization. ECM synthesis and degradation is a dynamic, highly organized process whereby MMP effects are balanced by tissue inhibitors of metalloproteinases (TIMPs). Several studies suggest the effects of these enzymes vary with the effector cell being modulated and/or soluble factors present within the tissue environment.

Selman et al. (79) have shown a higher interstitial expression of various TIMPs compared with interstitial MMPs in lungs of IPF patients. TIMP-1 was present in interstitial cells associated with fibrous tissue, whereas MMP-1 was noticeably absent. This balance presumably creates a ''prevailing nondegradative lung environment'' favoring deposition rather than resorption of interstitial collagen. TIMP-2 was almost exclusively expressed by myofibroblasts within fibroblastic foci—a characteristic morphological feature of IPF representing the leading edge of the fibrotic process.

One would expect to find low levels of MMPs in the IPF lung in accordance with the fibrotic phenotype. MMP-1 expression (collagenase 1), however, is increased in alveolar epithelial cells, whereas MMP 2 and MMP 9 (gelatinases A and B) were detected in subepithelial myofibroblasts and occasionally in areas of denuded basement membrane (79). MMP-2 and MMP-9 mRNA (and to a lesser extent TIMP-1) expression has also been shown to be upregulated in rat type II alveolar epithelial cells in response to hyperoxic injury (80). The role of these upregulated MMPs in pulmonary fibrosis, while counterintuitive, can perhaps be explained from studies in skin wounds.

In human wounds, MMP-1 is expressed by basal keratinocytes at the migrating front of repairing tissue and diminished in cells further away from the wound margin. Pilcher et al. have demonstrated that MMP-1 (collagenase-1) is required for keratinocyte migration on type I collagen by cleaving collagen to gelatin and providing a substrate more conducive to migration (81). The proteolytic activity of MMP-1 may also aid in dissociating keratinocytes from high-affinity collagen attachments (via integrin $\alpha 2\beta 1$), thereby enabling migration. Pilcher et al. have proposed that MMP-1, acting on type I collagen, provides direction for migrating keratinocytes during reepithelialization. When keratinocytes move off an intact basement membrane and are exposed to collagen, MMP-1 is activated, facilitating migration and reorienting the

direction of migrating cells. In support of this hypothesis, keratinocytes grown on Matrigel do not express significant levels of collagenase, whereas cells grown on type I collagen produce markedly increased levels of MMP-1 (82).

Although the keratinocyte interaction with underlying matrices seems to be the primary stimulus for MMP expression, soluble mediators appear to modulate this process. Keratinocyte MMP-1 production is stimulated by several growth factors including TGF-α, TGF-β, EGF, HGF, Interferase-α (IFN-α). In contrast, Pilcher et al. demonstrated that KGF inhibits keratinocyte MMP-1 expression through the FGFR-2-IIIb receptor (83). KGF is expressed during epidermal wound repair and accelerates wound healing (66,84). In theory, MMP inhibition by KGF can abrogate the degradation of reforming basement membrane or the aberrant destruction of underlying ECM, thereby facilitating epithelial repair. At the same time, in a seeming paradox, MMP-1 activity is required for epithelial cell migration, a critical event in wound closure (8). KGF inhibition of MMP-1 would thus seem to be counterproductive in promoting epithelial repair.

Pilcher et al. synthesized the following hypothesis elucidating the role of KGF/MMP regulation in epithelial injury (83). It is likely that KGF plays a dual role in wound repair; that is, impeding basement membrane degradation while concurrently facilitating migration. These effects are regulated through FGFR-2-IIIb (KGF receptor) expression. KGF receptors are expressed throughout intact skin. Wounding results in a dramatic reduction in KGF receptor expression in migrating keratinocytes at the wound edge. Proliferating basal cells just behind the migrating front, however, maintain prominent expression of the KGF receptor (85). Downregulation of the KGF receptor at the wound edge results in enhanced MMP-1 production facilitating migration. Concurrently, proliferating cells immediately behind the migrating front prominently express KGF receptors. KGF inhibition of MMP-1 in these cells prevents degradation of reforming basement membrane or aberrant destruction of underlying ECM.

In this context, a complex and delicate balance likely exists in lung injury whereby MMP regulation varies with effector cell type and location. MMPs may function in regulating migration of epithelial cells to areas of denuded alveolar basement membrane and favoring collagen degradation in these areas. Alternatively, overexuberant MMP production by alveolar epithelial cells may contribute to failure of reepithelialization through further degradation of the alveolar basement membrane, ECM remodeling, and transepithelial migration of inflammatory cells. In the lung interstitium and regions of fibroblastic foci, downregulation of MMPs and upregulated TIMPs may act to promote fibrosis. However, more studies are needed to determine if alveolar epithelial cells function similar to keratinocytes in regulating MMP expression and the protease/antiprotease balance in the alveolar microenvironment of the fibrotic lung.

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D. Inhibition of Fibroblast Activity

Various paracrine mediators are produced by alveolar type II cells. Some are profibrotic and proinflammatory and support the notion that the alveolar type II cell fuels fibrogenesis (see Sect. III). Conversely, alveolar type II cells release other regulatory factors that protect against pulmonary fibrosis by inhibiting fibroblast activity. One such mediator is prostaglandin E_2 (PGE_2).

PGE_2 has been reported to inhibit fibroblast proliferation (86), chemotaxis (87), and collagen production (88,89). Klien and Adamson (90) showed that alveolar epithelial cells exposed to silica secreted PGE_2. Serum free supernatants of these cells inhibited thymidine incorporation of fibroblasts and collagen synthesis. Pan et al. (91) further defined this relationship demonstrating that epithelial control of fibroblast proliferation in vitro involves reciprocal interactions between these two populations of cells and the autocrine production of PGE_2 by fibroblasts. These effects were enhanced by KGF and inhibited with indomethacin.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been implicated as a regulator of PGE_2 signaling protecting against pulmonary fibrosis. GM-CSF–deficient knockout mice developed more severe pulmonary fibrosis following bleomycin injury compared to wild-type mice (92). These animals also had reduced levels of PGE_2 in whole lung homogenates. This deficiency in PGE_2 was corrected with the exogenous administration of GM-CSF to GM-CSF null mice. Other studies have shown decreased mRNA expression of GM-CSF in alveolar type II cells isolated from rats following bleomycin injury (93).

E. Surfactant

Alveolar type II cells are responsible for the synthesis and storage of pulmonary surfactant. Pulmonary surfactant refers to a phospholipid and protein mixture comprising four distinct proteins (A–D). Surfactant proteins B and C (SP-B and C) are hydrophobic proteins that organize phospholipids and reduce lung surface tension and prevent collapse of alveoli (94), whereas SP-A and SP-D are hydrophilic proteins that attenuate and modulate host defense mechanisms. SP-A and D act as opsonins, enhancing clearance of inhaled pathogens (95,96). Furthermore, SP-A and SP-D are potent endogenous inhibitors of oxidative cell injury (97) and modulate the function of phagocytic cells both in vivo and in vitro (98–102). Surfactant composition is fairly constant in mammalian species and composed of approximately 80% phospholipids, 8% other lipids, and 12% protein. Phosphatidylcholine (PC) accounts for approximately 85% of phospholipid composition. Phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) comprise the remaining 15% of phospholipids (9, 3, and 2%, respectively). Both the lipid and the protein components of surfactant are likely altered in pulmonary fibrosis.

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In Ashbaugh and Petty's initial description of the acute respiratory distress syndrome (ARDS) (103), it was noted that the lung pathology resembled that of infant respiratory distress syndrome (IRDS). They postulated that deficient or defective surfactant may similarly contribute to the adult form of the disease. Qualitative alterations in phospholipid composition were also measured with a reduction in the relative percentages of PC and PG and increased percentages of PI and PE (104,105). These changes correlated with significant increases in surface tension. Reduced levels of SP-A and SP-D in BAL fluid of ARDS patients have also been correlated with disease morbidity and mortality (106). Subsequent studies also revealed reduced levels of SP-A and B in the BAL fluid (104,105). The efficacy of exogenous surfactant administration in neonates with infant respiratory distress syndrome is well established (107). Several small pilot studies have been performed examining the efficacy of the exogenous administration of various surfactant preparations in ARDS with mixed results (108). Controlled studies on larger patient populations are currently underway that will hopefully define the role of this therapy in this disease.

Marked surfactant disturbances have been noted in IPF. Patients with IPF had decreased levels of total phospholipid in BAL relative to normal volunteers (109). SP-A normalized to total phospholipid (SP-A/PL) was significantly reduced in the lavage fluid and correlated with a poor prognosis as defined by both 6-month follow-up clinical-radiographic-physiological (CRP) score and survival (110). Analysis of the surface-active material indicated compositional aberrancy with a reduced percentage of PG and an increased percentage of PI. Saturated levels of PC, considered an important modulator of alveolar surface tension, were not consistently altered (109,111,112). Serum levels of SP-A and SP-D are also increased in patients with IPF and inversely correlate with survival (113,114). Surfactant abnormalities may therefore prove to be a useful prognostic marker of disease severity in IPF. Gunther et al. demonstrated that the qualitative deficiency of phospholipids was associated with marked abnormal surface properties of surfactant from patients with IPF (112). In vivo studies by Enhorning et al. examined the effects of surfactant on airways and suggested that airway patency like alveolar patency is maintained by endogenous surfactant (115). It is possible that the altered surfactant in IPF leads to closure of small terminal airways and their reopening with inspiration produces Velcro-like rales.

IV. Evidence that Type II Cells Augment Fibrosis

A. Growth Factors

Over the last decade, significant advances have been made in understanding the idiopathic interstitial pneumonias. IPF is currently recognized as being

a distinct clinical subset associated with a histological pattern of UIP. As noted above, the inflammatory fibrosis hypothesis has been refuted in the pathogenesis of IPF. Katzenstein (116) has noted that inflammation is not a prominent histological feature of UIP, and studies have not demonstrated significant benefit with anti-inflammatory therapy (3). Selman et al. have suggested that IPF is an "epithelial-fibroblastic disease" characterized by a fibroproliferative disorder preceded by alveolar epithelial activation (5). Ultrastructural observations demonstrating direct cell-to-cell contact between alveolar type II epithelial cells and fibroblasts support this theory (117,118). Presumably, repeated multiple, focal microscopic sites of ongoing epithelial cell injury and activation induce the proliferation and migration of fibroblasts and myofibroblasts. These "fibroblastic foci" (see Fig. 2) are thought to represent the leading edge of the fibrotic process. It has been demonstrated that activated alveolar epithelial cells release several proinflammatory cytokines in the IPF lung, and contrary to the conventional notion that alveolar epithelial cells limit fibrosis, they may, in fact, promote fibrogenesis (see Fig. 4).

Immunohistochemical studies (119–121) on lung biopsies of IPF patients have demonstrated that hyperplastic alveolar type II cells constitute a source of upregulated TGF-β1 in the diseased lung. Macrophages likely remain the dominant source of TGF-β. TGF-β is a chemoattractant for macrophages and fibroblasts and perhaps the most potent promoter of ECM production and

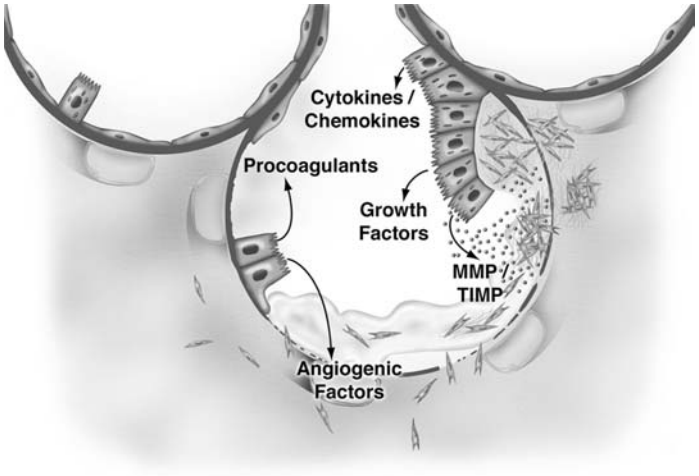

Figure 4 Hypothesis that alveolar type II cells augment fibrosis. This diagram depicts various profibrotic products secreted by alveolar type II cell, including (1) cytokines/chemokines, (2) growth factors, (3) MMP/TIMPs, (4) procoagulant proteins, and (5) angiogenic factors. The epithelial injury and profibrotic hypothesis are illustrated.

pulmonary fibrosis. In addition, TGF-β promotes the differentiation of myofibroblasts (122), the central cell type of the fibroblastic foci. Khalil demonstrated that the most intense staining for TGF-β occurred in hyperplastic type II cells and the alveolar cells lining honeycomb cysts in areas of advanced fibrosis (123). The critical role of TGF-β in inducing pulmonary fibrosis has been demonstrated by the TGF-β overexpression in fibrogenic-resistant TNF-α receptor knockout mice. TGF-β overexpression with an adenoviral vector induced pulmonary fibrosis in these mice (124).

TGF-α, a potent mitogen and chemotactic factor, is another growth factor secreted by alveolar type II cells that acts in the regulation of pulmonary fibrosis. Some of the growth factors expressed in hyperplastic type II cells are shown in Fig. 5. Studies with transgenic mice have demonstrated that overexpression of TGF-α by alveolar epithelial cells results in pulmonary fibrosis as well as areas of airspace enlargement (emphysema) (125). Increased TGF-α expression has been detected by immunohistochemistry in alveolar epithelial cells in lung tissue of patients with IPF (126). The induction of lung fibrosis after bleomycin injury is attenuated in TGF-α–deficient mice, providing further evidence for the contribution of TGF-α in fibrogenesis (127). Heparin-binding EGF (HB-EGF) is another growth factor that is similar to TGF-α and signals through the EGF receptor.

Platelet-derived growth factor (PDGF) is a profibrotic growth factor that acts as a chemoattractant for fibroblasts and smooth muscle cells and stimulates collagen synthesis. Various studies support a role for PDGF in stimulating pulmonary fibrosis. PDGF gene expression is upregulated in the lungs of patients with IPF (128) and in bronchoalveolar cells of rat lungs injured following asbestos exposure (129). In addition, inhibition of the PDGF receptor with specific chemical inhibitors attenuated collagen deposition in a rat model of lung injury (130). These cumulative data strongly suggest the alveolar epithelial cells are a source of profibrotic growth factors both in vivo and in vitro.

Conflicting data exist regarding the role of insulin like growth factor-1 (IGF-1) in pulmonary fibrosis. IGF-1 has been shown to be an important mitogen for alveolar epithelial cell proliferation in the developing postnatal rat lung (131). Targeted homozygous deletion of IGF-1 receptor in transgenic mice results in atelectasis, respiratory failure, and death in the immediate perinatal period (132). IGF is expressed principally by alveolar macrophages and only minimally expressed by alveolar epithelial cells in the normal lung. In contrast, the lungs of IPF patients demonstrate significant upregulation of IGF-1 expression (133). Similar increases of IGF-1 expression in rat alveolar epithelial cells has been demonstrated in models of hypereroxic lung injury (134,135). It is possible that IGF-1 limits pulmonary fibrosis by stimulating type II cell hyperplasia and restoring alveolar integrity of the injured epithelium. Other data, however, indicate that IGF-1 stimulates

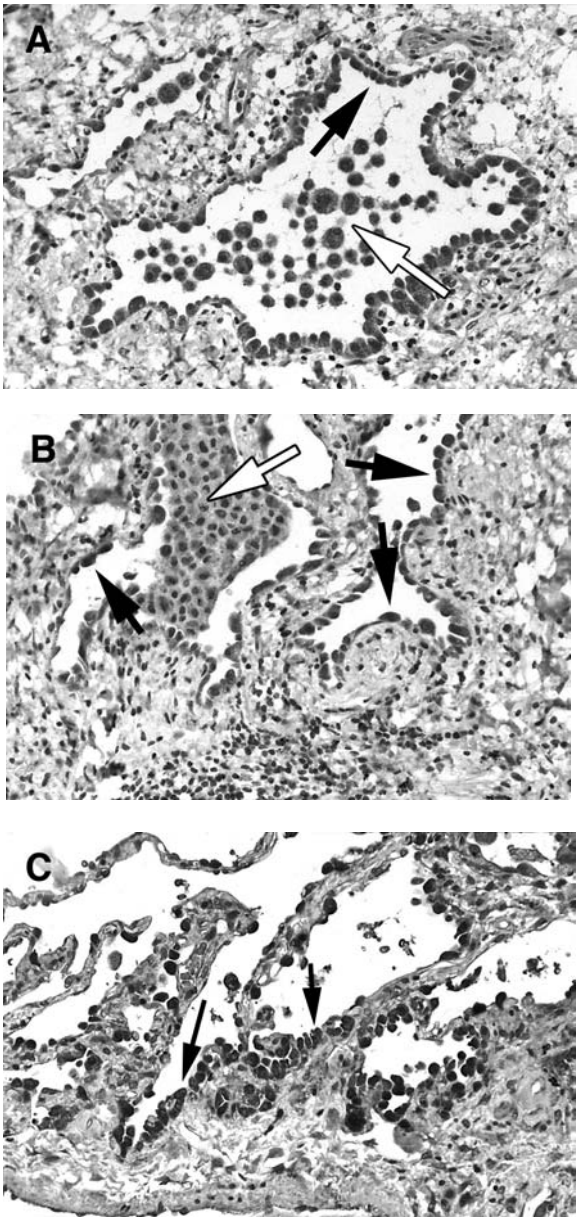

Figure 5 Immunocytochemistry for autocrine growth factors for the alveolar epithelial cells. Panel A shows immunostaining of hyperplastic type II cells and alveolar macrophages for HGF. The black arrow points to hyperplastic alveolar type II cells and the white arrow points to intraalveolar macrophages. Panel B depicts immunostaining

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fibroblast proliferation and collagen synthesis (136,137). One recent study (138) using a neonatal explant culture of rat lung demonstrated that hyperoxia led to an increase in IGF-1 in the alveolar and bronchial epithelial cells, whereas an increase in IGF-1 receptor was seen in fibroblasts. These studies suggest IGF-1 acts to augment pulmonary fibrosis through epithelia-mesenchyma interaction.

B. Cytokines

A variety of cytokines are produced by alveolar epithelial cells and aid in the regulation of pulmonary inflammatory and fibrogenic pathways. Families of chemokines (chemotactic cytokines) produced by many cell types, including alveolar epithelial cells, have been identified and their role in inflammatory responses elucidated. Chemokines are important mediators of host defense functioning in chemotaxis and activation of immune cells at sites of injury. Macrophage inflammatory proteins-l and -2 (MIP-1 and -2) are examples of such chemokines. In a rat model of silicotic lung disease, MIP-1α and MIP-2 gene expression was upregulated and attenuated with anti-TNF antibody (139). Based on these studies, MIP expression may be at least partially regulated by a network of cytokines including TNF.

Paine et al. (140) have shown that monocyte-chemoattractant protein-l (MCP-1), a chemoattractant for alveolar macrophages, secreted by alveolar type II cells cultured in vitro and may play a significant role in the initiation and maintenance of the inflammatory process. Transgenic mice deficient in CCR2, the major MCP-1 receptor, are protected from fibrosis in both fluorescein isothiocyanate (FITC) and bleomycin lung injury models (141). These mice have increased levels of GM-CSF and reduced levels of TNF-α compared to FITC-treated wild-type mice. Furthermore, MCP-1 mRNA is strongly expressed in alveolar epithelial cells of patients with IPF (142).

A number of other cytokines induce inflammation when expressed in the lung by various cells including alveolar epithelial cells. These include interleukin-6 (IL-6) (143), IL-1β (144), IL-8 (145), and TNF-α (146,147).

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of hyperplastic type II cells and macrophages for HB-EGF. The black arrows point to hyperplastic alveolar type II cells and the white arrow points to intraalveolar macrophages. Panel C shows immunostaining of hyperplastic type II cells and macrophages for TGF-α. The black arrows point to hyperplastic alveolar type II cells. These are the first micrographs showing staining for HB-EGF. The lung was removed for transplantation from a patient with IPF.

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TNF-α is an important proinflammatory cytokine, and compelling data suggest it is involved in pulmonary fibrosis. TNF-α mRNA is increased in the lungs of patients with IPF (148).Treatment with an anti–TNF-α antibody prevents bleomycin (149) and silica-induced pulmonary fibrosis (150). Administration of a soluble TNF-α receptor prevents bleomycin-induced pulmonary fibrosi (151). Murine studies have reported that TNF-α–deficient mice or knockout mice are protected from developing bleomycin- or asbestos-induced pulmonary fibrosis (152–154). However, chronic overexpression of TNF-α leads to emphysema and not pulmonary fibrosis (155). TNF-α may exert its profibrotic effects on fibroblasts through TGF-β or PDGF induction pathways (119) or by upregulating MMP activity and disrupting reconstitution of the alveolar basement membrane (156–158). Pirfenidone, an antifibrotic agent with anti–TNF-α properties, has been used successfully in the treatment of IPF patients (159).

Endothelins were first identified in endothelial cell supernatants and found to possess vasoconstrictor properties (160). Subsequent studies demonstrated that endothelin-1 (ET-1) possesses proinflammatory properties capable of activating neutrophils, mast cells, and monocytes and stimulating monocyte release of various cytokines including IL-1β, IL-6, IL-8, TNF-α, TGF-β, and GM-CSF (161). Furthermore, ET-1 can function as a profibrotic cytokine by stimulating fibroblast chemotaxis and proliferation (162,163). ET-1 gene expression is upregulated in epithelial cells of patients with IPF. In patients with scleroderma-associated pulmonary fibrosis, ET-1 levels in BAL fluid were fivefold higher than those of controls (164). In animal models of bleomycin lung injury, immunocytochemistry increased ET-1 staining in alveolar epithelial cells (165). These studies suggest a role for ET-1 in pulmonary fibrosis.

C. VEGF, Angiogenesis, and the Alveolar Epithelial Cell

Angiogenesis is an integral feature of tissue repair following injury. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that promotes endothelial growth (166), chemotaxis (167), and capillary permeability (168). In addition, VEGF protects against endothelial cell apoptosis both in vivo and in vitro (169–171). VEGF is constitutively expressed by human bronchial and alveolar epithelial cells (172), and its expression is significantly increased by hypoxia and exposure to TGF-β1 (173). Endothelial cells exposed to conditioned media from these cultures showed an increased growth rate, which was inhibited in the presence of neutralizing antibody to VEGF. These studies suggest that the alveolar epithelial cell, through VEGF production, participates in the endothelial repair and angiogenesis that occurs in lung injury.

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D. Coagulation and Fibrosis: Role of the Alveolar Epithelial Cell

In acute lung injury, plasma proteins leak into the interstitial and alveolar spaces triggering activation of the coagulation cascade and fibrin deposition. As the inflammatory exudate is converted into granulation tissue, fibroblasts migrate into the fibrin matrix and deposit collagen and other ECM components. Plasmin is the leading protein responsible for fibrin resorption. Persistence of fibrin fuels fibrosis and is regulated by a balance of procoagulant (tissue factor), fibrinolytic (plasminogen activators), and antifibrinolytic systems (plasmingen activator inhibitor—PAI-1 and -2).

Increased procoagulant and antifibrinolytic activity has been found in the lungs of patients with IPF (174). Tissue factor (175), PAI-1, and PAI-2 (174) levels are increased in the BAL fluid of IPF patients and strongly expressed on immunostaining by alveolar epithelial cells. Further evidence supporting the association of antifibrinolytic activity and fibrosis has been demonstrated in bleomycin-injured transgenic mice that either lack or overexpress the PAL-1 gene (176). Lungs of mice overexpressing the PAI-1 gene had significantly more collagen accumulation following injury. These data suggest alveolar epithelial cells are involved in establishing a hypercoagulable state in the lungs of IPF patients, inhibiting fibrin resorption and thereby promoting fibrosis.

E. SP-C Mutations

Another mechanism by which type II cells could contribute to interstitial lung disease is by producing a misfolded, inactive but fibrogenic form of SP-C. Recently, point mutations and deletions in SP-C have been associated with familial interstitial pneumonitis in young adults and children (177,178). In a recent report, one patient had UIP pathology with fibroblastic foci (6). In this kindred, a point mutation with a switch from T to A at position 128 resulted in a substitution of glutamine for a leucine and presumably leads to a misfolded protein that injures the cell and sets off a slow inflammatory and fibrogenic process. In addition, deletion of SP-C leads to a normal phenotype in some strains of mice but a fibrotic pattern in other strains. Clearly, the genetic background for the effects of these mutations and deletions is important. These will be disease-modifying genes that will need to be defined. When the bone morphogenic protein receptor mutation (BMPR-II) was found to be associated with familial pulmonary hypertension, it was only thought to account for a relatively few of the sporadic cases of pulmonary hypertension. However, this genetic abnormality has proven to be much more common than originally thought and could account for a significant portion of patients with primary pulmonary hypertension. The same might turn out to be true for the SP-C mutations or deletion. In addition, point mutations or heterozygous deletion of SP-B may also contribute to adult diffuse lung disease. The SP-C

point mutations may prove to be very important in our understanding of pulmonary fibrosis and even the relationship of UIP to fibrosing NSIIP.

F. Apoptosis

Inflammation is not a prominent histological feature in IPF. Epithelial injury and dysregulated repair appear to be the critical determinants in development of pulmonary fibrosis (56,179). Apoptotic alveolar epithelial cells have been identified in areas immediately adjacent underlying myofibroblasts in fibroblastic foci (180). Uhal et al. suggested that apoptosis may be an important mechanism in epithelial cell death, delaying epithelial repair and promoting fibrosis. This theory is supported by in vitro data where fibroblasts isolated from human IPF lung or from paraquat-injured rat lung release soluble factor(s) that induce apoptosis of alveolar type II cells in vitro (181). Angiotensin II has been implicated as the soluble factor responsible for inducing epithelial cell apoptosis (182). The clearance of apoptosic cells is associated with secretion of TGF-β, which would enhance the pulmonary fibrotic process. Although apoptosis of mesenchymal cells is considered to be an important mechanism in the clearance of granulation tissue from injured lung, apoptosis of epithelial cells may in fact impair restoration of normal architecture and promote fibrosis. However, apoptosis of alveolar epithelial cells is also required for the evolution from a hyperplastic epithelium to the normal type I and type II cell epithelium.

V. Gaps in Our Knowledge

As discussed in the previous sections, it is not absolutely clear how much the type II cell protects against or promotes fibrosis. What is known is that type II cells have the capability of doing both. Most likely both responses are involved at different stages of the disease process but could even occur within the same lungs at the same time. To resolve these issues and to provide new targets for therapy, there are some gaps in our knowledge that can be and are being addressed.

A. Regulation of Type II Cell Hyperplasia

There are two important processes that can theoretically produce the hyperplastic epithelium that is seen in pulmonary fibrosis. The first is direct stimulation of proliferation and the other is inhibition of transdifferentiation into type I cells. There are a number of growth factors that are potential candidates for stimulating proliferation. Although several families of growth factors have been identified as being potentially important,there is not a clear

relationship of growth factor expression and type II cell hyperplasia. To date, the growth factor(s) responsible for type II cell hyperplasia in pulmonary fibrosis has not been identified. Part of the problem is that many of these growth factors are bound to the ECM, and if these are the pools of growth factors that are used in the response to injury, there would not be a large increase in the mRNA or protein levels for these growth factors at the time of type II cell proliferation, which could be used to identify the growth factor responsible. Although there are some data on expression of individual growth factors in the human lung, a more detailed analyses needs to be done. In addition, a systematic evaluation of the precise local pathological changes in human lung that neighbors the hyperplastic epithelium is required to define the cellular milieu in which proliferation occurs. Does proliferation occur in sites of myofibroblasts, normal fibroblasts, macrophages, or other cell types, or does it occur only in areas of local expression of autocrine growth factors? Direct cell-cell contacts occur in the developing lung between fibroblasts and type II cells. Similar contacts occur in the fibrotic lung. These direct cell-cell contacts also need to be examined and their physiological role established.

Inhibition of transdifferentiation to type I cells can be theoretically very important, can lead the appearance of type II cell hyperplasia, and has not been the subject of much direct investigation. One of the reasons that this might be very important is that type I cells may be the alveolar epithelial cell phenotype that most directly inhibits fibroblast migration, proliferation, and matrix production. Type I cells likely also secrete PGE_2, which is a known inhibitor of fibroblast proliferation (183). In the fetal lung as the type I cells are formed, the alveolar wall thins out and there is a decrease in the relative number of fibroblasts. In addition, along the alveolar wall there are very few fibroblasts beneath type I cells.

The factors that regulate the transdifferention of type II cells into type I cells are not known. In vitro type II cells can be stimulated to express type I or type II cell markers depending on the culture conditions, especially the substratum on which the cells are grown. The interconversion of the type I cell and type II cell phenotype is reversible in vitro. In vivo it has not been proven that type I cells can revert to the type II cell phenotype. In vivo it is known that if type II cell hyperplasia is induced by KGF either as the recombinant protein or by an adenovirus that expresses KGF, the hyperplastic epithelium reverts back to the normal alveolar epithelium within a few days. This is accompanied by apoptosis of many of the type II cells. Therefore, it is possible that the type II cell hyperplasia that occurs in the fibrotic lung is due to the inhibition of apoptosis and the transdifferentiation to type I cells. Although apoptosis in the lung is traditionally evaluated by the TUNEL assay or similar methods, it is possible that apoptosis could also be measured by the appearance of type II cells in lavage

fluid, since apoptosis in an epithelium is accompanied by active extrusion of the apoptotic cells from the monolayer by their neighbors (184). One of the other functions of growth factors is their ability to suppress apoptosis, and hence they are also survival factors. The implication of this concept is that the resolution of pulmonary fibrosis may be accomplished by inducing apoptosis of the hyperplastic type II cells and stimulating this transdifferentiation of type II cells into type I cells. Clearly, for the type I cell to spread and assume their characteristic shape, many hyperplastic type II cells would have do undergo apoptosis. The importance of this concept will also require a better characterization of the type I cell phenotype to demonstrate conclusively that it is more antifibrotic than normal type II cells.

B. Products of Hyperplastic Type II Cells

Defining the secretory products of hyperplastic type II cells requires more investigation. Although it is clear that type II cells can express a variety of cytokines, growth factors, and chemokines, it is not clear the amount that is actually secreted nor the relevant contribution compared to other cells, especially macrophages. For example, there are in situ data that demonstrate type II cells express PDGF, but there are no studies that demonstrate the level of protein secretion. Similarly, there is evidence that dedifferentiated rat epithelial cells and hyperplastic type II cells in the IPF lung produce IGF-1, but the actual amount secreted by type II cells compared to other cells has not been reported. Parenthetically, it should be noted that determining the relative level of expression of chemokines or growth factors in type I cells in vivo may be extremely difficult, because in cross section, their cytoplasm is so thin and the correction for surface area in the sections may be difficult. However, with different culture conditions, it may be possible to measure the production and secretion of these growth factors, chemokines, and cytokines in alveolar epithelial cells that express either type II or type I cell markers. Similarly, the effect of coculture of these type II cells with macrophages or fibroblasts could easily be done to examine these important cell-cell interactions. It is highly likely that the response of alveolar epithelial cells will be determined by paracrine factors produced by macrophages, fibroblasts, and other cell types. Current culture techniques make these studies feasible.

The final issue is to determine if point mutations and misfolding of proteins within type II cells produce pulmonary fibrosis. This has been reported for SP-C (6). However, this could occur with other proteins as well. It is also probable that other mutations in the SP-C gene will be discovered. The intriguing issue is that the same mutation can produce a different histopathology within the same kindred.

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Acknowledgment

This work was supported in part by grants from the National Institutes of Health (HL-67671 and HL-07085).

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Surfactant Proteins in the Pathophysiology of Pulmonary Fibrosis

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I. Introduction

The existence of inflammatory reactions and a disordering of parenchymal cells are thought to be important for the evolution of pulmonary fibrosis. The fibrotic process may result from a complex interaction between fibroblasts, macrophages, lymphocytes, endothelial cells, and epithelial cells. This interaction is organized by much more complex network of cytokines and chemical mediators, which are produced by diverse cell types.

Alveolar type II cells, which serve as progenitor cells of type I cells covering 95% of the alveolar surface, are essential for repair of the alveolar epithelium in acute lung injury and pulmonary fibrosis. Alveolar type II cells are also multifunctional cells secreting myriad biological components such as pulmonary surfactant, which acts to prevent alveolar collapse by reducing surface tension. Since the persistence of alveolar-collapsed state may be a trigger of fibrosis in lung parenchyma, production of pulmonary surfactant is critical to prevent fibrosis. Recent studies have shown that some components of pulmonary surfactant downregulate the expression of certain proinflammatory cytokines including tumor necrosis factor-α (TNF-α) (1–3) which generate profibrotic growth factors such as transforming growth factor-β (TGF-β). Thus, pulmonary surfactant may play an important role to prevent progression of fibrosis.

Pulmonary surfactant is composed of phospholipids, cholesterol, and specific proteins. Four surfactant proteins (SPs) have been identified and are divided into two groups: hydrophilic proteins, SP-A and SP-D and the hydrophobic proteins, SP-B and SP-C. SP-A and SP-D serve important roles in

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innate immunity, whereas SP-B and SP-C play crucial roles in the generation and maintenance of a surface-active film in the alveoli. The cDNAs and specific antibodies have been shown to be useful tools to detect these proteins. Establishment of enzyme-linked immunosorbent assay (ELISA) for each protein also enables the determination of the absolute amount of the proteins (4–9). Since major clinical studies in pulmonary fibrosis have emphasized the hydrophilic proteins, this chapter will focus on the pathophysiological and clinical significance of SP-A and SP-D as biomarkers in this disorder.

II. Molecular Structure, Regulation, and Functions of SP-A and SP-D

The human SP-A locus is located on chromosome 10 and consists of two functional genes in opposite transcriptional orientation (10,11). They contain two highly similar SP-A gene products, SP-A1 and SP-A2, which are 95.6% homologous (238 of 249 amino acids are identical). The human SP-D is encoded by a single gene on the same chromosome in the region of 10q22.2-23.1 (12), which is located proximal to the centromere at approximately 80–100 kb from the SP-A2 gene (11).

SP-A and SP-D are structurally similar to the C1q component of complement and mannose-binding protein. These molecules are members of the collectin family, which contain a collagenlike amino-terminus and a C-type lectin carbohydrate-recognition domain (CRD) at the carboxyl-terminus (13,14) (Fig. 1). The structure for SP-A is very similar to that for serum mannose-binding protein (MBP-A) and C1q (15). Monomeric units are divided into an amino-terminal position, a collagenlike region, a neck, and a CRD. SP-A forms a ''flower bouquet'' structure composed of 18 monomers that are organized as 6 trimeric units with approximately 25 nm of length. SP-A trimers are thought to be composed of two SP-A1 molecules and one SP-A2 molecule (16). SP-D forms a symmetrical cruciform-shaped molecule composed of 12 monomers that are organized as 4 trimeric units (17,18). The distance between the CRDs in SP-D dodecamer is approximately 90 nm. The momomeric molecular mass of human SP-A and SP-D is 30–36 and 43 kD respectively. The molecular mass of the human SP-A octadecamer is reported to be approximately 650 kD by gel filtration analysis and that of SP-D dodecamer is approximately 540 kD (19).

SP-A, SP-B, and SP-D are synthesized by both alveolar type II cells and nonciliated bronchiolar (Clara) cells in the lung, whereas SP-C is produced solely by alveolar type II cells. Unlike SP-D, SP-A has a high affinity for phospholipid components of surfactant such as dipalmitoylphosphatidylcholine (DPPC) (20), which is the most abundant phospholipid in surfactant and serves the most central role for lowering surface tension. SP-D is not processed

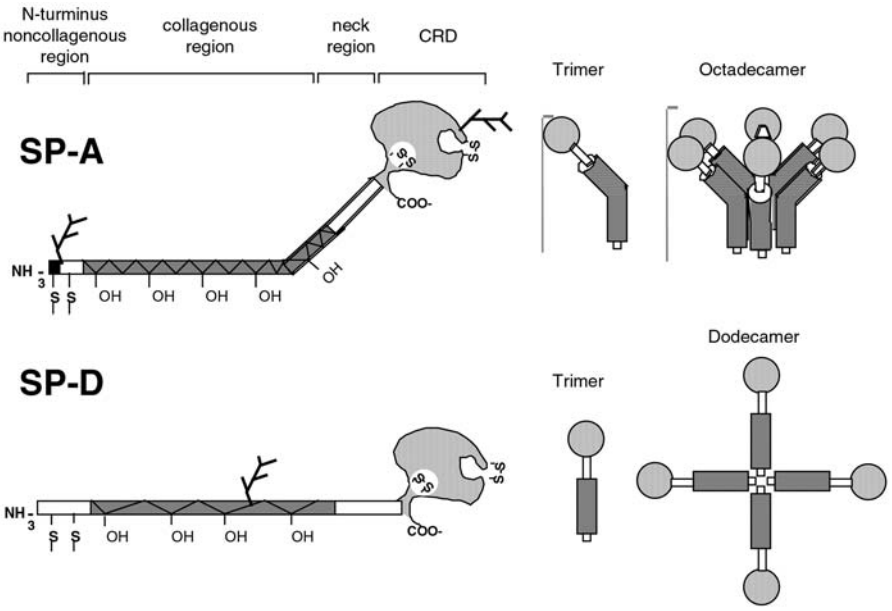

Figure 1 Monomeric and oligomeric structures of SP-A and SP-D. Monomeric structures can be conceptually divided into four major structural domains: a short N-terminal segment containing two intermolecular disulfide bonds, a collagenlike sequence of gly-x-y repeats, an acidic and hydrophobic neck domain, and a C-terminal carbohydrate-recognition domain (CRD). The CRD contains a calcium-dependent, carbohydrate-binding site. The matured molecule of SP-A and SP-D consists of six and four trimeric subunits, respectively. (Modified from Ref. 14.)

with the phospholipids of surfactant, and it is neither found in lamellar bodies (an intracellular form of mature surfactant) nor in tubular myelin (an extracellular form surfactant).

SP-A gene expression is regulated by several growth factors and hormones. SP-A levels are increased by prostaglandins (21), lipopolysaccharide (LPS) (1,22), interferon-gamma (INF-γ) (23) and epidermal growth factor (24). On the other hand, SP-A is decreased by insulin (25,26), TNF-α (27), and TGF-β (24). Glucocorticoids such as dexamethasone have a biphasic effect on the accumulation of SP-A and its mRNA; a stimulatory effect at lower concentrations, whereas an inhibitory effect at higher concentrations (28). Addition of 10^{-7} M dexamethasone antagonized the stimulatory effects of fibroblasts on expression of SP-A mRNA in type II cells, whereas expression of SP-D was unaffected (29). Dulkerian and colleagues (30) indicated that some inflammatory mediators such as TNF-α, which regulate SP-A, do not influence

SP-D. These above reports suggest that SP-A and SP-D are not coordinately regulated in response to lung inflammation. Shannon and colleagues (29) showed that mRNA levels of SP-A and SP-D in alveolar type II cells are significantly increased by coculture with lung fibroblasts and that keratinocyte growth factor (KGF), which is produced by lung fibroblasts, mimics the effects by lung fibroblasts on SP gene expression. These results suggest that alveolar type II cells synthesize SP-A and SP-D to inhibit the progression of fibrosis.

SP-A regulates the homeostasis of surfactant phospholipids and facilitates the lowering of surface tension in the alveoli. SP-A enhances the rate of formation of a phospholipid surface film (31), and SP-A binds to a receptor on type II cells and enhances the uptake of surfactant phospholipids for recycling. Surfactant clearance is also performed by alveolar macrophages, and this function is similarly enhanced by SP-A (32). SP-A inhibits the secretion of surfactant phospholipids by alveolar type II cells, suggesting that SP-A is involved in a negative-feedback loop that regulates surfactant homeostasis in the alveoli (33). SP-A also prevents the increase of surface tension by plasma proteins that have leaked into the injured alveoli (34), suggesting that SP-A attenuates collapse of alveoli promoted by the plasma proteins in interstitial lung injury and pulmonary fibrosis.

Recent studies using SP-A knockout mice (35) have revealed no major abnormalities in lung function. However, the SP-A geneknockout mice are more susceptible to infection with several pathogens examined. Therefore, the major role of SP-A in the alveoli is now recognized to relate to local host defense based on innate immunity. SP-A binds with a broad specificity to a variety of microorganisms including both gram-positive and gram-negative bacteria, herpes simplex virus type 1, *Pneumocystis carinii*, and *Aspergillus fumigatus* (36). SP-A is thought to bind to the pathogens via its CRD. This protein enhances the phagocytosis of *Staphylococcus aureus*, herpes simplex virus type 1, type A *Haemophilus influenzae*, *Mycobacterium tuberculosis*, and *Klebsiella* by alveolar macrophages.

SP-A inhibits the macrophage-derived TNF-α secretion induced by smooth LPS (2), which is a complete structural form of LPS and is possessed by gram-negative bacteria causing airway infection. Since TNF-α is a key cytokine which plays central roles for the progression of acute lung injury and resulting fibrosis, SP-A may possess an anti-inflammatory function in the lung. This anti-inflammatory effect is in part via the binding of SP-A to CD14, which functions as a pattern-recognition receptor for gram-negative bacterial ligands, LPSs. SP-A also inhibits the TNF-α synthesis by gram-positive bacteria via the interaction with SP-A and Toll-like receptors 2 (TLR-2) (3), which is mediating nuclear factor (NF-κB) induced by peptidoglycan (PGN), a major cell wall component of gram-positive bacteria. In the case of lung injury by noninfectious causes including toxic, irradiative, and autoimmune factors,

previous studies have not elucidated yet whether SP-A can elicit the excessive release of proinflammatory cytokines from immune cells that contribute to the adverse clinical manifestations. Another collectin, SP-D, also binds to microorganisms, opsonizes their phagocytosis by alveolar macrophages, and interacts with CD14. However, many aspects SP-D function remain to be defined.

III. SP-A and SP-D in Bronchoalveolar Lavage Fluids and Their Clinical Significance

SP-A and SP-D exist in the bronchoalveolar space under physiological conditions and their concentrations can be measured by ELISA (6,8,9,37). These proteins are known to modulate the production of TNF-α, a major proinflammatory cytokine, by alveolar macrophages (1–3). Therefore, alteration of these proteins in alveolar space may lead to exacerbation of inflammation.

The mean concentration of SP-A and SP-D is 3.5 ± 1.1 and $0.88 \pm 0.13\,\mu g/mL$, respectively. The content of SP-A to SP-D is approximately four times in bronchoalveolar lavage (BAL) fluids from the human lung. Interestingly, there is no correlation between the concentrations of SP-A and SP-D in BAL fluids. Although the reason for discrepancies between the concentrations of SP-A and SP-D in each subject remains unclear, this may be related to the independent regulation of synthesis and expression of these proteins.

Although it is difficult to determine the actual concentration in the epithelial lining fluid (ELF) of the alveoli, the calculated values of SP-A and SP-D range from 180 to 1.8 mg/mL and from 45 to 450 μg/mL, respectively. These values tend to be lower in smokers (38). The ratios of SP-A to phospholipid and SP-D to phospholipid are also decreased in smokers. The ratio of SP-A to SP-D in smokers (5.4 ± 2.3) is higher than that in nonsmokers (3.8 ± 0.8). The significant reductions of BAL fluid SP-A and SP-D in smokers might result in the modulation of host defense system in the lung, since these proteins belong to the collectin subgroup of the C-type lectin superfamily (13,14) and act in concert with phagocytes to enhance clearance of invading microbes and other substances (39,40).

There is an increase in surfactant proteins in BAL fluids from patients with alveolar proteinosis (6,8) and a decrease in BAL in acute respiratory distress syndrome (ARDS) and interstitial lung disorders.

The concentrations of SP-A and SP-D are significantly increased in patients with alveolar proteinosis (6,8), whereas they are decreased in patients with ARDS (41–44), idiopathic pulmonary fibrosis (IPF), and collagen vascular diseases with interstitial lung diseases (CVD-ILD) (8,45),

who showed decreased concentrations by 30–50% compared to healthy subjects. McCormack and colleagues (45) described that the mean SP-A/phospholipid ratio (to correct for total surfactant recovery) is significantly lower in patients with IPF who died than in those who survived (32.4 ± 2.6 vs $63.9 \pm 6.4\,\mu g/\mu moL$) and in patients who died within 2 years than in those who survived ≥ 2 years (23.4 ± 2.6 vs $37.5 \pm 4.2\,\mu g/\mu moL$). The SP-A/phospholipid ratio predicted 5-year survival better than any other lavage constituent measured, which included the cell count and differential (45). This study shows that the assay for SP-A in BAL fluids in patients with IPF has not only a diagnostic significance but also has a value of a biomarker, which predicts their prognosis when SP-A is normalized to phospholipid.

Gregory and colleagues (41) analyzed that the crude surfactant pellets recovered from BAL fluids of patients with ARDS and patients at risk to develop ARDS. SP-A, SP-B, and phospholipids were significantly decreased in patients with ARDS and also in at-risk patients. At-risk and ARDS patients had significantly decreased surfactant biophysical activity, suggesting that surfactant supplementation for at-risk patients might be beneficial in the prevention of ARDS.

A recent study by Greene and colleagues (44) demonstrated that the assay for SP-A, SP-B, and SP-D in BAL fluids from at-risk and ARDS patients may assess the risk for progression to ARDS and/or death after the onset of ARDS. They found that SP-A and SP-B concentrations are reduced in the lungs of patients at risk for ARDS even before the onset of clinically defined lung injury. Once ARDS was clinically apparent, SP-A and SP-B concentrations were low in BAL, and they remained low for as long as 14 days from the onset in patients with sustained ARDS. In contrast, the median concentration of SP-D in BAL was normal in most patients at risk and normal in most patients with established ARDS. On day 1 from the onset of ARDS, however, the concentration of SP-D was very low in the subgroup of patients who later died, and the BAL SP-D was directly related to the P_{IO_2}/F_{IO_2} ratio on days 1 and 3 of ARDS. These findings suggest that SP-D falls when lung injury is severe, which is consistent with destruction of alveolar type II cells in the lungs.

IV. SP-A and SP-D in Serum and Their Concentrations

Although surfactant components had been believed to exist solely in the lungs, Chida and colleagues (46) suggested that surfactant proteins could be found in the sera of newborn infants with respiratory distress syndrome (RDS) using a competitive ELISA with polyclonal antibody against SP-A or SP-B. However, the specificities of antibodies and the presence of the surfactant proteins in serum were not determined in this study. We developed monoclonal antibodies which exhibit high specificities against human SP-A and SP-D

(4,5,7,9). Western blot analyses using anti–SP-A antibody PE10 or anti–SP-D antibody 6B2 showed that these proteins exist in the bloodstream of healthy subjects (47).

The ELISA with two monoclonal antibodies (PC6 and PE10) to human SP-A has been applied to the sera from healthy volunteers and patients with diffuse lung disorders in our laboratory (6,48). This ELISA can determine the concentration of human SP-A at levels ranging from 2.0 to 250 ng/mL. The ELISA with two monoclonal antibodies (6B2 and 7C6) to human SP-D has also been applied to the sera from similar subjects. The reliable range for the determination of concentration is from 1.56 to 100 ng/mL. The mean level of SP-A in sera from 323 healthy subjects at ages of 30–70 years was 24.6 ± 9.6 ng/mL (48), and there was no difference in the levels based on gender and age (49). However, the SP-A level tends to be slightly higher in cigarette smokers (50). The mean level of SP-D in sera from 129 healthy subjects was 49 ± 24 ng/mL, and was similar between females and male (49 ± 26 and 49 ± 22 ng/mL, respectively); there was no correlation between the serum SP-D concentrations and ages (correlation coefficient; $r = 0.07$) (9).

V. Aberrant Levels of Serum Concentrations

Previous studies have demonstrated that the levels of SP-A and SP-D are elevated in the sera from patients with IPF (6,8,9,48,51,52), CVD-ILD (9,47,53,54), radiation-induced pneumonitis (55,56), pulmonary alveolar proteinosis (PAP) (6,8), and ARDS (37,44,57). The mechanism of this increase in the bloodstream is not known but may reflect a combination of alveolar type II cell hyperplasia and a concomitant increase in the synthesis of both proteins and overleakage into the bloodstream due to the disturbance of the air-blood barrier based on the damage of epithelium, endothelium, and/or basement membrane. A preliminary study (58) showed irradiated rats developed subacute lung injury with the overproduction of both proteins, patchy loss of basement membrane in the alveoli, and elevation of serum levels of SP-A and SP-D.

The serum SP-A concentrations were increased in patients with IPF, PAP, and ARDS: mean values of 77.6 ± 47.6 ng/mL (mean SD, $n = 57$) for IPF, 55.3 ± 37.9 ng/mL for 36 patients of CVD-ILD; 74 ± 45.7 ng/mL for 8 patients with PAP, and 86–634 ng/mL for ARDS. The mean SP-D concentrations were also high in these patients: 303 ± 220 ng/mL for IPF, 208.7 ng/mL for CVD-ILD, 461 ng/mL for PAP, and 406–995 ng/mL for ARDS. When the cut-off values (mean $\pm$ 2 SD of healthy control subjects) were set at 43.8 ng/mL for serum SP-A and 109.8 ng/mL for SP-D, IPF patients showed high sensitivities for SP-A (78%) and SP-D (87%). These values were extremely high in comparison with (17%) lactate dehydrogenase (LDH) activity, which is a

simple serum marker that appears to reflect changes of disease activity in patients with IPF. LDH is not specific to the lung and released from many organs. The high sensitivity and specificity, which SP-A and SP-D provide, demonstrate that these markers are outstanding tools for detecting IPF.

The levels of SP-A in sera from patients with pneumoconiosis and pulmonary tuberculosis also showed statistically significant increase, but sensitivities were low (less than 20%). The levels of SP-D in pulmonary tuberculosis and pulmonary sarcoidosis were significantly increased with very low sensitivity (12.5% each). There was no significant increase of SP-A and SP-D in bacterial pneumonia, bronchiectasis, chronic pulmonary emphysema, or bronchial asthma.

In addition, patients with hypersensitivity pneumonitis showed high concentrations of serum SP-A and SP-D, which fell in response to successful therapy (unpublished data).

The most important prognostic factor for IPF is acute exacerbation, which shortens survival periods of patients with IPF. High-resolution computed tomographic (HRCT) scan is the most important examination for detecting acute exacerbations, but it is not always possible frequently to repeat examinations by HRCT. Our studies showed that the serum levels of SP-A and SP-D increase without exception when patients with IPF developed respiratory failure due to acute exacerbation. In contrast, IPF patients developing respiratory failure due to bacterial infection seldom show high levels of SP-A and SP-D. Therefore, the measurements of these markers may support the differential diagnosis for the cause of acute respiratory failure. Moreover, in many patients, the levels decline promptly concomitant with clinical improvement (e.g., oxygenation), suggesting that these proteins may be a reliable monitoring marker.

VI. Relation Between HRCT Findings and SP-A and SP-D

HRCT scanning is widely recognized to be a gold standard to determine disease activity and disease extent of pulmonary fibrosis. Although a differential diagnosis of IPF should be confirmed by a histopathological findings of usual interstitial pneumonia (UIP) (59), recent retrospective and prospective studies have suggested that radiological findings, using HRCT scanning, are highly specific for IPF and can be used to make a diagnosis without a lung biopsy in some patients (60–62).

The HRCT pattern of IPF commonly shows patchy, predominantly peripheral, subpleural, bibasal reticular abnormalities. There may be a variable amount of ground-glass opacity (GGO) and alveolar opacity (AO). In areas of more severe involvement, there is often reticular opacity, traction bronchiectasis (TBE), and subpleural honeycombing (HCMB). The GGO

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seen on HRCT in patients with IPF can be associated with alveolar inflammation (63), patchy and mild fibrotic thickening of alveolar septa, and intra-alveolar young granulation tissue called intraluminal fibroblastic foci (64). Areas of GGO often progress to reticular opacity or HCMB on follow-up evaluation (65).

Radiological findings of IPF are complicated, since it reflects the pathological abnormality characterized by a heterogeneous appearance with alternating areas of normal lung, interstitial inflammation, fibrosis, and honeycomb change. Most patients with IPF reveal elevated levels of SP-A and SP-D in the sera, and the elevation may mirror certain pathophysiological abnormalities, which closely relate to the process of inflammation and fibrosis. The relation between elevated serum levels of SP-A and SP-D and HRCT features may provide insight into pathophysiological events in IPF.

We evaluated HRCT findings from 49 IPF patients (51) to analyze the correlation between scoring of HRCT findings and serum levels of SP-A and SP-D. In that study, the extent of GGO correlated significantly with serum levels of SP-A and SP-D (SP-A: $\rho = 0.791$, $P < .0001$; SP-D: $\rho = 0.446$, $P = .0034$, when analyzed with Spearman's rank correlation test), whereas the extent of HMCB did not correlate with levels of either surfactant protein (Fig. 2). GGO is related in part to alveolitis, which is a potentially reversible and treatable abnormality (66–68). In contrast, HCMB reflects end-stage of fibrotic change, which seldom regresses even with therapy. Our study shows that increased SP-A and SP-D levels may reflect the extent of potentially reversible moieties in IPF. Since the possibility of most favorable therapeutic management requires a precise tool for discriminating between the reversible and irreversible forms of IPF, assays for SP-A and SP-D could help in selecting therapies. Although the efficacy of corticosteroid therapy for IPF is controversial, corticosteroids may reduce GGO in some patients with idiopathic interstitial pneumonias, and this reduction parallels improvement in pulmonary function (69). Moreover, novel agents including pirfenidone, relaxin, suramin, INF-γ, and angiotensin II receptor antagonists are tested for treatment of IPF and interstitial lung disorders associated with collagen vascular diseases (70). Therefore, it is expected that assays for SP-A and SP-D will be used to estimate the efficacy of these agents.

As stated above, SP-A was similar to SP-D in the relationship with GGO, but the correlation coefficient between SP-A and SP-D was not large ($r = 0.491$, $P = .0003$). This suggests that the mechanisms of the increases in the two proteins may differ, and that some factors other than the extent of GGO are independently involved in the increase in SP-A or SP-D in IPF. We divided the IPF subjects into two subgroups: GGO-dominant type and parenchymal collapse-opacity (PCO), which was defined as air bronchiolograms with intense lung attenuation with parenchymal collapse, often accompanied by thickened vessels and traction bronchiectasis, on the basis

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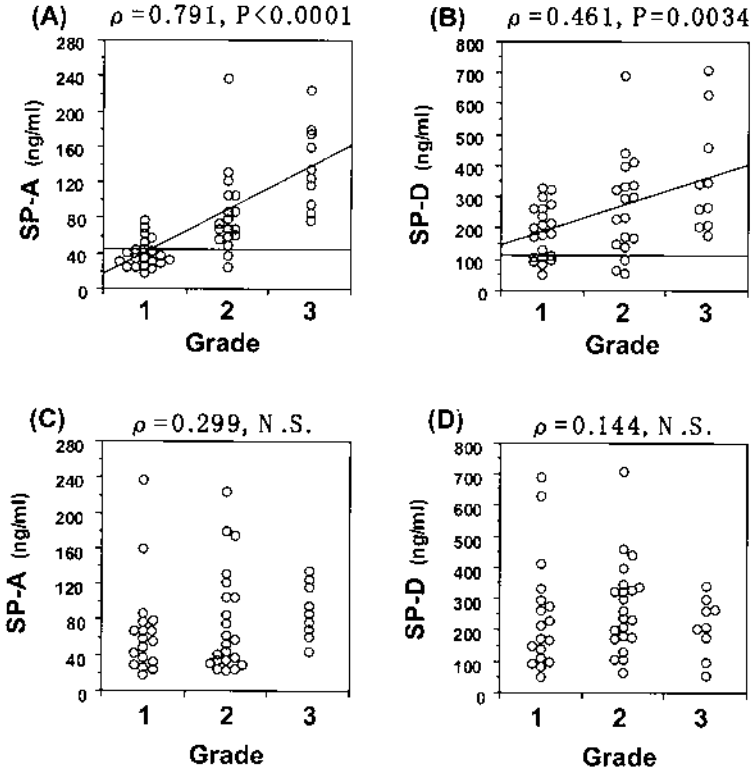

Figure 2 Relationship between concentrations of SP-A and SP-D and GGO (A and B) or HCMB (C and D) on HRCT from patients with IPF. Horizontal lines are cutoff levels of SP-A and SP-D. Grades 1, 2, and 3 correspond to 0 to <5%, 5 to 25%, and >25% extent of involvement, respectively. The correlation was analyzed with Spearman's rank correlation test. The statistical comparison was done with a post hoc test using Scheffe's F. $*P < .01$. (From Ref. 51.)

of the observations of Nishimura and colleagues (64). PCO may reflect collapsed and fibrotic abnormalities in peripheral airspaces of alveoli and bronchioles. SP-A levels (51.3 ± 33.3 ng/mL) in the PCO-dominant type were significantly ($P = .0003$) lower than those (98.3 ± 55.8 ng/mL) in the GGO-dominant type, whereas SP-D levels were not significantly different for two types (GGO-dominant type: 243.1 ± 142.4 ng/mL; PCO-dominant type: 266.6 ± 161.1 ng/mL). The sensitivity of SP-A (52%) was inferior to SP-D (83%) in PCO-dominant group. PCO is observed more often in patients with collagen vascular disease than in patients with IPF. This may explain SP-D is superior to SP-A to detect mild interstitial changes in patients with systemic scleroderma (SSc) (47).

VII. SP-A and SP-D as Determinants of Progression and Survival in IPF

The typical findings of pulmonary function tests (PFTs) in patients with pulmonary fibrosis are consistent with restrictive impairment, of which key variables are vital capacities (VC) and total lung capacity (TLC) (71). We previously attempted to assess whether deterioration in PFT results can be predicted by measuring the concentrations of SP-A and/or SP-D in sera from patients with IPF (51). We found that the levels of SP-D at the initial time of study, unlike those of SP-A, correlated with the velocity of decline in VC and TLC. Most subjects studied, even many who showed high levels of SP-D, did not have dyspnea at the initial time of study. Nevertheless, our results clearly indicate that high levels of SP-D predict subsequent declines in %VC and %TLC. Recent data are is shown in Table 1. Interestingly, high levels of SP-D did not correlate with either VC or TLC at the initial time of study, suggesting

Table 1 Relationship Between Serum Concentrations of SP-A and SP-D and Percentage Change in Parameters of Restrictive Pulmonary Disturbance

	Serum concentrations at the initial time			
	SP-A		SP-D	
	r	*P*	r	*P*
Values at the initial time of the study				
%VC	−0.005	0.0819	−0.108	0.6460
%TLC	−0.036	0.8777	−0.129	0.5026
%D_{LCO}	−0.319	0.1604	−0.074	0.7522
Percentage change in the parameters				
Δ%VC	0.106	0.6513	−0.551	0.0085
Δ%TLC	−0.003	0.9900	−0.460	0.0349
Δ%D_{LCO}	0.193	0.4081	−0.352	0.1188
Percentage change per year in the parameters				
Δ%VC/y	0.106	0.6511	−0.513	0.0163
Δ%TLC/y	−0.045	0.8480	−0.410	0.0649
Δ%T_{LCO}/y	0.215	0.3534	−0.300	0.1891

Twenty-seven patients with IPF subjected had serial PFTs over a period of more than 24 months. The follow-up interval was 35 ± 7 months. The PFTs were performed at least twice: at the initial time and at the end of the observation period. For example, the percentage change in VC was calculated according to the formula: $\Delta\%VC = (\%VC\ final - \%VC\ initial)/\%VC\ final \times 100$. The percentage change per year in each functional parameter was calculated as $\Delta\%VC/yr = \Delta\%VC/$ number of follow-up months $\times 12$. Statistically significant correlation coefficients ($P < 0.05$) were found in the relation with SP-D, but not SP-A.

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that increase of serum SP-D is not caused by the completed fibrosis but by the ongoing epithelial damage and the successive process of fibrosis.

IPF is characterized by a median survival of 3–5 years after diagnosis (72–74). However, there is no absolute determinant predicting survival in patients with IPF. However, there is no absolute determinant predicting survival in patients with IPF. Reported prognostic indicators of survival in patients with IPF have been inconsistent in various studies. Schwartz and colleagues (74) found that dyspnea, sex, and diffusing capacity were independent predictors of survival. In contradistinction to this report, a recent study (75) denied reliability of these variables and suggested that survival was significantly related to age at presentation, the presence or absence of finger clubbing, cigarette smoking history, profusion of interstitial opacities, and evidence of pulmonary hypertension on the chest radiograph, reduced lung volume, and gas exchange abnormalities with exercise. One histopathological study (76) demonstrated that the most valuable determinant of poor prognosis is the extent of small aggregates of proliferating myofibroblasts and fibroblasts (fibroblastic foci). Although this insight seems to be very important in pathogenesis of IPF, surgical lung biopsy is not always performed. Serological determinants of survival have infrequently been studied.

The previous (51) and our recent data (Fig. 3) demonstrated that measurements of SP-A and SP-D in sera from patients with IPF at the initial time of diagnosis are invaluable to assess prognosis. One of the major causes of death in IPF is severe respiratory failure caused by an acute exacerbation. Eleven patients who died of acute exacerbation of IPF before the end of the 3-year follow-up period (nonsurvivors) were compared with 47 patients who were still alive (survivors) for study of the relationship between survival and SP-A or SP-D (51). There was no significant differences in sex, age, cigarette smoking, or Pao2 between survivors and nonsurvivors. All of the 11 nonsurvivors exhibited levels of both SP-A and SP-D that were higher than the cutoff level. In contrast, all of the 16 patients exhibited lower SP-A and/or lower SP-D than each cutoff level were survivors. When all patients were divided into two groups at twofold cutoff level (mean + 4SD), 7 of 23 patients, who showed SP-A above the level, died within 3 years after the initial time of diagnosis. In addition, 10 of 34 patients, who showed SP-D above the level, died within 3 years. Moreover, 7 (39%) of 18 patients, who showed both values of SP-A and SP-D above the twofold cutoff, died within 3 years. In contrast, only 4 (9%) of 40 patients, who showed SP-A and/or SP-D lower than the twofold cutoff, died. Therefore, a combination of SP-A and SP-D assays may predict prognosis better than only one parameter. At the initial time, most of the 11 nonsurvivors did not show abnormal pulmonary function on gas exchange. Nevertheless, they died within 3 years, suggesting that some high-risk subjects are mixed in with patients with IPF who are judged to be mild on standard examinations. These data suggest that clinicians can detect subclinical

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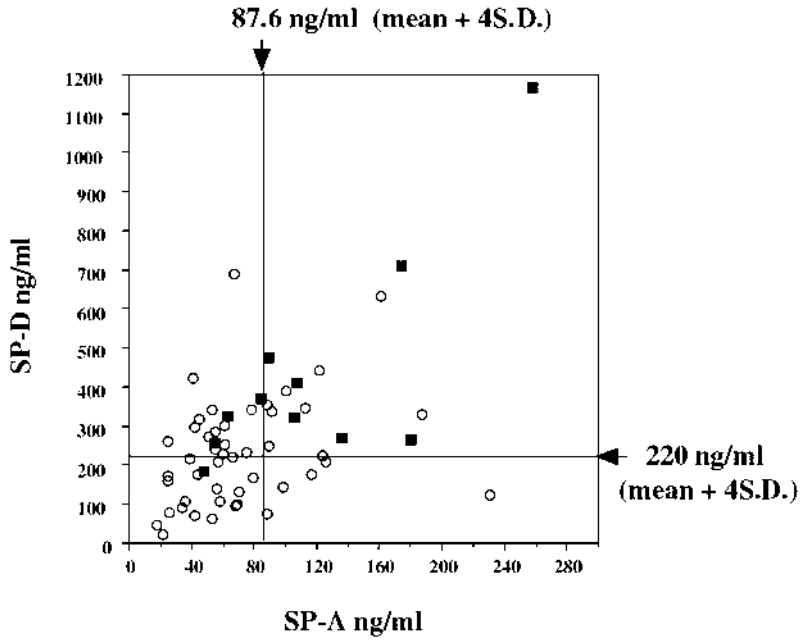

Figure 3 Relationship between serum concentrations of SP-A and SP-D at the initial visit of 58 patents with IPF and survival during 3-yr follow-up. Each value from 47 survivors (open circle) and 11 nonsurvivors (closed square) were plotted. Vertical and horizontal lines are mean + 4SD (two times cutoff level) of SP-A and SP-D, respectively. The mortality (39%) of high-risk subjects, who showed both values of SP-A and SP-D above the lines, was significantly higher than that (9%) of the other remaining subjects, who showed SP-A and/or SP-D lower than the lines.

high-risk subjects by using SP-A and SP-D assays. Several reports (77,78) suggest that it may be more effective to start treatment for IPF before the manifestations of severe pulmonary fibrosis occur. Moreover, recent studies have proposed novel therapeutic strategies using agents that inhibit the evolution of fibrosis (79), correct Th1-Th2 lymphocyte imbalance (80), or enhance apoptosis of lung fibroblasts (81) rather than agents suppressing inflammatory processes such as corticosteroids. Our results raise the possibility that assays of SP-A and SP-D may guide novel therapies.

VIII. SP-A and SP-D in Other Diseases

ARDS may complicate a wide range of serious medical and surgical conditions, only some of which involve direct pulmonary injury. The characteristic histological feature of ARDS is an intense inflammatory process

in the lungs, which may progress to fibrosis. This morphological variant, termed diffuse alveolar damage (DAD), has been subdivided into sequentially occurring exudative, proliferative, and fibrotic phases. The earliest physiological characteristic is an increase in the protein permeability across the endothelial and epithelial barriers of the lungs. The increased permeability allows protein-rich fluid to move into the alveoli and leads to the organization of intraluminal exudates, which dominates the histological feature in the proliferative phase and establishes the framework for subsequent fibrous remodeling of the lungs.

As stated in Section III, patients with ARDS exhibit significantly lower SP-A, SP-B, and SP-D concentrations in BAL fluids than those from healthy subjects (41,44). The BAL SP-A was significantly lower even in patients at risk who later developed ARDS, suggesting that the assays for SP-A may identify patients who are at low risk for developing ARDS. The BAL SP-D was significantly lower in patients who died than in patients who lived when the concentrations were measured at an onset of ARDS (44). These data suggest that SP-D, unlike SP-A, may identify patients with the most severe epithelial injury in the lungs and those with the worst overall lung injury. Taken together, data suggest that low concentrations of surfactant proteins promote a process of proliferative and fibrotic phases in the lungs of ARDS patients and result in poor prognosis.

The levels of *serum* SP-A, SP-B, and SP-D are elevated in patients with ARDS (37,44,57,82). In the analysis of outcome, the serum concentrations tends to be higher at the onset of ARDS in patients who died, but the rage was broad and the differences did not reach statistical significance (44). Bersten and colleagues (82) proposed the clinical application of the assay for plasma SP-B, which is a hydrophilic protein and is closely associate with a phospholipid function lowering surface tension. They found the initial plasma SP-B, but not SP-A, was a useful indicator for discriminating at-risk patients who later developed ARDS, particularly when it was applied to patients suffering a direct lung injury with a sensitivity of 85% (95% CI: 55–98%) and specificity of 78% (40–97%) at a cutoff of 4994 ng/mL, whereas the initial lung injury score was not predictive of ARDS.

Collagen vascular diseases (CVDs) are heterogeneous immunologically mediated systemic disorders. Although CVDs are frequently associated with pulmonary involvements including pulmonary fibrosis, alteration of the function of surfactant proteins and their association with pulmonary fibrosis is poorly understood. Early studies (8,53) demonstrated the clinical utility of serum SP-A and SP-D in CVDs. Recent studies (47,54,83) focused on systemic sclerosis (SSc), since it shows the highest frequency of pulmonary involvements in CVDs. Takahashi and colleagues showed serum SP-A and SP-D levels in SSc patients with pulmonary fibrosis ($n = 30$) detected by chest HRCT (including six patients with normal chest radiographs) were significantly higher

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than those in SSc patients without pulmonary fibrosis (n = 12) and healthy subjects (n = 108). In contrast, LDH activity, a classic serum marker for fibrosis did not differ between the patient groups. SP-D levels had a high sensitivity (77%) for detecting pulmonary fibrosis, whereas the sensitivities of SP-A and LDH activity were low (33% and 17%, respectively). Moreover, five of six SSc patients with positive CT scan but negative chest radiographs showed elevated concentrations of SP-D. Asano and colleagues (54) suggested serum SP-D levels are also useful for evaluating early pulmonary fibrosis associated with normal chest radiographs in patients with SSc. Even in all four patients with early pulmonary fibrosis (defined as restrictive abnormalities on pulmonary function test or alveolitis on HRCT), serum SP-D levels were significantly higher than patients without pulmonary fibrosis. When serum SP-D levels were examined longitudinally over a period of 1–10 years, 9 of 10 patients, in whom the pulmonary fibrosis was exacerbated, showed significant increases of SP-D, whereas 18 of 25 patients, in whom the pulmonary fibrosis was unchanged or improved, showed stable SP-D levels. The incidences of decreased %DLCO and of decreased %VC were greater in patients with elevated levels of SP-D.

Radiation pneumonitis (RP) is the most common complication of radiotherapy for thoracic tumors. RP sometimes leads to pulmonary fibrosis. In some cases, radiation pneumonitis develops into diffuse widespread pneumonitis affecting the contralateral lung and leads to progressive respiratory insufficiency, sometimes resulting in death. Alteration of the surfactant system is one of the earliest detectable changes following lung irradiation (84). In patients with RP, saturated phosphatidylcholine, an essential lipid component for surfactant function, decreases according to the progression as shown by radiography. This alteration in the surfactant system may cause a collapse of alveoli and result in fibrosis. SP-A levels in BAL fluids were also decreased after irradiation, and the concentrations showed a negative correlation with the severity of radiographic changes. On the other hand, SP-A levels in sera are increased significantly (55,56). The difference between BAL fluids and sera from patients with RP is similar to patients with IPF (45,51) or ARDS (44). Takahashi and colleagues analyzed 12 patients with RP detected by chest HRCT and showed that both SP-A and SP-D concentrations in sera from patients with RP were significantly higher than in patients without RP eventhough only 3 of the 12 patients with RP had abnormalities on chest radiographs. When the concentrations (post) at the occurrence of RP were compared to those (pre) at the initiation of radiotherapy and the post/pre ratios of more than 1.6 considered to be positive, both the SP-D and SP-A assays showed an 83% sensitivity and an 85% specificity, with positive and negative predictive values of 83 and 85%, respectively. The relative risk for the complication in the presence of both assays post/pre ratios of greater than 1.6 was 5.4. When SP-A and SP-D assays were combined, a high sensitivity (92%)

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was obtained for detection of RP by HRCT. Another report (56) demonstrated that serum SP-A and SP-D monitoring is a practical and useful method for the early detection of RP. Early and accurate diagnosis of RP is important, since intensive strategies and dose-escalation trials are under investigation for the treatment of lung cancer. Serial assays of these novel markers can prevent clinicians from overlooking the early stage of lung damage, at which time corticosteroid therapy can be still effective.

IX. Note Added in Proof

A hydrophobic surfactant protein, SP-C, is expressed solely in alveolar type II cells. Recent reports (85,86) indicated that mutations in the gene encoding SP-C were identified in association with familial interstitial pneumonias including usual interstitial pneumonia, nonspecific interstitial pneumonia, and desquamative interstitial pneumonia. The gene mutations lead to the production of aberrant SP-C proprotein, probably resulting in the dysfunction and damage of alveolar type II cells. Thus, the cell damage based on the mutation may underlie the pathogenesis of pulmonary fibrosis.

Acknowledgments

The writer wishes to express his appreciation to Yoshio Kuroki, M.D., Toyoaki Akino, M.D., Yasuhito Honda, M.D., and Shosaku Abe, M.D., for their valuable advice concerning the basic and clinical aspects.

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Bronchiolar Epithelium in Idiopathic Pulmonary Fibrosis/Usual Interstitial Fibrosis

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I. Introduction

Although the pathogenesis of idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP) is mainly centered on the progressive loss of alveolar parenchyma with eventual interstitial remodeling and with pulmonary function tests which mainly reveal restrictive impairment, some pathological features suggest that more proximal airway structures, and in particular the respiratory bronchioles, can also be involved in this disease. In this chapter, we will review the morphological and molecular features that characterize conductive airway abnormalities in IPF/UIP, and will describe recent data obtained by our group also showing how bronchiolar epithelium may represent a crucial target of injury and abnormal regeneration, thus contributing significantly to the pathogenesis of this disease. Our recent data are mainly based on the demonstration of the peculiar molecular features of UIP samples analyzed in situ by immunohistochemistry, using an extended panel of antibodies. This approach can help in deciphering the

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molecular complexity of the pathogenesis of IPF/UIP, by reconciling the data provided by molecular and cellular biology with those obtained by traditional histology.

The pathogenesis of IPF/UIP has been the focus of many studies, and several different, not mutually exclusive, schemes have been proposed (1). Recently, the widely accepted ''inflammatory theory'' has been challenged (2), and new pathogenetic hypotheses have been suggested that focus on the abnormal proliferation of fibroblasts and myofibroblasts (1,2). Moreover, new evidence has been provided pointing to a major role of dysregulated regeneration of epithelial components in pulmonary fibrosis, influencing stromal and vascular remodeling in affected areas (3–5). Nevertheless, the centrality of fibroblasts/myofibroblasts in IPF/UIP still remains controversial and unproven. In addition, little is known about the precise nature, timing, and primary target of tissue injury, as well as the role of proliferative events of both the epithelial and stromal components following tissue damage. Recent attention has been focused on models describing the pathogenesis of respiratory disorders in ways closely related to basic molecular pathways involved in lung ontogeny (6). In order to address these crucial points, we are currently investigating both the expression of some molecular mechanisms in IPF/UIP that are responsible for the regulation of morphogenesis and for the renewal of pulmonary tissue and the interdependence of mesenchymal and epithelial components. Our efforts have been directed at characterizing in situ patterns of expression of the p63 gene, and the beta-catenin signaling involved in the *wnt* pathway, as well as the expression of products of the p53-p21^{waf1} pathway. These molecules are known to exert important roles in the regulation of cell cycle progression and apoptosis after cell injury, and they also have regulatory functions in lung development and morphogenesis.

The possible involvement of airways in IPF/UIP has attracted little attention in the past. In fact, this disease has an insidious onset centered in the early phases at the pulmonary bases, and mainly involves alveolar interstitium, pursuing a chronic course that eventually culminates in honeycomb lung. It is widely accepted that honeycombing represents a poorly specific end-stage phenomenon following extensive pulmonary damage, but in our opinion, this notion is not entirely correct, since honeycomb lesions can be observed very early during the natural history of the disease, and are part of the spectrum of diagnostic parameters of UIP. In our experience, most early as well as late honeycomb lesions in IPF/UIP can be recognized as bronchiolar in nature and can thus represent direct proof of bronchiolar involvement in this disease. In addition, a number of relevant molecular abnormalities have been demonstrated in bronchioles in IPF/UIP, as described in detail in sections II and V.

Finally, at variance with less harmful interstitial pneumonias such as diffuse interstitial pneumonia (DIP), in which arterial hypoxemia is mainly

due to restriction of gas exchange because of limited diffusion as a result of alveolitis and interstitial thickening, the presence of honeycomb cysts and airway distortion in IPF/UIP can cause ventilation-perfusion mismatch, which significantly contributes to hypoxemia and functional impairment recalling that observed in chronic obstructive pulmonary disease (COPD) (7).

II. Morphological Evidence of Bronchiolar Involvement in IPF/UIP

The morphology of UIP has been known ever since the work of Liebow (8), and it has been clearly separated from other interstitial pneumonias, including desquamative interstitial pneumonia/bronchiolitis-associated insterstitial lung disease (DIP/RB-ILD), nonspecific interstitial pneumonia (NSIP), bronchiolitis obituars–organizing pneumonia (BOOP), and acute interstitial pneumonia (AIP) (9,10). The main features characterizing UIP are a variegated appearance due to the coexistence of deeply affected areas, alveolitis, and intervening residual islands of normal lung reflecting the temporal variability that characterizes the evolution of this disease from early lesions to eventual fibrosis and remodeling (11–13). The interstitial modifications are mainly due to varying degrees of fibrosis and to a variable, often inconsistent, inflammatory infiltrate. Fibrosis characteristically affects different zones, and it can be either diffuse or patchy (fibroblast foci, FF). FF indicate that injury and repair are actively ongoing and represent one of the hallmarks of UIP. They are usually intramural and arranged with their long axis parallel to the long axis of the epithelial septa (11). FF are often covered by epithelial cells that line the luminal surface and have various morphological features. The characterization of the epithelial cells associated with FF is crucial for understanding the pathogenesis of UIP, since it can define the target of early and/or ongoing injury. ''Bronchiolization'' is another morphological pattern frequently described in IPF/UIP. This pattern, although not fully clear in its definition and pathogenic significance, clearly highlights the role of bronchiolar epithelium in IPF/UIP pathology.

III. Bronchiolar Cells at Bronchioloalveolar Junctions Are Among the Targets of Injury in UIP—Nature, Location, and Significance of Fibroblast Foci

FF are discrete collections of myofibroblasts, and accordingly express contractile molecules such as α-smooth muscle actin (α-SMA) and desmin, but completely lack h-caldesmon (14,15). They also contain elevated levels of tenascins, a family of extracellular matrix glycoproteins involved in development and morphogenesis, as well as wound healing, cell adhesion,

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cytokine entrapment, and immunomodulation (16–19). Tenascin-immunostaining is considered to be a good marker for FF, and can help reveal them on tissue sections (16,20). During development, tenascin is expressed by epithelial cells at sites of active growth of bronchial tubes (21), but in normal lung, its expression is restricted to basal membranes. Interestingly, increased tenascin expression, especially its accumulation under metaplastic bronchiolar-type epithelium, has been associated with a shortened survival time in UIP (22).

FF are considered to represent repair processes following alveolar damage and collapse, and it is currently accepted that they primarily form within injured alveoli, and are eventually incorporated in the interstitial spaces, thus significantly contributing to lung remodeling (23,24). Nevertheless, some evidence suggests that alternative mechanisms of mural formation of FF can also occur in IPF/UIP. In BOOP, intraluminal FF (also known as inflammatory polyps or fibromyxoid lesions) can be mainly demonstrated within alveoli and bronchiolar lumens (23). In UIP, demonstration of intraluminal FF is less evident, and most FF are found intramurally in association with abnormal epithelial structures. Interestingly, FF occurring in UIP are not extensively capillarized, which is at variance with those characterizing BOOP (25). In acute interstitial pneumonia fibrosis (AIP), another fibrosing pneumonitis sharing features with UIP, alveolar damage is diffuse, but myofibroblast proliferation is mainly contained within interstitial spaces. It is then possible to argue that FF formation in IPF/UIP may occur within the pulmonary interstitium following peculiar tissue injury (e.g., affecting the capillary walls as observed in diffused alveolar damage (DAD) and AIP), and as a consequence of endogenous noxious agents entering through the capillary bed. In this regard, UIP shares a variety of features with autoimmune diseases (26,27), and abnormal interstitial neovascularization has been described in human and experimental pulmonary fibrosis (5,28,29).

In many UIP samples, FF appear as leaning against enlarged structures lined by cuboidal epithelial cells. The nature of the epithelial cells lining the luminal surface of FF has not been fully clarified so far, probably because previous studies utilized only morphological criteria. The FF-associated epithelial structures are generally described as being either formed by hyperplastic type II pneumocytes or enlarged bronchioli, sometimes defined as "metaplastic alveoli." In our experience, when investigated using a panel of specific markers for alveolar pneumocytes (surfactant protein SP-A), Clara cells (CC10), or bronchiolar basal cells (p63), many of these structures can be clearly defined as being bronchiolar. This is not a trivial point, since, as stated above, the site and cellular target of injury can be relevant for understanding the pathogenic scenario and timing leading to lung tissue effacement. In fact, it has been hypothesized that myofibroblasts in lung fibrosis are able to produce factors inducing apoptosis of epithelial cells, so that their accumulation at

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particular sites cannot be merely considered as an abnormal repair process following epithelial injury but rather as exerting an active role in the pathogenesis of IPF/UIP (30,31). This is particularly true if we consider the expected heterogeneity of renewal mechanisms utilized by alveolar and bronchiolar compartments, as discussed in this chapter.

IV. Bronchiolar and Alveolar Compartments Utilize Different Molecular and Cellular Strategies for Development and Regeneration

Many data suggest that during development and organogenesis, and also after birth, the proximal (bronchi and bronchioles) and distal (alveoli) compartments of the lung use different cellular and molecular strategies for tissue maintenance and renewal. In fact, transgenic models have demonstrated that progenitor cells of the distal lung parenchyma differentiate early in lung development from embryonal rudiments of endoderm, and depend on specific reciprocal interactions exchanged with corresponding mesenchyme (32–34). The dynamics of cell turnover of proximal and distal lung epithelia are significantly different, as are their physiology, biochemistry, and functions. Conducting airways are slowly renewed, whereas alveoli have a higher turnover. The stem cells in the trachea, bronchi, and bronchioles are strictly compartmentalized in the basal zone of the mucosa (35–38), whereas the stem cell pool of alveoli is dispersed over a large surface, since it corresponds to type II pneumocytes.

A. Conductive Airway Stem Cells

Basal cells in conductive airways are specialized cells that remain in close contact with basal lamina through hemidesmosomes and are characterized by a peculiar morphology and phenotype, including the expression of high molecular weight cytokeratins 5 and 14, and a specific repertoire of adhesion molecules (37,39–41). The contact to basal membrane and mesenchymal cells is of paramount importance not only in maintaining the integrity of the mucosa by anchoring columnar epithelial cells and promptly forming an epithelial barrier after epithelial shedding (42,43) but also by providing the necessary signals for maintaining the stem cell phenotype and functions related to that particular microenvironmental niche (44). Consensus on the stem cell nature and function of airway basal cells is not evident in the literature, probably because of ambiguity in the commonly accepted definition of stem cells (cells with extensive self-replicating potential and the ability to produce differentiated progeny) and a lack of reliable markers for their precise recognition. In fact, over the years, not only basal cells but also other cell types, including secretory cells, Clara cells, neuroendocrine cells, and ciliated cells, have been

thought to partcipate equally in regenerating the airway mucosa (37,42,45–48). All these apparently discordant data can be reconciled in the light of a strict definition of stem cell subtypes in accordance with recent criteria (49,50). Accordingly, all epithelia contain cells that are capable of maintaining tissue integrity by repopulating lost cells after injury, but among them it is possible to distinguish between slowly dividing stem cells and "transit amplifying cells" committed to restricted differentiation after a finite number of divisions (49,50). Multipotent, dormant stem cells need to be activated only when extensive damage occurs, whereas unipotent stem cells are mainly involved in steady-state tissue renewal (50). Stem cells need to exert their functions within specialized microenvironments (stem cell niches), and involving a complex interplay of the stem cells, their daughters, and neighboring cells and extracellular matrix (ECM) (44). In conductive airways, the basal cells have all the features needed for being recognized as bona fide multipotent stem cells. In addition, their molecular profile is identical to that characterizing the stem cells in other stratified epithelia, including the constitutive nuclear expression of p63 (51). The p63 protein is the product of a gene which is essential for regenerative proliferation in limb, craniofacial, and epithelial development, and is considered to be a reliable specific marker of stem cells in a variety of epithelial tissues, including skin, cornea, esophagus, prostate, lung, and others (51–57).

B. p63 Gene, a Marker of Bronchial and Bronchiolar Stem Cells, Is Involved in the p53 Pathway

Activation of the p53 pathway induces growth arrest and apoptosis following DNA damage and various stress stimuli (e.g., cytotoxic drugs, gamma irradiation, heat shock, hypoxia, osmotic shock, and nucleotide depletion), promoting cell cycle arrest to enable DNA repair or apoptosis to eliminate defective cells (58–60). In normal cells, nuclear concentration of p53 is low or undetectable, since it is strictly regulated by proteolytic removal after ubiquitination under the control of a complex molecular network including, for example, mdm2, p14ARF, JNK, and pRB, (60,61). The transcriptional activating functions of p53 have been revealed and its principal molecular targets characterized. Cell cycle arrest is mainly mediated by transactivation of $p21^{waf1}$, a potent inhibitor of cyclin-dependent kinases, and is also dependent on expression of GADD45 and cyclin G. Proapoptotic target genes of p53 include *Bax*, Igf-Bp3, PIG, CD95, *Noxa*, and others (60,61). Further complexity in the fine tuning of these processes is provided by regulatory molecules which can positively or negatively modulate the action of p53, such as the recently described molecules belonging to the p53 family: p73 and p63 (57,62,63).

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C. p63 Is a Key Regulator of the Physiological Maintenance and Function of Epithelial Basal Cells

The human p63 gene is a member of the p53 tumor suppressor gene family identified in human and experimental animals (52,62–65). It is able to activate p53-responsive promoters, upregulating relevant target genes involved in the modulation of cell proliferation and apoptosis, including, for example, the CDK inhibitor $p21^{waf1}$, the transcription factor NF-Y, and the receptor tyrosine kinase EphA2, (62,63,66–69). In addition, p63 can have unique functions such as repression of epidermal growth factor receptor (70). Despite its similarity to p53, the p63 gene is not a tumor suppressor gene, since p63 mutations are extremely rare in human malignancies, and loss of heterozygosity is not frequent in the chromosome 3q27-29 region where the p63 gene is located (71,72). Recent studies show that p63 is far more complicated than p53 at the molecular level (52,73). In fact, the p63 gene can be transcribed from two different promoters generating a complete p63 molecule (TA-p63) with a transactivating domain or a N-terminal truncated protein (ΔN-p63) produced when the p63 gene is transcribed from the cryptic promoter in intron 3; both isoforms undergo further complex alternative splicing, generating at least six p63 isoforms (52,57,64). Interestingly, these different molecular species are able to exert contrasting effects on the same molecular and cellular targets. TA-p63 molecules have functions similar to p53 in inducing cell cycle arrest and apoptosis, whereas the N-terminal truncated proteins can act as dominant negative agents toward transactivation by p53 and p63 itself, inhibiting their activity (52,57,64). Curiously, a mutual influence between p53 and p63 has been recently demonstrated, generated by the suppressing activity of p53 on dominant negative functions of ΔN-p63 (74).

The main physiological role of p63 in normal stratified epithelia, where it is highly expressed in the nuclei of basal reserve cells, seems to be related to the p53 antagonizing and modulating functions necessary to maintain the regenerative quality of epithelial stem cells (53,56). On the other hand, when abnormally overexpressed owing to gene amplification, truncated p63 variants can behave as oncogenic molecules (75–77). In a recent investigation, we demonstrated a striking heterogenity in the p63 isoform expression in bronchiolar and alveolar compartments (51,78). We demonstrated, in fact, that only bronchial and bronchiolar basal cells constitutively express truncated ΔN-p63 isoforms, whereas all other cells within the airways and alveoli are negative. Interestingly, in lung samples where alveolar regeneration was evident (including UIP, AIP, and BOOP) nuclear expression of the transactivating TA-p63 isoforms could be demonstrated within a proportion of regenerating type II pneumocytes. The possible significance of this expression and its possible role in inducing pneumocyte apoptosis remain to be investigated.

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D. Alveolar Stem Cells

After injury, alveolar cells are reintegrated by proliferating type II pneumocytes that can function as reserve/stem cells and can respond to alveolar loss by proliferating and migrating to repair the damaged epithelium (79,80). Cuboidal epithelial cells, which serve as reserve cells, appear during embryonal lung development, and are accompanied by the activation of genes encoding surfactant proteins. In adult mammalian lung, alveolar basement membranes possess functional and structural domains that determine sites at which type I and type II cells localize, and that can provide suitable microenvironmental niches for stem cells (81). Surprisingly, recent data point to the possible concurrence of bone marrow stem cells in the alveolar renewal (82). In conclusion, since the mechanisms of cell renewal between bronchioles and alveoli are different, it is possible to argue that these compartments should heterogeneously respond to injury with diversified repair mechanisms. An interesting comparison can be made between the bronchioloalveolar junctions and squamocolumnar junctions of the gastroesophageal tract, as well as the cervical transition zone (83,84). In these tissues, in fact, two epithelial components characterized by different renewal strategies (one based on the presence of basal cells expressing ΔN-p63) are joined together in transitional regions characterized by increased metaplastic and neoplastic transformation potential.

V. Bronchiolar Abnormalities in IPF/UIP

A. Morphological Features: Bronchiolectasis, Bronchiolar Hyperplasia, Squamous Metaplasia, Alveolar Bronchiolization, and Honeycombing

Bronchiolar abnormalities have been occasionally described at histological analysis in IPF/UIP, but evidence of these features was not usually provided, since attention was mainly focused on alveolar damage and interstitial fibrosis. The most evident morphological abnormalities include bronchiolectasis, hyperplasia, squamous metaplasia, and honeycombing (Fig. 1).

The number of bronchioles per microscopic field is often increased in UIP when compared with normal lung, and hyperplasia is striking in a number of samples. In addition, bronchioles exhibit significant morphological abnormalities, including enlargement, basal cell hyperplasia, squamous metaplasia, and focal aspects of atypia. Airway enlargement is evident at both radiological and histological analyses, and has been mostly interpreted as "traction bronchiectasis" (85). Many dilated bronchioles in IPF/UIP specimens are distorted and filled with inspissated mucus containing macrophages and neutrophils, and exhibit the morphological features of honeycomb cysts. Accordingly, at spiral volumetric computed tomographic (CT) analysis, the

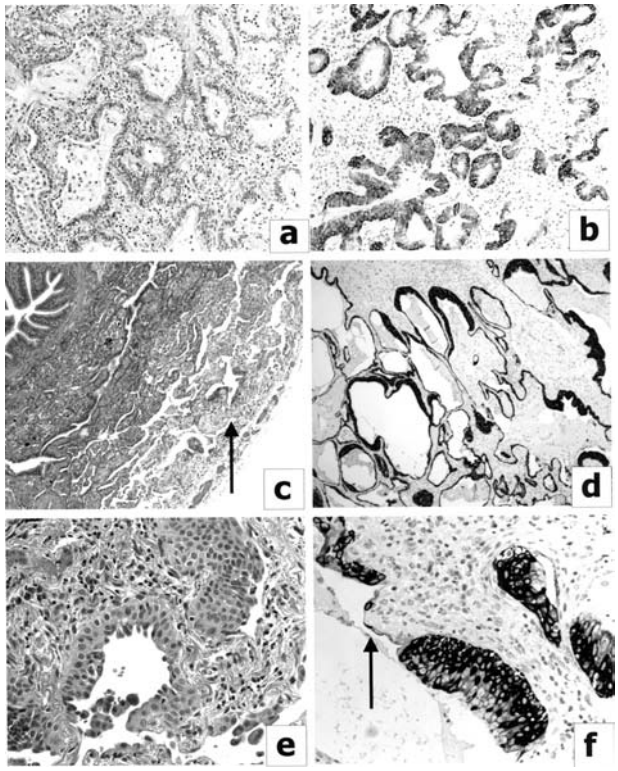

Figure 1 Morphological abnormalities of bronchioles in UIP samples: bronchiolectasis, hyperplasia, squamous metaplasia, and honeycombing are shown in different cases of UIP. (a) UIP case 1, bronchiolar hyperplasia: many enlarged bronchioles occupy a distal lung portion showing abnormal remodeling (H&E staining); (b) UIP case 1, a consecutive section of the same sample showing 34βE12 cytokeratin immunostaining in distorted bronchioles; (c) UIP case 2, abnormal bronchioles extending close to the pleural surface (arrow) (H&E staining); (d) UIP case 3, a large portion of distal lung showing abnormal proliferation of metaplastic bronchioles (cytokeratin 8/18 immunostaining); (e) UIP case 4, a proliferative epithelial lesion in an area of remodeling (H&E staining); (f) case 4, alternating segments of squamous metaplasia and monostratified epithelium (arrow) in a large honeycomb lesion (34βE12 cytokeratin immunostaining).

majority of honeycomb cysts represent dilated bronchioles that communicate with proximal airways (86). Although honeycomb lung is considered the end-stage manifestation of a variety of lung diseases, cystic lesions are an early finding in almost all biopsies of IPF/UIP, and are considered to be a hallmark of this disease with diagnostic and prognostic significance. The precise

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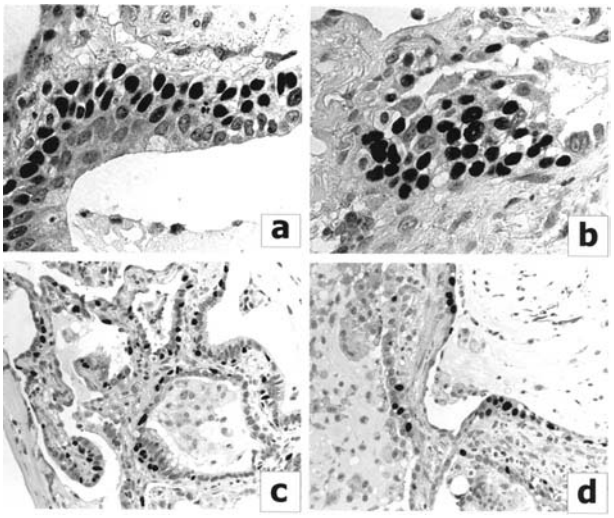

Figure 2 Basal cell abnormalities as evidenced by ΔN-p63 immunostaining in UIP. (a and b) Crowding of ΔN-p63+ basal cell nuclei in basal cell hyperplasia and squamous metaplasia; (c) ΔN-p63+ basal cells in a proliferative bronchiolar lesion with bronchiolization; (d) ΔN-p63+ cells in a honeycomb lesion.

mechanisms leading to the formation of enlarged spaces is not entirely clear. The nature of the epithelial cells forming honeycomb cysts has been generally described as being either bronchiolar or hyperplastic-alveolar on the basis of morphological features. When investigated in situ with antibody markers, the majority of these lesions can be recognized as being abnormal bronchioles on the basis of their cytokeratin profile, the lack of surfactant SP-A expression, and the presence of a significant component of cells expressing nuclear ΔN-p63 (Fig. 2) (78). In our experience, cysts formed by alveolar epithelial cells often appear as dilated spaces lined by normal-appearing pneumocytes, similar to emphysematous bubbles, or smaller cysts lined by hyperplastic cuboidal pneumocytes. The alveolar-type cysts can be easily recognized by the expression of SP-A and lack of ΔN-p63+cells.

Other proliferative features characterizing abnormal bronchioles and honeycomb cysts in UIP include basal cell hyperplasia, squamous metaplasia, and bronchiolization. Metaplasia and basal cell hyperplasia often appear to be segmental in affected bronchioles with zones of metaplastic epithelium alternating with segments of either normal-appearing ciliated or abnormal cuboidal epithelium. Bronchiolar basal cell hyperplasia is also segmental with focal nuclear crowding and atypia. Proliferative bronchiolar lesions in UIP, akin to those occurring in the bronchi of tobacco smokers, have long been

considered to be precancerous, and their occurrence is more frequent in UIP patients complicated by lung carcinoma (87–89). In bronchial metaplastic lesions induced by smoking, squamous stratified epithelium substitutes for the ciliated cylindrical epithelium, recapitulating the potential differentiation of embryonic endoderm rudiments. Metaplasia in epithelial tissue commonly occurs in association with damage and regeneration, and can be interpreted as changes in the states of determination of the stem cells rather than changes between already differentiated cell types (90). In metaplastic lesions, multipotent stem cells do not generate the characteristic cell types of their own tissue, but change their developmental commitment producing differentiated cells of another tissue, which is generally polyclonal (50,91). In metaplastic lesions observed in IPF/UIP, it is possible to hypothesize that basal cells, the multipotent stem cells of conducting airways, can change their physiological commitment, producing squamous epithelium instead of bronchiolar epithelium.

Bronchiolar abnormalities observed in UIP occur mainly distally at the transitional zones, where nonciliated cuboidal cells are progressively substituted for ciliated cells. In these bronchioloalveolar junctions, it is possible to demonstrate features of bronchiolization, the process of migrating bronchiolar cells progressively colonizing alveolar spaces. Alveolar bronchiolization is a histologically distinct change which can be considered to be within the spectrum of epithelial proliferative responses to alveolar injury after exposure to noxious substances such as ozone (92,93). Bronchiolization (also known as bronchiolar metaplasia of proximal alveolar ducts) occurs in vivo in various conditions including IPF/UIP, lung carcinoma, and experimental lung injury (93). The histogenesis of alveolar bronchiolization is still uncertain, since it can either arise from colonization of alveolar walls by bronchiolar precursors or from the metaplastic transformation of alveolar pneumocytes into bronchiolar epithelium (94). In line with the first hypothesis are the recent experimental pulmonary engraftments in nude mice, where human cells exhibiting the basal cell phenotype are able to migrate and reconstitute differentiated respiratory epithelium (95,96). In UIP lesions, the mobilization of bronchiolar basal cells necessary for the progressive colonization of alveolar spaces could be mediated by metalloproteinases, as demonstrated in experimental animals exposed to bleomycin (97). Finally, further support for the migration theory is provided by the demonstration of superficially located ΔN-p63+ cells at the bronchiolar-alveolar junctions (Fig. 2.) (78).

B. Molecular Abnormalities: p53 Pathway

Recently, molecular studies have focused attention on some abnormalities occurring not only in affected alveolar parenchyma but also in bronchiolar structures of IPF/UIP patients. In particular, abnormalities of p53 gene

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expression have been described using different experimental approaches (78,98,99). The occurrence of p53 nuclear accumulation, demonstrated in situ by immunohistochemical analysis, is considered to be a reliable marker of cell injury due to DNA-damaging agents. After DNA damage, in fact, p53 gene is activated, and both the amount and the half-life of nuclear proteins are increased (100). Wild-type p53 is able to transactivate a discrete array of genes involved in the regulation of the cell cycle and apoptosis (see also Sec. IV. B.). It can be hypothesized that p53 overexpression in UIP is determined by either acute or chronic DNA damage, as is also confirmed by the concomitant expression of $p21^{waf1}$, the downstream product of wild-type p53 (98,99). A direct confirmation of this hypothesis is further provided by the presence of GADD45 and $p21^{waf1}$ among the few genes induced early after bleomycin administration at global analysis of gene expression in experimental pulmonary fibrosis (101).

Overexpression of both p53 and $p21^{waf1}$ has been clearly demonstrated in UIP epithelium. According to several reports and our personal experience, numerous cells exhibiting abnormal p53 nuclear immunostaining can be demonstrated within damaged alveoli in affected areas, showing the morphological features of regenerating type II pneumocytes. Moreover, a significant number of p53-positive cells are clearly localized in bronchiole-related proliferative lesions such as squamous metaplasia, bronchiolization, and honeycomb cysts, as well as in the basal layer of a number of normal-appearing bronchioles (78,98,102–104). As a consequence of chronic damage and regenerative stimulation of p53 expression, mutations of p53 and other tumor suppressor genes can eventually occur. Accordingly, heterogeneous point mutations of the p53 gene have been demonstrated in IPF/UIP patients (102), which are often centered on metaplastic lesions (104). In addition, frequent genetic alterations can be demonstrated in the sputum of patients with IPF, including microsatellite instability and loss of heterozygosity (105). Loss of heterozygosity of the *fragile histidine triad* (FHIT) gene is another abnormality recently demonstrated in IPF/UIP (103). FHIT is a tumor suppressor gene that has been reported to be mutated, deleted, or hypermethylated in lung cancer as well as in preneoplastic lesions (106). All these findings can obviously be related to the high incidence of lung cancer, especially peripheral-type squamous carcinomas, occurring in IPF/UIP (107–110). Interestingly, a large proportion of p53-expressing cells in proliferative lesions in UIP samples coexpress ΔN-p63 (78) (Fig. 3). This finding is relevant, since an identical pattern of p53 distribution is observed in squamous metaplasia of the bronchial epithelium, which is considered to be a preneoplastic condition. In bronchial lesions, in fact, abnormal p53 expression is mainly localized in basal cells (111). In addition, the concomitant expression of both p53 and p53-antagonizing p63 isoforms in the same abnormal basal cells in UIP could represent an important factor in the pathogenesis of cancer

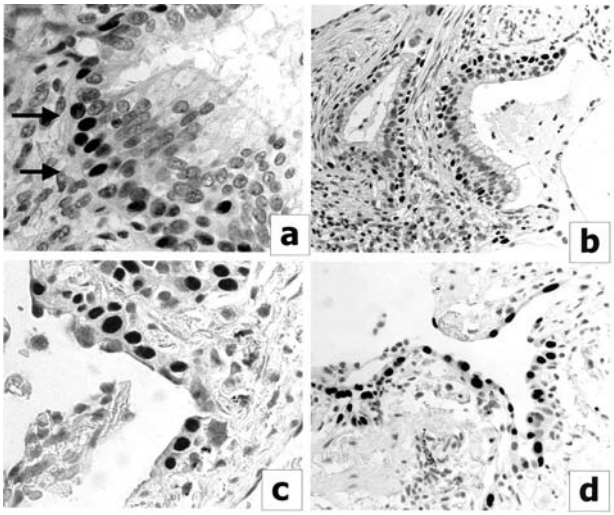

Figure 3 p53 Abnormal expression in bronchiolar lesions in UIP. (a) Nuclear accumulation of p53 in basal cells of a bronchiolar segment; (b) many p53 + cells in the abnormal epithelium lining a honeycomb cyst; (c) many abnormal ΔN-p63 expressing cells in a consecutive section of the same cyst; (d) abnormal cells expressing $p21^{waf1}$ in a consecutive section of the same cyst.

development in metaplastic proliferative lesions. In fact, activation of the p53 pathway after tissue injury does not seem sufficient to increase the risk of cancer progression, since p53 gene activation has been demonstrated in a variety of pulmonary diseases other than IPF/UIP such as DAD, BOOP, and collagen lung disease, as well as in experimental conditions such as acute immune complex alveolitis or following bleomycin administration (98,99,101,112–114). At variance with all these conditions, IPF/UIP is characterized by the occurrence of p53 abnormalities affecting basal cells, a cell type which constitutively expresses ΔN-p63. It is then possible to argue that ΔN-p63 in IPF/UIP can contribute to the proliferative dysregulation of basal cells by counteracting the diverse functions of wild-type p53, and also act as a potent oncogene by inducing unchecked survival of abnormal clones, the emergence of genetic alterations, and the eventual development of malignancy (75,102,105). This could be particularly true for cells expressing ΔN-p63 that become abnormally located in a nonbasal (superficial) position, as those forming honeycomb cysts and bronchiolization. In fact, these cells can represent exceedingly exposed targets for exogenous carcinogenic substances (e.g., in patients who smoke), with a further increased risk of malignant transformation. In normal bronchial and bronchiolar basal cells, the hidden

basal position could represent a means of avoiding contact with potentially noxious agents transported by respiratory gases. In this regard, it is interesting to note that squamous carcinoma, a cancer type which can be considered as being typically derived from basal cell transformation and is characterized by amplification of the p63 gene (AIS) (75), is among the malignancies frequently complicating IPF/UIP (115).

C. Molecular Basis of Mesenchymal-Epithelial Interactions in Lung Development and Fibrosis

There is extensive experimental evidence that normal lung development and morphogenesis are under the influence of a complex network of reciprocal epithelial-mesenchymal interactions determined by important molecular signaling pathways including *Fgf*, *Wnt*, *smoothened*, *Hox*, *smad*, *sprouty* (6,116–119). From flies to mammals, the growth, branching, and differentiation of different airway segments is progressively regulated by a variety of positive and negative signals provided in turn by mesenchymal and epithelial components modeling each other. Interestingly, similar or identical molecular schemes seem to be utilized in physiological and pathological regeneration of pulmonary tissue after injury, so that investigation of the expression profiles of these pathways may provide important information which may explain unresolved pathogenic issues in lung diseases, including IPF/UIP, where the occurrence of abnormal mesenchymal-epithelial cross talk seems to be crucial.

In this regard, it has been clearly demonstrated that mesenchymal signals are necessary to induce the tracheal epithelium to both branch and differentiate into the alveolar epithelium (120). Mesenchymal cells are able to fine tune the regional morphogenesis by expressing molecules that are able to play opposing roles in the vicinity of peripheral lung bud epithelium, where epithelial cells express corresponding receptors (6,121,122). Secreted mesenchymal proteins can induce the expression of transcription factors, such as thyroid transcription factor-1 (TTF-1), in epithelial cells, where these factors are necessary for a correct development, positioning, and differentiation (123–126). In addition to *Hox*, *Bmp*, and *Wnt* genes, whose sequential expression is involved in lung development (118), the interaction between epithelium and mesenchyme is critically dependent on more basic nuclear factor-κB (NF-κB)–mediated transcription signals, acting on the expression of important members of the transforming growth factor-β (TGF-β) and Fgf families (127). Fibroblast growth factor (FGF) signaling is absolutely necessary for a correct development of the pulmonary system, as clearly shown by experimental FGF-deficient mice (121,128). Fgf expression can be tuned by complex regulatory loops with *wnt* signaling by means of beta-catenin (129,130). Mesenchymal interactions are also necessary to induce the expression of p63 and the correct

development of basal cells, as demonstrated in müllerian tissue morphogenesis (84). Another example is given by early development of the thymus where neural crest–derived mesenchymal signals are necessary for the differentiation of the endodermal epithelial rudiment (131). Interestingly, thymic epithelial stem cells also constitutively express ΔN-p63 (132). On the other hand, epithelial cells are able to secrete molecules influencing fibroblast proliferation, migration, and activation, such as insulinlike growth factor-1 (IGF-1) and TGF-β1.

Beta-Catenin Is Abnormally Expressed in Basal Cells and Proliferative Lesions in IPF/UIP

In our ongoing investigation of the expression of molecules involved in lung development and morphogenesis in IPF/UIP samples, we have analyzed the *wnt* signaling pathway by searching in situ for abnormal cytoplasmic and nuclear localization of beta-catenin (the terminal effector of the *wnt* signaling pathway) by immunohistochemistry (133). The immunohistochemical approach is particularly informative, and has been extensively utilized to demonstrate abnormal involvement of the *wnt* pathway in human malignancies and preneoplastic lesions (134–138). According to our findings, beta-catenin is abnormally expressed (cytoplasmic/nuclear accumulation) in proliferative bronchiolar lesions in most IPF/UIP cases, which is at variance with normal lung and all other investigated lung diseases (Fig. 4) (133). This finding was particularly evident in areas of bronchiolar basal cell hyperplasia, squamous metaplasia, and honeycomb cysts, where increased expression of beta-catenin target genes (including cyclin-D1 and matrilysin) was also demonstrated (133). On the other hand, cytoplasmic/nuclear beta-catenin accumulation was never observed in bronchial and bronchiolar epithelia in samples from patients with DAD or BOOP. Interestingly, in these diseases, where pneumocyte hyperplasia occurs, nuclear localization of beta-catenin was observed in alveolar pneumocytes characterized by type II morphology and phenotype. We can then argue that beta-catenin activation is involved in the physiological repair processes of alveolar epithelium after injury, but is engaged in bronchiolar epithelium only in peculiar pathological conditions such as IPF/UIP. This finding is in line with recent data demonstrating an accumulation of beta-catenin in the nuclei of alveolar epithelium in the developing mouse lung, and also with the demonstration of enhanced expression of *wnt* target genes, including c-myc and cyclin D1 in lung tissues where extensive pneumocyte renewal is needed, as in DAD (116).

Notably, we could observe nuclear accumulation of beta-catenin also in fibroblasts of intramural foci, which is at variance with FF of BOOP samples. These FF were associated with epithelial proliferative lesions also characterized by abnormal beta-catenin expression, thus suggesting a zonal activation

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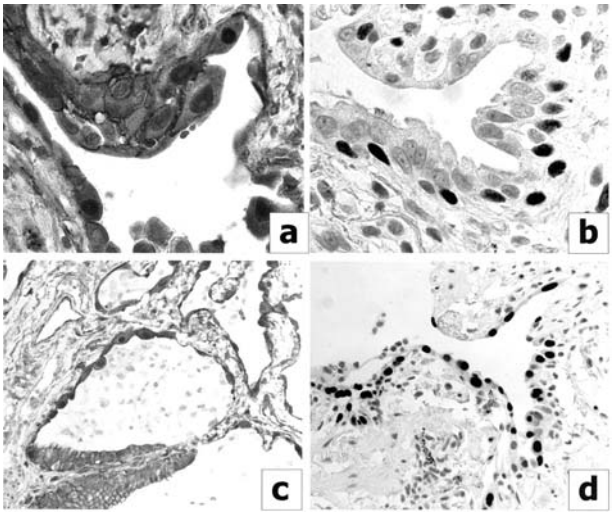

Figure 4 Abnormal cytoplasmic and nuclear accumulation of beta-catenin in UIP. (a) A proliferative lesion with many epithelial cells exhibiting intense nuclear immunostaining for beta-catenin; (b) ΔN-p63 expression provides evidence of the bronchiolar nature of the same lesion on a consecutive section; (c) abnormal expression of beta-catenin in a small cyst; (d) nuclear expression of beta-catenin in myofibrasts forming a fibroblast focus associated with an epithelial lesion.

of beta-catenin. It can be tentatively hypothesized that both FF and proliferative epithelial lesions occur in microenvironmental foci where the *wnt* signaling pathway is abnormally stimulated. It is worth noting that nuclear accumulation of beta-catenin is a central feature in the pathogenesis of aggressive fibromatosis (desmoid tumor), as a consequence of beta-catenin gene mutations (139). Interestingly, proliferation of aggressive fibromatoses can be modulated by cyclooxygenase-2 (COX-2), an important factor also involved in human and experimental lung fibroses (140,141). Investigations of the possible occurrence of beta-catenin gene mutations in proliferative epithelial lesions and/or fibroblast foci of IPF/UIP are needed fully to understand the significance of these observations. In conclusion, all these findings suggest that alterations of the *wnt* pathway and the abnormal subcellular localization of beta-catenin can exert a significant role in the pathogenesis of IPF/UIP.

wnt *Pathway in Lung Development and Morphogenesis*

Evidence suggests that genes involved in the *wnt* signaling pathway are important for cell fate decisions and differentiation in lung development

together with other gene family members produced by embryonal mesenchyme such as *Hox* and *Bmp* (118). In mouse prenatal lung, cytoplasmic/nuclear localization of beta-catenin is observed together with expression of beta-catenin–binding molecules (Tcf/Lef), and sFRPs (soluble *Frizzled*-related proteins, which are soluble proteins that bind to *wnt* and interfere with *wnt* signaling), in undifferentiated primordial epithelium, in differentiating alveolar epithelium, and also in adjacent mesenchyme (142).

Wnt/*wingless* proteins are highly conserved cysteine-rich secreted glycoproteins that control development and organogenesis from old metazoan to mammals by regulating the subcellular distribution of beta-catenin between the cytoplasm and the nucleus (143,144). *Wnt* proteins are secreted at discrete locations, and act by binding as ligands to specific Fz receptors (the *Frizzled* family of *wnt* receptors; 10 in the human genome), expressed on the membranes of target cells, eliciting different responses at various distances from the secreting cells (145). Once *wnt* ligands bind to their fz receptors, a complex molecular cascade is triggered that is able finally to induce the translocation of beta-catenin in the nuclei of target cells, where it can activate important genes involved in cell proliferation, as well as cell-cell and cell-matrix interactions (146). Beta-catenin is a key regulatory protein of the *wnt* signaling pathway, but it also participates in the assembly of cell-to-cell adherens junctions by binding cadherins to the cortical actin cytoskeleton, thus promoting epithelial architecture (147,148). When beta-catenin degradation is limited or impaired, it enters the nucleus and forms a heterodimer with one of the four members of the T-cell factor (TCF) family, also known as lymphoid enhancer factor (LEF). These complexes control the expression of specific *wnt* target genes (including c-myc, c-jun, fra-1, cyclin D1, matrilysin, and others) involved in the regulation of cell proliferation and apoptosis, as well as cell-cell, and cell-matrix interactions (145,149–153). Matrilysin (MMP-7) is one of the metalloproteinases, secreted proteinases needed to remodel extracellular matrix and facilitate cell migration (154,155). Matrilysin is constitutively expressed in conducting airways, and its upregulation is required for repair of airway epithelial injuries (156). Activation of beta-catenin and upregulation of matrilysin contribute to the invasive phenotype of colon carcinoma (157), and may also have a role in the process of alveolar colonization (bronchiolization) described above. Accordingly, another metalloproteinase, gelatinase B, is required for alveolar bronchiolization after intratracheal bleomycin administration in mice (158).

In the absence of *wnt* receptor stimulation, the nonjunctional pool of beta-catenin is phosphorylated, ubiquinated, and degraded by proteasome after binding to a cytoplasmic multimolecular complex consisting of Axin, glycogen synthase kinase GSK-3β, the serine/threonine phosphatase PP2A, and the product of the APC tumor suppressor gene (adenomatous polyposis coli) (146). Axin is a scaffold protein that binds to several molecules to create a multienzyme complex. *wnt* Signals are able to activate *Dvl* (the mammalian

homologue of *fly dishevelled*), a cytoplasmic molecule that is able to bind to Axin and to antagonize GSK-3β–dependent phosphorylation of β-catenin, thus releasing it from Axin complex–mediated degradation. Abnormal beta-catenin nuclear accumulation and aberrant activation can occur when the Axin complex is nonfunctional (e.g., for APC gene mutations), or when the beta-catenin gene itself is mutated, or when *wnt* signals are aberrantly activated.

Beta-catenin is considered to be a potent oncogene product, and its uncontrolled nuclear accumulation is implicated in a variety of human tumors, including, for example, colon carcinoma, melanoma, aggressive fibromatosis (desmoid tumors), trichofolliculoma, and lung carcinoma (159–164).

Abnormal Molecular Cross Talk: TGF-β and IGF-1

Additional layers of complexity are added by other important interactions occurring between the *wnt* pathway and basic molecular systems known to be involved in IPF/UIP pathogenesis. In fact, an important molecular crossroad is that provided by interactions between the *wnt* and TFG-β pathways. Direct interactions occur between the beta-catenin/TCF complex and Smad4, an essential mediator of TGF-β signaling (165–167). Cooperation between these signaling pathways is crucial during development, and can also have a role in the pathogenesis of IPF/UIP. The TGF-β superfamily is, in fact, an important class of mediators involved in the regulation of cell fate and proliferation during development, tissue maintenance, and fibrosing diseases (119,168–173). In particular, TGF-β1 is produced in excess by bronchiolar epithelium in IPF/UIP (174), and its antiproliferative and antibranching effects on epithelial cells can significantly contribute to the alveolar loss characterizing this disease. Another important target of *wnt* signaling is the PEA3 family of transcription factors, which are involved in the expression of COX-2 (97,153,154,175). COX-2 abnormalities are common in human and experimental pulmonary fibrosis (140,176). IGF-1 is a potent growth factor for fibroblasts that has been widely implicated in the pathogenesis of pulmonary fibrosis, and is produced in fibrotic lung by a variety of cell types (177,178). Recently, evidence has been provided that IGF-1 can induce the dissociation of beta-catenin from E-cadherin followed by its relocation to the cellular cytoplasm (179). Interestingly, the IGF-1–induced disruption of adherens junctions can be associated with the promotion of epithelial cell motility and migration (180). It is possible to argue that in IPF/UIP tissue, the local excess of IGF-1 may contribute, together with metalloproteinases, to increased bronchiolar basal cell motility and bronchiolization as described above.

Further studies are needed to investigate the possible role of other molecular pathways that interact with the *wnt* pathway during development, and which might also be involved in lung pathology. Among these, the

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homeodomain-containing genes (HOX genes), a set of master transcription factors involved in the control of patterning, differentiation, and proliferation during development (181).

Interactions Between the p53 and the wnt *Pathways*

Several data suggest that these two important molecular pathways can interfere with each other. Excess of beta-catenin can induce an accumulation of active p53, apparently through interference with its proteolytic degradation (182), and p53 in turn is able to down regulate beta-catenin, directly inducing *Dickkopf-1*, an inhibitor of the *wnt* signaling pathway (183,184). As recently demonstrated, overexpression of beta-catenin induces apoptosis independent of its transactivation functions (185,186). Moreover, p73β, another member of the p53 gene family, is able to enhance *wnt*/beta-catenin signaling (187). Thus, beta-catenin can directly interfere with important functions of the p53 pathway; namely, cell cycle progression and apoptosis. It is possible to speculate that in bronchiolar lesions in IPF/UIP, the abnormalities described above are related to the concurrent accumulation in the same cell nuclei of p53 and beta-catenin together with the constitutive expression of ΔN-p63 (see above), and that the eventual cell fate (proliferation versus apoptosis, and/or cell cycle inhibition) in a given cell is likely to be modulated by the final balance of a variety of proapoptotic and proliferative signals.

VI. Mesenchymal Remodeling and Smooth Muscle Cell Hyperplasia in IPF/UIP

Interstitial remodeling in IPF/UIP is produced by the haphazard accumulation of ECM molecules and fibers, collagens, and elastic fibers, as well as increased numbers of contractile cells, which are usually absent in normal distal lung. Thick collagenous fibrosis and interstitial smooth muscle hyperplasia are frequently associated within injured areas of lung parenchyma and honeycombing in IPF/UIP. The nature and genesis of smooth muscle hyperplasia are not completely clear (14,188,189), but some morphological and immunophenotypical evidence suggests that different contractile cell types are involved, including reactive myofibroblasts and smooth muscle cells of uncertain origin (189,190). In order to clarify the nature and significance of smooth muscle cells in IPF/UIP, we have investigated the expression profile of contractile molecules in cells forming clusters and well-organized bundles in remodeling areas. Recent research has clarified the fact that smooth muscle cell phenotypes are transcriptionally regulated and follow maturation and differentiation of smooth muscle cell subtypes (191). Specific markers have been identified which allow for easy distinction between airway smooth muscle and reactive myofibroblasts (192,193). On the basis of the expression of h-caldesmon and

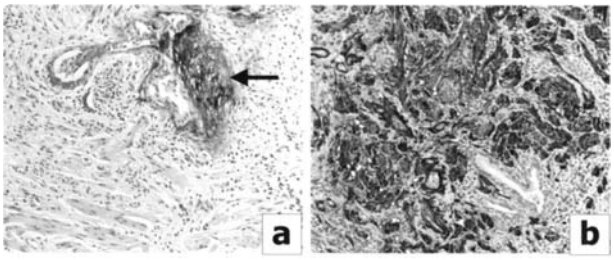

Figure 5 Fibroblast foci and smooth muscle hyperplasia in UIP. (a) A fibroblast focus immunostained for tenascin is shown stuck to a bronchiolar proliferative lesion (arrow). Note the smooth muscle hyperplasia in the surrounding zone of remodeling (bottom); (b) Extended smooth muscle hyperplasia charactrerized by strong expression of h-caldesmon.

smooth muscle myosin heavy chain, we were able to demonstrate that contractile cells in IPF/UIP have an immunophenotypic profile corresponding to that of the bronchiolar wall, which is at variance with that characterizing myofibroblasts (Fig. 5) (15).

What possible interpretation can be given to the nature and significance of smooth muscle hyperplasia in IPF/UIP? Recent studies have provided evidence that smooth muscle cells exhibit a high degree of phenotypic plasticity, and their differentiation is highly dependent on environmental influences, including molecular signals released by neighboring cells and extracellular matrix. In line with this view is the demonstration that IGF-1 and laminin, a constituent of basement membranes, can induce a differentiated phenotype in cultured visceral smooth muscle cells (194). It is then possible to hypothesize that in IPF/UIP, smooth muscle hyperplasia and abnormal extracellular matrix deposition occur under the influence of abnormal signaling provided by overgrowing bronchiolar epithelium in an attempt to reconstitute its natural scaffolding. Interestingly, a similar mechanism has been implicated in the abnormal airway remodeling observed in asthma (195). In normal bronchioles, the muscular scaffolding acts as mechanical support, maintaining airway function and structure, and is, of course, absent in alveolar wall.

VII. Conclusions: The Role of Proliferative Bronchiolar Lesions in IPF/UIP—A New Piece in the Pathogenic Puzzle

In conclusion, on the basis of all these findings, the abnormal wound healing model for IPF/UIP pathogenesis recently proposed by Selman and coauthors (2) could be further defined by including new evidence. First, we propose that

central to the pathogenesis of IPF/UIP is the abnormal activation of the *wnt*/beta-catenin pathway. The abnormal activation of this pathway in bronchiolar basal cells and bronchiolar proliferative lesions can, in our view, account in part for the abnormal proliferative features exhibited by the bronchiolar component in IPF/UIP, including basal cell hyperplasia, squamous metaplasia, bronchiolization, and honeycombing. All these proliferative lesions in UIP could be considered as being responses to peculiar engagement of bronchiolar stem cells after tissue injury. Second, the activation of beta-catenin nuclear accumulation at sites of ongoing injury and repair (focused on bronchiolar-alveolar junctions) could be triggered by abnormal local production of *wnt* mediators and other growth factors. The deviation from normal tissue repair can be related, in our model, to the heterogeneity of stem cell dependence and proliferative responses utilized by bronchiolar and alveolar components after injury. In fact, a crucial pathogenic feature of IPF/UIP might be represented by the repetitive injury and repair at the ''junctional'' zones. This mechanism (with the possible concurrence of genetic predisposition) could trigger unbalanced renewal of epithelial (bronchiolar versus alveolar) and mesenchymal components in an attempt to reconstitute the microenvironmental organization of normal lung. The variety of important mediators and transcription factors previously described in IPF/UIP can find a place in this complex scheme, since they can directly or indirectly interact with the *wnt* pathway. The dominant negative activity of ΔN-p63 counteracting p53's proapoptotic function, and the multiple roles of beta-catenin, as previously discussed, are likely to be involved in these contrasting effects.

Several points remain to be further elucidated, including the possible role of soluble *wnt* signals, and their ''field'' effect on different cell targets. This is a crucial point, since it could suggest possible pharmacological means to counteract the irreversible remodeling of the pulmonary interstitium.

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Role of Viruses in the Pathogenesis of Pulmonary Fibrosis

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I. Introduction

Idiopathic pulmonary fibrosis (IPF) is by definition a disease of unknown etiology. The prognosis is poor, with essentially no response to therapy (1,2). Many potential etiologies have been put forth, including environmental exposures, circulating autoantibodies, and infection. Frequently, individuals date their original symptom onset to a viral infection or other upper respiratory tract symptoms. Based on these observations, it has therefore been proposed that IPF may be an infectious disease triggered by a response to a virus.

Early on, with the initial pathological descriptions of the pulmonary changes associated with IPF, many investigators identified dense particles within the nucleus or the cytoplasm of lung cells that potentially represented viral inclusion bodies (3,4). A case report of Hamman-Rich syndrome in an infant also identified particles suspicious for virus (5). With further refinement of microscopical techniques, these dense particles were felt to be more consistent with nuclear membrane invaginations or nuclear degenerative processes (6,7) or membranous cytoplasmic bodies. In part, this was because cultures were uniformly negative. In addition, control samples from lungs without fibrosis were also noted to contain these same densities, although there were more in the patients with fibrosis.

One important observation related to these studies is that a diagnosis of IPF, as it is defined today would not have been made in many of the ''IPF'' patients evaluated in older studies. In this regard, new refinements in the

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pathological diagnosis of IPF have recently highlighted the differences in IPF compared to other fibrotic diseases. In a recent review, we have noted that the pathological hallmark of IPF is a ''geographically and temporally heterogeneous parenchymal fibrosis against a background of mild inflammation'' (2). There are also relatively specific lung computed tomographic findings in patients with IPF (8).

However, a viral etiology for IPF has a basis in studies looking at the impact of viral gene products on the immune system. Viruses can alter immune responses either directly through activation of the immune system or through upregulation of important molecules that can indirectly affect the immune response. Viruses have been shown to increase the expression of both class I and class II HLA genes on cells with a subsequent increase in inflammation (9). Viral gene products have also been shown to impact on adhesion molecules (10). In addition, some viruses have been shown to carry genes that code for human protein homologues, thus producing a form of molecular mimicry that might result in an autoimmune response (11). Viral gene products can function as transactivators of cellular genes, including cytokines (12–17) and protooncogenes (18). This chapter will review some of the data suggesting that viruses may play a role in the etiology of IPF, as well as point out the inconsistencies of the viral theory of pathogenesis.

II. Viruses that May Play a Role in IPF

A. Epstein-Barr Virus

Epstein-Barr Virus (EBV) is a gamma herpesvirus that is ubiquitous in the general population (19). EBV is associated with the development of lymphoproliferative diseases (20). It can also exist in a latent state and can reactivate under conditions of immunosuppression (20). In 1984, Vergnon and colleagues implicated EBV in the pathogenesis of IPF (21). In this study, the researchers noted the prevalence of EBV-positive serology among patients with IPF. Given the ubiquitous nature of the infection, this in itself was not particularly intriguing. However, they also demonstrated elevated levels of immunoglobulin (Ig) A to the viral capsid antigen, which is a more sensitive test for the presence of replicating virus. In a subsequent study, Egan and colleagues used immunohistochemistry directed toward lytic phase proteins to demonstrate the presence of EBV (22). Fourteen of 20 open lung biopsy samples in patients with IPF stained positive for both the viral capsid protein and the membrane antigen gp340/220, both of which are expressed during active viral replication. In comparison, of 21 control tissues resected from individuals with lung cancer, only 2 stained positive for both antigens. This study is important, as it demonstrated the presence of actively replicating virus. Other studies have demonstrated EBV protein in lung tissue, but they used

antibodies directed toward proteins associated with latency and not with active replication. Another important issue relates to the cell type being studied. Actively replicating virus and the expression of antigens such as the viral capsid antigen are more likely to occur in terminally differentiated epithelial cells as compared to B cells in which latent infection is more likely to occur.

Using more sensitive techniques, Stewart and colleagues have demonstrated the presence of EBV DNA in tissue from patients with IPF (23). Looking at 27 patients with IPF, polymerase chain reaction (PCR) for EBV DNA was positive in 13 cases. In contrast, only 4 of 28 controls were positive by PCR. When analyzed by immunohistochemistry to lytic phase proteins, 12 of 27 IPF and 4 of 28 control samples were positive for EBV. Eleven IPF patients, but none of the controls, were positive using both techniques. However, the sensitive nature of PCR leads to some difficulties with interpretation, as the greater the sensitivity of the test, the more likely EBV will be found given its ubiquitous nature. In summary, it appears that there may be some association between the presence of EBV in the lung and the presence of IPF. However, given the new pathological information regarding IPF, it is also possible that some of these patients were misclassified as having IPF but may actually have had other forms of interstitial lung disease (ILD). More importantly, many of the IPF patients were treated with agents likely to reactivate latent EBV, whereas controls did not receive this therapy. Thus, the presence of EBV may simply reflect the therapy of IPF and not be the cause of the disease.

B. Adenovirus

Adenovirus is also a ubiquitous virus in the general population. It can exist as a latent virus, but it is also a frequent cause of upper and lower respiratory tract infections in individuals with both intact and compromised immune function. Adenoviral DNA has been shown to be present in the lower respiratory tract of normal individuals through the use of PCR for adenoviral DNA (24). Using a more sensitive PCR technique, nested PCR, Kuwano et al. have demonstrated the presence of adenoviral DNA in the lower respiratory tract of individuals with IPF (25). In this study, 16% of the cases were shown to be positive for the adenovirus E1A gene. This rate of positive response is too low to make the case for adenovirus being the cause of IPF. However, with the process of injury and repair that occurs in patients with IPF, it is possible that the primarily infected epithelial cells were removed through this repair process and unavailable for testing. Also, as noted above, IPF is a geographically heterogeneous disease. The study by Kuwano et al. used transbronchial biopsies for their PCR analysis. It is possible that the appropriate tissue was missed, and that the virus is only located in areas of active disease. This issue was addressed in the study by the Matsuse group. They demonstrated that positive staining for adenovirus

alternated with areas of negative staining when looking at serial biopsy sections from open lung biopsies (24). This suggests that sampling error may play a role in reporting of positive staining. It was also interesting to note that the incidence of positive samples in Kuwano et al.'s study was higher in patients receiving steroid therapy, suggesting that there is a higher viral load in individuals who are immunocompromised. This again suggests that the therapy for IPF may have reactivated the virus. The observation of increased viral presence could also suggest that antiviral therapy may be beneficial in these individuals who also have IPF. However, in studies done to evaluate therapeutic response, there was no benefit noted from aerosolized ribavirin (26). In summary, adenoviral proteins have been shown to be present in lung tissue from patients with IPF. Again, the concerns regarding true classification of IPF versus other forms of ILD hold for these studies. It will be interesting to evaluate future studies as we become more sure of pathological diagnosis of this disease.

C. Influenza Virus

Influenza is a seasonal viral disease that can have significant pulmonary effects during its course. There have been case reports evaluating the effects of influenza virus infection on the development of IPF. In 1981, Pinsker and colleagues reported on two individuals who developed changes consistent with usual interstitial pneumonitis after influenza infection (27). In one case, the course was rapid, requiring mechanical ventilation over a short time period. That patient recovered but was left with a restrictive pattern on pulmonary function testing and lower lobe scarring. This patient most likely had acute interstitial pneumonitis (AIP), as opposed to IPF, during the acute phase and then was left with residual scarring in the lung. The other patient had a more subacute course of disease, presenting with dyspnea 4 months after an acute influenzal pneumonia. Open lung biopsy demonstrated usual interstitial pneumonia (UIP). Despite therapy, her symptoms did not improve, nor did she progress. Although the biopsy was thought to represent UIP, it would be unusual for UIP not to progress. Similar case reports came from Philadelphia, reporting on the sequelae of influenza in three children. In all three children, the biopsy findings were reported to be consistent with UIP (28). However, it would be necessary to review these biopsies in light of our current understanding of the pathology of IPF.

Jakab and associates have reported several studies related to the role of influenza in the development of ILD. Using animal models, they noted a link between the development of usual interstitial pneumonitis after chemical exposure (29). Interestingly, this disease was markedly exacerbated by subsequent infection with influenza virus, suggesting an oxidant-induced progression of lung disease. In a second experiment, Jakab looked at

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the long-term effects of viral infection (30). He demonstrated a persistent alveolitis in mice given influenza virus through the nasal route. This alveolitis persisted in the absence of clear ongoing influenza replication with resultant pulmonary fibrosis. However, these animals developed a form of pulmonary fibrosis that likely was not IPF.

D. Human Immunodeficiency Virus

Pulmonary complications in human immunodeficiency virus (HIV) infection are a hallmark of the disease (31,32). Many of these pulmonary complications relate to the development of opportunistic pulmonary infections from other etiological agents. However, there have also been studies demonstrating the development of diffuse lung disease independent of detectable pulmonary infection. Ramaswamy et al. presented data from 12 individuals with HIV infection who developed a variety of pathological lung findings, including diffuse alveolar damage and interstitial fibrosis (33). It was unclear whether these changes were due to the HIV infection or the result of repeated secondary infections at other times. Other investigators have demonstrated nonspecific interstitial pneumonitis (NSIP) in patients with HIV infection without other infectious agents (34,35). The classic noninfectious pulmonary disease in this patient population is NSIP, which differs from UIP in the degree of inflammation and fibrosis, as well as the uniformity of the disease. Inflammation tends to be a more prominent feature of NSIP as compared to UIP. Therefore, association of lung disease with HIV appears to be more related to NSIP as opposed to IPF.

E. Cytomegalovirus

Cytomegalovirus (CMV) is a beta herpesvirus that is a common pathogen in the population. Many individuals have asymptomatic infection early in childhood. Like EBV, CMV becomes latent and can reactivate under conditions of immunosuppression. In a single study, Jiwa et al. were able to demonstrate the presence of CMV DNA in the lungs of individuals with IPF using several different methods of detection (36). There appeared to be differences in the sensitivities of these techniques in the detection of the virus. Once again, we would caution that the pathological diagnosis of IPF may be in doubt in many of these cases. It is clear that the lung is a major reservoir for CMV (37). Whether it is an active pathogen versus a bystander has been raised with regard to HIV infection and pneumocystis pneumonia (38,39). Much like the studies described for EBV, as previously discussed, and in the case of hepatitis C, as discussed below, the high incidence of CMV in the general population precludes making a strong argument for the role of CMV in the development of IPF.

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F. Hepatitis C Virus

Hepatitis C virus (HCV) has recently been determined to be the infectious etiology of non-A non-B hepatitis. Once infected, 50–70% of individuals are expected to develop chronic infection. Whether HCV is associated with IPF is open for debate. In a study from Japan, 66 patients with IPF were evaluated for the presence of HCV infection using an enzyme-linked immunosorbent assay (ELISA) (40). Twenty-eight percent (19 of 66) of these patients were HCV antibody positive. When a more sensitive test, recombinant immunoblotting assay (RIBA), was used, 12 of those 19 patients were also positive. However, another study by Irving et al. looked at sera from 62 patients with the clinical diagnosis of IPF (41). They utilized more sensitive ELISA and RIBA tests to evaluate the presence of HCV in this patient population. In this study, only one patient had detectable HCV antigens using the more sensitive tests. When using confirmatory reverse transcriptase PCR on this individual, no detectable HCV was found. It was felt that the discrepancy in these findings was related to the difference in sensitivity of the tests used in the first study compared to the second. In an Italian study, 60 patients with IPF were studied (42). A control group of 130 patients with nonfibrotic disease were also evaluated for the presence of HCV. Using the more sensitive tests described above, 6 of the 60 (13%) patients with IPF were positive for HCV. However, 6% of the control patients were also HCV positive by the same tests. In addition to the difference in the sensitivities of the tests used, the investigators in this last study pointed out the importance of the background rate of HCV infection in the general population. In western Europe, the background infection rate ranges from 1.0 to 3.4%, whereas in Japan, the background rate is as high as 30%. It was therefore felt that the high rates of infection seen in the Japanese study may simply reflect the background rate of infection and not a causal link between HCV infection and IPF.

III. Conclusions

Although an attractive theory, a viral pathogenesis for IPF has not been demonstrated. Clearly, viral infections can cause some forms of pulmonary fibrosis, such as AIP and NSIP. Some of the earlier studies of viruses and IPF are difficult to interpret, since it is not clear that the patients studied had IPF using our current diagnostic criteria. Also, many of the viruses that are thought to be associated with IPF are latent viruses. These viruses can be reactivated as a result of immunosuppressive therapy that is often used initially to treat IPF. Clearly, further studies into this area are needed for better clarification of the role viruses may play in the disease process we call IPF.

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Current Treatment Options

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I. Introduction

The management of idiopathic pulmonary fibrosis (IPF) has evolved little over decades. The development of novel treatments was limited by an incomplete knowledge of the underlying pathophysiology and epidemiology. Recently there have been a number of new insights, both epidemiological and histopathological, that will likely impact on how we currently approach the management of IPF. Studies now suggest that IPF is more common, with both the incidence and prevalence of IPF being up to 10 times higher than originally supposed (1). Also, we now recognize that IPF is a distinct clinical entity associated with the histopathological pattern of usual interstitial pneumonia. Pathologically, IPF is characterized by inflammation, fibroblastic proliferation, and collagen deposition, with the current most prevalent focus being on fibrogenesis and the concept of aberrant wound healing. With regard to treatment options, we are currently at a crossroads as we move away from traditional anti-inflammatory therapies that have had, disappointingly, only minimal impact on improving disease outcome, toward a more targeted antifibrotic approach. As our understanding of the pathogenesis of lung fibrosis has evolved, we are now better positioned to develop future interventional strategies. However, despite an increased understanding of the cellular and molecular mechanisms underlying IPF and the emergence of a number of more targeted treatments, we still have no proven therapy for IPF.

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II. Importance of Clinical Phenotype: Diagnosis of IPF

Until recently, the definition of IPF included a heterogeneous collection of idiopathic interstitial lung diseases. Clinically, IPF was a single entity characterized by progressive breathlessness and cough, end-inspiratory fine crackles on auscultation, impaired gas exchange and restrictive physiology seen on pulmonary function tests, and basal interstitial shadowing on chest radiographs. Further diagnostic refinement is dependent upon histological examination. In 1969, Liebow (2) differentiated these conditions histopathologically into a number of subsets: usual interstitial pneumonia (UIP), desquamative interstitial pneumonia (DIP), bronchiolitis obliterans with interstitial pneumonia (BIP), lymphoid interstitial pneumonia (LIP), and giant cell interstitial pneumonia (GIP). This classification has been updated recently by Katzenstein and Myers (3), who dropped LIP and GIP, given that such cases were often not truly idiopathic, and added acute interstitial pneumonia (AIP) and nonspecific interstitial pneumonia (NSIP). Taking into account these reclassifications, the American Thoracic Society (ATS) and European Respiratory Society (ERS) have recently published a position paper on the classification of IPF (4), as well as the diagnosis and treatment of IPF (5). The term *idiopathic pulmonary fibrosis* is now reserved for those patients who, in the appropriate clinical-radiographic setting, on biopsy have the histological pattern of usual interstitial pneumonia (UIP), or on transbronchial biopsy $\pm$ bronchoalveolar lavage, findings that exclude alternative diagnoses.

III. Treatment Approaches

Previous beliefs pertinent to the pathogenesis of IPF led to management strategies that evolved around the central tenet that inflammation precedes the development of pulmonary fibrosis. In this regard, treatment strategies that have incorporated anti-inflammatory therapies, particularly corticosteroids, have remained largely unchanged over the past 50 years. Despite this, IPF remains a chronic, generally progressive and usually fatal disease. The recent publication of an international consensus paper on the management of IPF (5) reviewing the historical treatment of the condition concluded, "... no data exist that adequately document any of the current treatment approaches improves survival or the quality of life for patients with IPF." Current and future clinical trials are moving away from studies of anti-inflammatory treatments toward alternate approaches that target the earliest pathological steps that lead to lung injury and the final common pathway, fibrogenesis. Currently, antifibrotic therapies that prevent or inhibit the fibroproliferative response to lung injury are yielding encouraging results.

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A. The Problems of Previous Trials

The absence of an efficacious treatment for IPF can in part be accounted for by the inadequacies of previous trials that have been set up to evaluate treatment responses. The weaknesses of such studies must be acknowledged: first, as they may account for the absence of an observed treatment effect, and second, in that they can provide guidance on how to construct more rigorous trials in the future. Reviews of historical trials that have evaluated different therapies in the management of IPF highlight a number of weaknesses that are common to many of the studies.

Considerations of treatment options in IPF can only be made armed with knowledge of the reclassification of the idiopathic interstitial pneumonias, thus avoiding the issue of diagnostic contamination. Any assessment of potential treatment effects is only meaningful if the likely natural history of the underlying lung condition is taken into account. Furthermore, previous reports of treatment efficacy would have included patients with a variety of interstitial lung conditions with differing histological subtypes. Previous studies most likely included subtypes of idiopathic interstitial pneumonias other than UIP. Thus, in reviewing previous studies of the treatment of IPF, outcomes measured were likely those of a heterogeneous group of diffuse lung diseases that had predominantly, but not exclusively, the histological pattern of UIP. It is likely that the widely differing clinical outcomes described also reflect the different histopathological subtypes.

Early studies did recognize that different histological subtypes had vastly differing clinical outcomes. Carrington et al. (6) followed 93 patients with pulmonary fibrosis over a period of 20 years, and observed that patients with UIP had a 66% mortality rate, whereas those with DIP had a mortality rate of 27%. The changing nomenclature and previous grouping of the diffuse lung diseases into a single clinical entity thus compromises the ability to make valid comparisons between studies performed today with those done previously given the major differences in outcome in relation to the pathological classification.

The effect of reclassification can also be demonstrated in measuring outcome variables. The 5-year median survival of IPF described in previous studies is reduced to 2.8 years in current studies that exclusively follow patients with the histological pattern UIP (7–10). The prognostic implications of the histopathological subtypes are shown by Nicholson et al. (10). In this study, two pulmonary histopathologists reevaluated the surgical lung biopsies of 78 patients who had been labeled as having IPF between 1978 and 1989. They were reclassified to show UIP (47%), NSIP (36%), or DIP/respiratory bronchiolitis–associated interstitial lung disease (RB-ILD) (17%). After a median follow-up of 3.5 years, mortality rates were UIP (89%), NSIP (61%), and DIP/RBI-LD (0%). A worse prognosis is seen in those who

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are elderly, male, have poor lung function at presentation, or show a recent deterioration in pulmonary function tests (11).

Selection bias has been an issue in a number of studies that have recruited patients who have failed to respond to first-line therapy (usually with corticosteroids) and typically have advanced disease characterized by extensive honeycombing on computed tomographic (CT) imaging reflecting severe fibrosis. Treatment is likely to be more efficacious if instigated early in the natural history of the condition, and the same is likely to be true of new agents.

The incidence of IPF is increasing but remains relatively low by comparison with other chronic pulmonary inflammatory diseases such as asthma or chronic obstructive pulmonary disease (COPD). Previous single-center trials have, therefore typically enrolled small numbers of patients, thus limiting the power of an individual study to demonstrate the efficacy of a therapy. The progressive, often irreversible, natural history of IPF means that only marked therapeutic benefits will be observed in such small studies, and that smaller changes in clinical course that may have impact on survival will often be missed. It follows that if a disease progresses slowly, it is essential that patients be followed up over a protracted length of time in order that a treatment benefit can be identified. Multicenter studies are currently evaluating the role of a number of different pharmacological therapies, and future studies will need to follow a similar approach. Multicenter studies are costly to run and require both pharmaceutical industry investment and interest, which in the past has not always been forthcoming given the relative rarity, although increasing incidence, of IPF.

Further recognized problems of older IPF therapeutic trials include lead-time bias; differing study design and follow-up; differing study medication, dose, and route of administration; dissimilar outcome variables; lack of enrolled patients; and the absence of a placebo arm.

Future studies will need to be international collaborative efforts that are based upon a unifying template of study design, thus ensuring enrollment of sufficient patient numbers with a defined clinical phenotype of IPF. Only by incorporating patients with accurate diagnosis can trials be set up truly to assess the treatment responsiveness of the different idiopathic interstitial pneumonias.

IV. Anti-Inflammatory Agents

A. Corticosteroids

Since the 1950s corticosteroids have been first-line treatment for IPF (12,13) and continue to be widely prescribed despite the absence of prospective, randomized, double-blind, placebo-controlled trials to evaluate their efficacy.

Compounding the absence of clinical trials demonstrating improved survival or quality of life following treatment with corticosteroids are concerns that the treatment of IPF may actually be associated with considerable morbidity (14,15). Despite the lack of documented efficacy, corticosteroids remain first-line therapy for UIP in most nontertiary centers (4,16).

Retrospective Studies

The majority of studies have been retrospective reviews of treatment with corticosteroids. In the earliest studies a symptomatic improvement was reported in 41–57% of patients (12,13), but objective improvements were less impressive, with a 10% increase in forced vital capacity (FVC) being observed in only 16–37% of patients. In the study by Turner-Warwick et al. (12), there was no survival benefit in the corticosteroid-treated arm after controlling for age and sex. It is likely that the improvement reported in the initial studies is skewed by a small subset of patients with a steroid-responsive pattern such as DIP, RB-ILD, or NSIP.

After the initial reports, larger studies have failed to demonstrate any survival benefit following treatment with corticosteroids or other anti-inflammatory regimens (5–11,17). In the largest of the retrospective studies, Douglas et al. reviewed 487 patients with UIP confirmed either on surgical open lung biopsy (20%) or high-resolution computed tomography (HRCT) (95%), who had been managed at a single center over a 3-year period (11). Treatments received included prednisolone alone (n = 54), prednisolone plus colchicine (n = 71), colchicine alone (n = 167), and no therapy (n = 157). Oxygen was prescribed to 133 patients. Following univariate analysis, both prednisolone therapy and oxygen therapy were associated with worse survival, whereas after multivariate analysis, older age, male gender, low diffusing capacity for carbon monoxide (D_{LCO}), and recent deteriorating pulmonary function were associated with worse survival. After adjusting for these variables, there was no significant difference between those patients treated with corticosteroids and those who were not.

In a British study (10), the open lung biopsies of 78 patients with "cryptogenic fibrosing alveolitis" obtained between 1978 and 1989 were reclassified by two pulmonary pathologists as UIP (47%), NSIP (36%), and DIP/RB-ILD (17%). Only 3 (11%) of the 28 patients with UIP who were treated with prednisolone responded to the therapy, and after a median follow-up of 42 months, 89% of those with UIP had died. Better clinical outcomes were seen in those patients with histological subtypes other than UIP. In the majority of studies that have followed patients where the underlying histology has been identified as UIP, the disease invariably follows an aggressive course that is unaltered by treatment with corticosteroids (6–9,14,18–22).

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Prospective Studies

There have been few prospective studies. Of these, many are limited by small patient numbers, no placebo control arm, and incomplete classification of the underlying histological pattern, but taking into account these caveats, a number of studies have shown clinical improvement following corticosteroid therapy.

Gay et al. (23) studied 38 patients with surgical biopsy-proven interstitial lung disease (37 with UIP, 1 with DIP). Patients were treated with 3 months of high-dose corticosteroids (1 mg/kg/day) and were graded before and after treatment using a clinical, radiographic, and physiological (CRP) composite scoring system. A clinical response was seen in 10 of 38 (26%) patients after prednisolone therapy, whereas 37% remained stable.

The same group (23) has recently published a second prospective study evaluating the risk and potential benefit of high-dose corticosteroid treatment. Using the same CRP scoring system, of the 41 patients studied, 27% improved and 46% remained stable following 3 months of corticosteroid treatment (14). These studies demonstrated that a response to corticosteroid treatment is associated with better survival, but in a later paper, it was stated that most responders upon reclassification had NSIP and not UIP (24). The improvement seen in the responders may also reflect milder disease rather than a true treatment effect.

Douglas et al. (25) entered 26 patients with suspected UIP (25 diagnosed by HRCT, 1 diagnosed by surgical open lung biopsy) into a randomized prospective trial comparing high-dose prednisolone with colchicine. The study is weakened by the lack of histological information, but the appearance on HRCT in most enrolled patients was of largely fibrotic disease. There was no clinical improvement seen with treatment; neither therapy with prednisolone nor with colchicine affected disease progression. Patients on corticosteroids experienced more adverse events compared with those on colchicine.

There are no studies that have adequately compared differing doses of prednisolone. Most studies have treated patients initially at high doses of prednisolone (50–100 mg/day) followed by tapering to lower doses. In two small randomized studies, the use of high-dose intravenous methyl prednisolone did not improve clinical outcome compared with conventional treatment with oral prednisolone (26,27), but this approach may have a role in the management of the accelerated exacerbation of IPF (28).

Predictors of Response

Clinical responses following high-dose corticosteroid treatment have been shown in patients with IPF, who have an increased lymphocyte count on bronchoalveolar lavage BAL (29–31). More recent studies, however, demonstrate conflicting results, indicating that once significant confounding

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variables such as clinical status are analyzed, BAL cell counts fail to predict response to treatment (32,33).

The detection of ground-glass changes on HRCT may reflect alveolar and interstitial inflammation (34), and the extent of ground-glass opacities may correlate with the degree of alveolitis in the diffuse infiltrative lung diseases (35). The impression that corticosteroids may benefit those patients who appear to have an inflammatory component to their disease resulting in improved survival is suggested in a number of studies (23,36–40). Wells et al. (37) studied patients with either lone cyptogenic fibrosing alveolitis or fibrosing alveolitis associated with scleroderma and graded the HRCT appearances into either cellular, mixed, or predominately reticular patterns. A response to corticosteroid treatment was seen most frequently in those patients with a cellular pattern on HRCT. It is probable that some of the improvement was in patients who had NSIP and not UIP, although some patients with UIP will exhibit mixed patterns of cellular and fibrotic disease.

In a study by Lee et al. (36), the degree of ground-glass opacification, as assessed on HRCT, was reduced following treatment with corticosteroids. The importance of such changes should not be overstated given that the resolution of ground-glass attenuation may not always be reflected by disease outcome (41), particularly if ground-glass changes are present adjacent to areas of established fibrosis as evidenced by the presence of traction bronchiectasis and reticulation (21,35). In IPF, areas of ground-glass opacification may also progress over time to reticular opacities and honeycombing (38,41).

The belief that a cellular biopsy is predictive of a response to corticosteroid treatment is questioned by a recent study by King et al. (19). The aim of the study was to review the surgical lung biopsies of 87 steroid-naïve patients with UIP to identify histopathological features that predicted survival. The strengths of the study included the exclusion of the other idiopathic interstitial pneumonias. They concluded that the extent and severity of interstitial fibrosis or cellularity were not predictive of survival, but the relative absence of fibroblastic foci was associated with survival. They hypothesize that therapy should move away from anti-inflammatory regimens that aim to resolve areas of alveolitis, but rather focus on treatments that inhibit or delay the fibroproliferative response.

Complications of Corticosteroid Treatment

The adverse effects of treatment with corticosteroids have long been recognized in the context of respiratory disease (42). Given the lack of efficacy of corticosteroid therapy in the management of IPF, any improvement that patients achieve is likely to be offset by potential toxicities of the treatment. The potential adverse events are protean and include weight gain, hypertension, glucose intolerance, myopathy, infection, peptic ulcer disease, cataracts,

and cushingoid changes. Close monitoring to identify early potential musculoskeletal complications such as osteoporosis, compression fractures, and aseptic necrosis of the femoral head is vital, especially if the patient is to be considered for lung transplantation. All patients being started on corticosteroids should receive prophylactic therapy with calcium and vitamin D (43), whereas those patients with confirmed osteoporosis or previous bone fractures should be offered bisphosponates (44).

Bone density scans should be incorporated into review visits. The potential psychological effects include anxiety, mania, depression, and commonly insomnia. Between 19 and 66% of patients with pulmonary fibrosis treated with corticosteroids will develop side effects of the treatment (6,12,45). The likelihood of adverse events are dose related (46) and are more likely to occur in the elderly (14,15,47). In the absence of proven alternative therapies for the management of IPF, research continues into newer corticosteroids that have an improved therapeutic ratio (48).

B. Cyclophosphamide

The belief that IPF was an inflammatory disease characterized by a cellular alveolitis made treatment with immunosuppressant regimens logical. Although early studies suggested that treatment with combined cyclophospamide/prednisolone conferred a survival benefit compared to prednisolone alone (45), these results have not been substantiated in later trials. The trial performed by Johnson et al. was limited by the lack of histological data on the treated patients, and the inclusion of cases of idiopathic interstitial pneumonia other than UIP may account for the tantalizingly promising results. There are no studies comparing cyclophosphamide directly with corticosteroids or other immunosupressive drugs. In open studies, pulsed treatment with intravenous cyclophosphamide has been shown to improve pulmonary function (49,50), particularly in patients with recently diagnosed IPF who have relatively preserved lung volumes (51), but survival benefits have not been documented.

The role for cyclophosphamide has recently been questioned in a study by Zisman et al. (52). They prospectively followed 19 patients with biopsy-proven UIP who had previously either failed to respond or developed side effects to corticosteroid therapy. The majority of patients developed toxicity to the treatment, whereas of the 19 patients studied, only 1 patient improved, 7 stabilized, and 11 deteriorated following 6 months of treatment with oral cyclophosphamide. Further studies have demonstrated disappointing outcomes when cyclophosphamide is given to patients who have previously failed to respond to prednisolone (18,53). These disappointing results may reflect that cyclophosphamide is often given as second-line therapy in patients who have advanced disease.

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Our own practice is to give cyclophosphamide at doses of 600 mg/m^2. Mesna (sodium 2-mercaptoethane sulphanate) should be considered in order to attempt to reduce bladder toxicity. Potential adverse events associated with cyclophosphamide include hemorrhagic cystitis, bone marrow suppression, and opportunistic infections. To minimize toxicity, some centers advocate the use of intravenous rather than oral routes of administration. Complicating the management of patients with IPF treated with cyclophosphamide is that deteriorating pulmonary function may reflect the development of cyclophosphamide-induced interstitial pneumonitis rather than progression of the underlying lung condition, although this is uncommonly seen in dosages used for diffuse lung disease (54).

C. Azathioprine

There have been very few studies evaluating the use of azathioprine in IPF, and most are anecdotal and retrospective in design (55,56). The routine use of azathioprine in the management of the IPF became more established following a report by Raghu et al. (57), who prospectively followed 27 patients and randomized them into either treatment with combined azathioprine/prednisolone (n = 14) or prednisolone alone (n = 13). At 1 year of follow-up, there was no difference in lung function and survival between the two groups, but at long-term (9 years) follow-up, there was a significant survival advantage, with 43% of patients who had received combined azathioprine/prednisolone treatment dying compared to 77% in the prednisolone-treated arm. The same group (57) had previously described a favorable response to combined azathioprine/corticosteroid therapy in a more heterogeneous group of patients who had a number of different types of idiopathic interstitial pneumonia (58).

Patients who receive azathioprine commonly complain of nausea, vomiting, and gastrointestinal upset. Bone marrow suppression and hepatotoxicity are more serious side effects. At our center, we start all patients on a dose of azathioprine 50 mg and initially monitor a full blood count every week for four weeks. At this point, if the patient has tolerated the drug and blood tests remain unchanged, we increase the azathioprine to a maintenance dose of 2.5 mg/kg body weight (maximum 200 mg).

V. Antifibrotic Agents

The lack of substantial clinical improvements or significantly enhanced survival following conventional anti-inflammatory treatment with corticosteroids and cytotoxic agents has led investigators to reexamine the underlying pathophysiological mechanisms that underpin IPF. The tenet that chronic inflammation precedes fibrosis is now being questioned (3). Evidence is emerging that pulmonary fibrosis develops as a consequence of abnormal

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wound healing following repeated alveolar epithelial injury (59). A number of groups have developed and tested drugs that are targeted to inhibit the fibroproliferative response.

A. Interferons

The potential therapeutic uses of the type 1 helper T cell (Th1) cytokine, IFN-γ, are suggested by its ability to suppress fibroblast proliferation and the production of extracellular connective tissue matrix (60). It also downregulates the expression of the type 2 helper T cell (Th2) cytokines (61), and shifts the balance away from a Th2 phenotype, (interleukin-4 (IL-4), IL-5, IL-10), which mediate inflammatory and profibrotic responses, to a Th1 phenotype. IFN-γ also inhibits transforming growth factor beta-1 (TGF-β1) receptor signal transduction and triggering of upstream IFN-γ–stimulated gene response elements. The Th1/Th2 balance is thought to regulate the immune response (62). There is also evidence that patients with IPF have low blood and tissue levels of IFN-γ compared to levels of Th2 cytokines (63,64). It was thus a favorable candidate for further clinical trials.

Promising results in an initial pilot study (65) were followed by a prospective study testing the efficacy of long-term treatment with IFN-γ and low-dose prednisolone in patients with IPF (66). Eighteen patients who had previously failed to respond to corticosteroid or other immunosuppressant drugs and whose lung function had deteriorated over the previous 12 months were enrolled. At baseline, all patients had unrecordable lung tissue levels of IFN-γ (obtained by transbronchial biopsy). The results of the study were impressive. All nine patients who received IFN-γ/prednisolone had improved pulmonary function tests and an increase in the arterial partial pressure of oxygen. Of the patients who received prednisolone alone, eight of the nine deteriorated as assessed by the same variables. Given the striking nature of the results, a rigorous debate has ensued with respect to the interpretation of the study (67,68). Concerns raised included (1) selection bias given that the sickest patients would not be fit for surgical biopsy; (2) not all patients had IPF, demonstrated at subsequent independent review of the biopsy and other data; (3) the excellent survival figures seen in all 18 patients given the usual poor prognosis of IPF; and (4) the lack of a total placebo arm. Following the publication of an international position paper on the diagnosis and management of IPF (5), the results were reanalyzed by an independent panel of experts. The panel felt that of the original 18 patients, 9 had definite IPF, 6 had probable IPF, and 3 did not have IPF. They then reanalyzed the data excluding those patients who did not have IPF and were able to verify the results of the original preliminary study, albeit with much smaller patient numbers. The need for a larger phase III clinical trial to validate the initial study was recommended. The results from a large (>300 patients) recently completed

multicenter randomized, double-blind trial are awaited, and when published will better define the role of IFN-γ in the management of IPF.

B. Colchicine

Colchicine has been shown in animal models to have both anti-inflammatory and antifibrotic properties (69,70). Douglas and colleagues in a series of studies compared colchicine to prednisolone in the treatment of IPF (25,71,72), concluding that although colchicine was no more efficacious than prednisolone, it had a preferable side effect profile. In a more recent study (73), the same group of investigators retrospectively reviewed 487 patients who had been treated at a single center over a 3-year period. Treatments received were colchicine alone in 167, no treatment in 157, prednisolone alone in 54, combined colchicine/prednisolone in 71, and other regimens in 38. On univariate analysis, mortality was higher among patients on combined therapy, and there was no difference in mortality between patients on colchicine alone compared with those on no therapy. In a nonrandomized prospective study comparing colchicine and/or penicillamine with prednisolone in a group of patients with IPF who were treated with prednisolone, there was no improvement in pulmonary function or a survival benefit in those patients treated with colchicine (74). In animal models with bleomycin-induced lung injury, there was no improvement in lung inflammation or fibrosis following colchicine therapy (75). At present, we do not recommend the routine use of colchicine in patients with IPF.

VI. Other Agents

A. Cyclosporine A

A number of the retrospective studies that have evaluated the response to treatment with cyclosporine A have typically demonstrated an initial response that is not, however, usually sustained (76,77). Cyclosporine A acts as an immunomodulator predominantly by suppressing Th1 lymphocyte function and proliferation. Its lack of efficacy in IPF is thus not unsurprising, but it may have a role in cases of idiopathic interstitial pneumonia that are characterized by a lymphocytic alveolitis (78). Its clinical use is further restricted by the high incidence of adverse events, and it is not routinely recommended.

B. D-Penicillamine

D-penicillamine prevents collagen accumulation in animal models (79). Those studies that have suggested a potential role in the management of IPF are weakened by small numbers and are not controlled (56,74,80,81). The data do not support the use of D-penicillamine in the treatment of IPF.

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C. Pirfenidone

In animal studies, pirfenidone has been shown to be anti-inflammatory (82), but in addition, it has a number of antifibrotic actions. In the bleomycin animal model, pirfenidone reduces lung fibrosis (83) and downregulates production of both growth factors (84) and procollagens I and III (85). Pirfenidone remains an experimental drug, but a potential future role in the treatment of IPF has been suggested following a study by Raghu et al. (86). Pirfenidone was given to 54 patients with advanced IPF who had either not responded to ($n = 46$) or had failed to tolerate ($n = 8$) corticosteroids. Given the severity of the initial mean DLCO (34%), the 1- and 2-yr survival figures (78 and 63%, respectively) were encouraging. The treatment was largely well tolerated. Further studies are required to delineate further the role of pirfenidone in IPF, but the drug is not currently available.

D. N-Acetylcysteine

The antioxidant N-acetylcysteine prevents free radical–induced epithelial cell injury by promoting the replenishment of the antioxidant glutathione, the levels of which are low in patients with IPF (87). In animal models, it has an antifibrotic effect (88,89). In an open prospective study, 3 months' treatment with high-dose N-acetylcysteine (1800 mg/day) increased lung antioxidant levels and improved pulmonary function tests in patients already taking corticosteroids (90). A large multicenter placebo-controlled study is currently assessing the role of N-acetylcysteine in the management of IPF in Europe (91).

VII. Other Management Approaches

The benefit of supplemental oxygen for patients with IPF remains a vexed question. Long-term oxygen therapy (LTOT) being given for greater than 16 hr/day has been shown to reduce right heart strain (92,93), and supplementation during exercise improves performance (94); however, a survival advantage has not been demonstrated (95). Given the potential benefits of using antioxidant therapy (90), there are concerns that the use of oxygen may result in the production of free oxygen radicals that may exacerbate tissue damage.

There are some limited data on the benefits of enrolling patients with IPF in a pulmonary rehabilitation course (96), and the international consensus panel advocates its consideration in well-motivated patients (5).

Cough is a common symptom associated with fibrotic lung disease and unfortunately is often resistant to therapy. Suggested remedies include nebulized saline, steam inhalation, humidified oxygen, codeine preparations, and low-dose morphine. No approach is universally successful, and the symptom is often intractable.

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VIII. Lung Transplantation

Patients with IPF whose disease is progressive despite optimal medical management should be considered for single-lung transplantation (97). The age of the patient is often a limiting factor, and many centers only consider patients under the age of 60 years. The timing of referral to transplantation is crucial given that the natural history of the disease suggests a median survival of 2.8 years (7), which has to be reconciled against the reality that most patients once listed will have a substantial wait until they are transplanted. The high rates of mortality seen in patients with IPF who are awaiting transplantation suggests that patients are often referred too late for consideration of lung transplantation, and thus earlier referral of suitable candidates should be encouraged (98,99). Patients with IPF who receive a transplant show survival rates of 80% at 1 year and 55% at 3 years (100), with the development of chronic allograft rejection (obliterative bronchiolitis) accounting for most of the longer term deaths following transplantation.

IX. Treatment of Other Types of Idiopathic Interstitial Pneumonias

A. NSIP

The NSIP pattern of histopathology is common to a number of disparate clinical conditions that have different natural histories and thus prognosis (4). It does not represent a single well-defined clinicopathological entity, thus rendering it difficult to compare the limited number of trials that have assessed the treatment of patients with NSIP. Response to treatment is often variable and is dependent upon the balance between the degree of inflammation and of established fibrosis (9). Initial retrospective studies suggested that NSIP responded to corticosteroid treatment and conferred a better prognosis compared to UIP (7–9,20,101), but this benign natural history has been questioned in a recent study which showed that patients with fibrotic NSIP, presenting with the clinical pattern of IPF, only had a 45% 5-year survival (10).

The NSIP pattern of fibrosis is common in patients who have an underlying connective tissue disease. The treatment of NSIP in the setting of systemic sclerosis has been addressed in a number of studies investigating the role of corticosteroids, usually in association with either cyclophosphamide or D-penicillamine (102–104). A number of patients either improve or stabilize on such treatment regimens. Unfortunately, most of these studies have been open studies that have retrospectively evaluated outcome measures. Two randomized placebo-controlled prospective studies are currently underway in the United Kingdom and United States to evaluate the efficacy of corticosteroids in combination with cyclophosphamide or azathioprine. Until such trials are completed, there are insufficient data to define the appropriate

treatment of patients with scleroderma-associated lung disease. Our practice is, however, initially to use prednisolone (0.25 mg/kg) in combination with cyclophosphamide (600 mg/m^2) as first-line therapy for the diffuse lung disease of systemic sclerosis.

B. DIP/RB-ILD

DIP and RB-ILD are linked by their association with cigarette smoking, and there is some evidence that they may exist as overlapping subtypes within a single entity (4). The prognosis of patients with smoking-related interstitial lung disease is generally good (10,20), with many patients improving upon cessation of smoking (105,106). In patients with DIP, there is often a dramatic response both clinically and radiologically to the introduction of corticosteroids (6,107). There are fewer reports with regard to the efficacy of corticosteroid treatment in patients with RB-ILD, which is a more heterogeneous group than DIP, but prednisolone therapy may be helpful (108). Relapses can occur and we, therefore, advocate long-term follow-up of patients with smoking-related interstitial lung disease.

C. AIP (Hamman-Rich Syndrome)

AIP is an aggressive form of idiopathic interstitial pneumonia that is associated with rapid onset of respiratory failure in previously normal lungs, and is characterized by the histological pattern of diffuse alveolar damage (DAD). The important differential diagnoses are an accelerated phase of UIP and acute respiratory distress syndrome (ARDS). In order to confirm the diagnosis, a comprehensive approach to investigation, using BAL, mainly to exclude infection, transbronchial biopsy, with and without surgical biopsy where indicated, is advocated. There are limited trials evaluating treatment options. Despite one study failing to demonstrate a survival benefit using corticosteroids (109), we use a high-dose methylprednisolone regimen (1 g intravenously on days 1, 2, and 3) in the early stages of the disease, usually supported by intravenous cyclophosphamide given every 2–3 weeks (at 600 mg/m^2 intravenously). Treatment is otherwise supportive. The mortality remains high (>60%) (110), and survivors are often left with irreversible fibrosis.

D. Cryptogenic Organizing Pneumonia

Corticosteroids are the mainstay of treatment of cryptogenic organizing pneumonia (COP) (111). There are no trials to evaluate the optimal dose. We advocate the use of high-dose intravenous methylprednisolone in patients who have severely impaired pulmonary function on initial presentation followed by intermediate doses of prednisolone (0.25 mg/kg) thereafter. In less acute presentations, we advocate the use of oral prednisolone. Given the tendency for relapse, treatment should be continued for at least 1 year (112).

Most patients with COP treated with corticosteroids will demonstrate complete radiological resolution, but it is increasingly recognized that a subset of patients progress to fibrotic disease (113). Fibrotic COP is often resistant to treatment and can be fatal. There is little guidance in the medical literature as to how such patients should be managed, although some centers have used immunosuppressants (114).

E. LIP

Corticosteroids are first-line treatment in LIP (115). Most patients respond to prednisolone therapy, but up to one-third progress to fibrotic disease, which is often seen in combination with small cysts on HRCT. Spontaneous resolution can occur. Hydroxycholoroquine has been used to treat LIP with reported efficacy (116).

X. Which IPF Patients Should Be Treated?

Prevailing treatment regimens using anti-inflammatory agents to suppress the inflammatory process that is putatively central to the pathogenesis of IPF have not been shown significantly to delay or reverse the natural history of the fibrotic process. Despite this, treatment regimens incorporating currently available anti-inflammatory drugs remain the mainstay of therapy for patients with a new diagnosis of IPF. The ATS recommends that therapy should only be introduced if there is evidence of a clinical deterioration or a drop in pulmonary function (5). Clinical evaluation should involve a global assessment of clinical, radiographical, and physiological variables. Factors that may indicate which patients are more likely to respond to treatment and thus demonstrate improved survival have been identified (Table 1).

For other idiopathic interstitial pneumonias, less definitive information is available and treatment recommendations are based on peer consensus (5,117,118).

XI. Current Recommendations for Treatment of IPF

The international consensus statement on IPF (5), although concluding that there are insufficient data from previous clinical trials suggesting that any individual regimen improves survival, does suggest that combined therapy be prescribed to informed patients who are more likely to respond to therapy (see Table 1). The current recommendations are summarized in Table 2. Therapy should continue for at least 6 months before any definitive conclusion about response can be made and should remain unchanged if the clinical course is stable or improving. Assessment of possible adverse events associated with treatment should be made at every clinic visit. The ATS guidelines of what constitutes a treatment response include: (1) reduced symptoms, (2)

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Table 1 Features that Influence Treatment Responsiveness and Survival

Age
Worse survival in the elderly (10)
Early Presentation
Improved survival in patients with relative preservation of lung function (19)
More Ground-Glass Changes on HRCT
Better response to treatment but this may also suggest a diagnosis other than IPF (23,37)
Bronchoalveolar Lavage
A BAL lymphocytosis often predicts treatment responsiveness, however, such patients may have an idiopathic interstitial pneumonia other than UIP (14)
Histology
On biopsy, the number of fibroblastic foci is inversely related to survival (33)
Response to Treatment
Initial response to therapy confers a more favorable prognosis (12,30)
Cigarette Smoking
Improved survival and reduced interstitial cellularity in current smokers: explanation unclear but may be ascertainment (33)

Table 2 Current Treatment Recommendations for Idiopathic Pulmonary Fibrosis

Corticosteroid (prednisone or equivalent)
0.5 mg/kg LBW/day orally for 4 weeks
0.25 mg/kg/day for 8 weeks
Taper to 0.125 mg/kg/day or 0.25 mg/kg on alternate days
Plus
Azathioprine
2–3 mg/kg LBW/day
Maximum oral dose 150 mg daily; occasionally up to 200 mg
Dosing should begin at 25–50 mg/day, increasing by 25-mg increments every 1–2 weeks until the maximum dose is achieved
Or
Cyclophosphamide
2 mg/kg LBW/day
Maximum oral dose 150 mg/day
Dosing should begin at 25–50 mg/day, increasing by 25-mg increments every 1–2 weeks until the maximum dose is achieved

Source: Ref. 6.
LBW, lean body weight.

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radiological resolution or improvement, (3) improved pulmonary function ($\geq$10% increase in total lung capacity or forced vital capacity, $\geq$15% increase in the DLCO), (4) improved oxygen saturation during exercise. Treatment should be discontinued if there is no evidence of a clear objective improvement or if the patient develops adverse events to the prescribed therapy. Early assessment of the patient's suitability for lung transplantation should be made in patients who develop worsening pulmonary function despite optimal medical management.

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Future Directions in the Treatment of Idiopathic Pulmonary Fibrosis

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I. Introduction

Despite significant investigative effort over the last 5–10 years, the management of patients with pulmonary fibrosis, particularly those with idiopathic pulmonary fibrosis (IPF), remains a frustrating and seemingly increasing management problem. Although the progress made over the past decade concerning the classification and pathobiology of the disease exceeds that made over the previous 40 years combined, unfortunately this has not translated into improved survival in the patient with IPF (1). In fact, when carefully evaluated, it actually appears that the outcome for IPF has actually worsened during the last decade. This is likely due to the reclassification of the idiopathic interstitial pneumonias (2). Histopathologies that previously were classified as IPF, such as respiratory bronchiolitis–associated interstitial lung disease (RB-ILD), desquamative interstitial pneumonia (DIP), cryptogenic organizing pneumonia (COP) or bronchiolitis obliterans–organizing pneumonia (BOOP), and nonspecific interstitial pneumonia (NSIP) have been distinguished and shown to differ from IPF both in clinical manifestations and outcome. Excluding these alternative diagnoses, the true outcome of IPF becomes clear (3,4) (Fig. 1). Today, the prognosis of IPF with its underlying histology of usual interstitial pneumonia (UIP) differs little from that of lung cancer (5) (Table 1).

This difficulty in identifying a beneficial therapy for these patients was appreciated 50 years ago when Silverman and Talbot wrote, ''Unfortunately, the rarity of diffuse interstitial pulmonary fibrosis, the extreme difficulty

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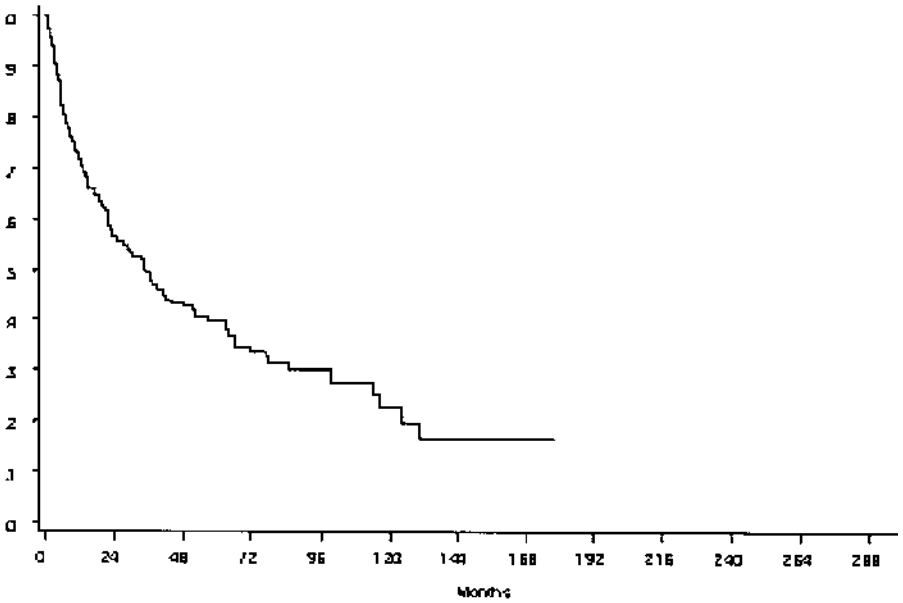

Figure 1 Kaplan-Meier plot of survival of 238 surgical lung biopsy proven cases of IPF from time of initial visit. Median survival – 35.2 months (modified from King T et al, Am J Resp Crit Care Med, 164:1171–1181, 2001).

Table 1 Comparative Mortality: IPF vs Other Chronic Diseases

IPF	50% at 3 years
	65% at 5 years
COPD $FEV_1 < 30\%$ predicted	50% at 3 years
Lung cancer	85% at 5 years
Breast cancer	20% at 5 years

Source: Modified from Ref. 5.

in establishing an early diagnosis and, finally, the complete ignorance as to its etiology present almost insurmountable obstacles in evaluating any form of therapy in this bizarre condition'' (6). Fortunately, recent investigation has offered insights into the pathogenesis of IPF that suggest novel therapeutic approaches. The changing focus in treatment from intense anti-inflammatory to antifibrotic therapy is one result, and offers an opportunity to evaluate the effectiveness of both new and old therapies in a controlled fashion.

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II. Natural History

An understanding of the natural history of IPF offers us the only way to interpret the uncontrolled treatment trials available in this disease. The natural history assists the clinician in determining when and in whom to initiate therapy and defines the therapeutic response. Moreover, it dictates the primary endpoints, number of subjects, and necessary length of any placebo-controlled treatment trials. Since the opportunity prospectively to define the natural history of the untreated disease is gone, only historical data allows us insight into the outcome.

In 1978, Carrington described 53 patients with open lung biopsy–proven UIP (7). While 11 patients were noted to have collagen vascular disease and 3 to have drug toxicity, the majority seem to have had an idiopathic illness consistent with IPF. Forty-eight patients were untreated for at least 1 year. When evaluated by a combination of clinical, physiological, and radiographic criteria, no patient improved and 85% showed disease progression. In 1980, Turner-Warwick described 220 patients who met the prevailing definition of cryptogenic fibrosing alveolitis (CFA), 118 of whom had had biopsy or autopsy confirmation of their disease (8). Seventy-seven of these patients were untreated and survived a median of 54 months. Current interpretation of this study is complicated by the enrollment of patients with known autoimmune disease and the assured presence of patients with NSIP. More recent data on the natural history of untreated patients come from a retrospective study from the Mayo Clinic. Of 487 patients identified, 157 received no treatment. Median survival was 3.2 years for all patients and 3.8 years for those in whom the index visit was also the date of diagnosis. Comparing the untreated IPF group to those treated with colchicine alone ($n = 167$), prednisone alone ($n = 54$), or the combination ($n = 71$), evaluation on an intention-to-treat basis revealed no survival benefit provided by either active therapy (9). These and other studies (3,4,10) suggest that the median survival of patients that meet the current criteria for the diagnosis of IPF is approximately 3 years.

Although the overall prognosis in IPF is poor, a small number of patients stabilize for long periods regardless of treatment status. In a recent study from Spain (11) of patients who met the current American Thoracic Society (ATS) criteria for the diagnosis of IPF, 29 were treated with corticosteroids with or without azathioprine and 14 patients received no treatement. Untreated patients were crossed over to the treatment arm if progressive dyspnea occurred. Patients in the initial treatment group were more ill at baseline. Of the untreated patients, 7 of 13 required therapy an average of 12 months following enrollment. The remaining six patients (15% of the total cohort) remained clinically and physiologically stable and without therapy after 2 years of follow-up. No significant differences in physiology or gas exchange were noted between the treated and untreated patients at the end of the study.

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This intrinsic variability in outcome is underscored by a study from nine centers within the Trent region of England. Prevalent cases (n = 168) had a median survival of 9 years, whereas in the incident cases (n = 76), it was only 2.9 years (12).

Concerning the natural history of treated IPF, the survival for the majority of patients appears to be dismal regardless of treatment. A recent study of 238 surgical lung biopsy–proven cases of IPF that followed patients for up to 18 years revealed a median survival of 2.9 years from the time of the initial visit (4). However, variability in survival was apparent, as over 10% of the patients were alive at 10 years or more. Most of the patients in this study were treated with corticosteroids and/or cyclophosphamide, but no apparent survival differences were noted between treated and nontreated patients. New data regarding the expected changes in clinical, physiological, and gas exchange parameters were presented at the 2002 American Thoracic Society International Conference. Eighty-one patients with surgical lung biopsy–proven IPF prospectively enrolled in a longitudinal natural history study were reviewed and changes in clinical, physiological, and gas exchange variables over time were correlated wtih survival (13). All patients were treated with an immunosuppressive regimen. When follow-up evaluations at 6 and 12 months were compared to baseline, increasing dyspnea as measured on the standardized ATS dyspnea scale, a falling forced vital capacity (FVC), and a rising alveolar-arterial (A-a) gradient all correlated with a significant increase in mortality. In contrast, decreasing dyspnea, a rise in FVC, and a fall in A-a gradient correlated with prolonged survival. Thirty of the patients were dead at 1 year. At 6 months, 9 of 81 (11%) of the patients had improved their FVC by at least 10% of predicted (e.g., from 50% of predicted to 60% of predicted or more), whereas 13 patients (16% of the original cohort or 25% of those still alive) showed an improvement. Interestingly, these changes were found to be independent of baseline values. A second study of 55 biopsy-proven cases of IPF was presented at the same conference. All patients were treated with an immunosuppressive regimen. Mortality at 1 year was 17%. Six patients (11% of the original cohort or 15% of those remaining alive) had an improvement in FVC of 10% or greater at 1 year, whereas the rest had stabilized or deteriorated (14). These studies confirm the logic that progressive symptoms, continued physiological restriction, and worsening gas exchange are poor prognostic indicators, and an improvement in clinical, physiological, and gas exchange parameters are in fact possible in a small number of treated patients.

III. Predictors of Outcome

Although the natural history of IPF, is only now being fully characterized, there are a number of patient-related variables that have a significant impact on prognosis and the rate of disease progression (Table 2). The reasonable

Table 2 Predictors of Outcome

Histological features
Age
Gender
Smoking status
Duration and severity of symptoms
Radiographic features
Pulmonary physiological abnormalities
Initial response to therapy

hypothesis that patients with features suggestive of a more favorable outcome are more likely to benefit from therapy is often expounded, and if treatment is to be initiated, it is for these patients that it is recommended. Patients with late-stage disease and poor prognostic indicators are unlikely to respond to any of the currently available therapies, so consideration should be given to forgoing treatment for these patients, particularly if the risk of side effects is high.

A. Histological Features

Historically, the histopathological features of an open lung biopsy specimen have been the best predictor of clinical outcome among patients with an idiopathic interstitial pneumonia. In large part, this is explained by our failure to distinguish between what are now considered distinct histopathological disorders. IPF is characterized and histopathologically defined by the usual interstitial pneumonia (UIP) lesion. Once alternative histological diagnoses are excluded, the commonly identified histological features of UIP, such as degree of fibrosis, inflammatory cell infiltration, or honeycombing, may not correlate with either prognosis or response to therapy (15). However, this view has been challenged (16). There are new data suggesting that the number of fibroblastic foci (geographically defined regions of fibroblasts, myofibroblasts, and young connective tissue which are characteristic of UIP) is associated with a poorer prognosis (15), as well as worse radiographic and gas exchange abnormalities (17,18).

B. Age

IPF is a disorder of older adults. The mean age of diagnosis is between 60 and 70 years. To establish a diagnosis in a patient under the age of 50 years, surgical lung biopsy confirmation of UIP is required. There is a clear prognostic relationship with age at diagnosis—the older the patient the poorer the outcome (9). The median survival of a 70-year-old diagnosed with IPF is <2 years, whereas that of a 50-year-old is >5 years (4).

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C. Gender

In Turner-Warwick's study of the relationship between clinical features and survival in patients with IPF, males had a poorer outcome (8). Although this early study included patients with autoimmune disease and other patients that would not meet the current diagnostic criteria, other studies with stricter diagnostic criteria seem to support this finding (9,19). On the other hand, studies have not shown a significant survival advantage of gender in UIP confirmed by open lung biopsy (4).

D. Smoking Status

Cigarette smoking has been identified as a potential risk factor for the development and progression of IPF (19). However, there are recent data suggesting that active cigarette smoking may provide an unexplained protective effect (4). Actively smoking patients in this study were younger and had more normal initial physiological function. Given this new information, confirmation and explanation of the role of cigarette smoking in IPF requires further studies.

E. Degree and Duration of Dyspnea

Dyspnea ultimately develops in all patients with IPF. Both the severity (8,20) and the duration of the dyspnea appear to be associated with outcome, with greater degrees of breathlessness and a longer duration of symptoms associated with a poorer prognosis (21). With treatment, improvements in dyspnea are not uncommon (22), and a decrease in breathlessness is associated with a better prognosis (13).

F. Radiographic Features

Radiographic features on both the plain chest radiograph and high-resolution computed tomographic (HRCT) scan have prognostic significance in IPF. More severe pulmonary involvement as quantified by the International Labor Organization (ILO) criteria on chest radiograph is associated with a worse prognosis (8,23). Moreover, both the pattern and extent of changes on HRCT scan are particularly useful. Three features have been shown to correlate with outcome: ground-glass opacity (GGO), reticular opacities, and honeycombing. These likely correspond to different disease stages. GGO can revert to a normal appearance or progress to reticular opacities. Reticular opacities correlate histologically with collagenized lung and variable amounts of chronic inflammation. This is an irreversible change which overtime generally progresses to honeycombing. Honeycomb cysts are areas of end-stage lung disease and are irreversible. The GGO predominant pattern, which is the least frequently encountered, is associated with longer survival, whereas a predominance of reticular abnormality and the presence of honeycombing

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are both accurate predictors of a poorer survival (24,25). Extensive reticulation or honeycombing is a particularly poor prognostic sign, and may be more predictive of outcome than physiological, histological, or the combination clinical/radiographic (using plain chest radiograph)/physiological (CRP) scoring system (26).

G. Pulmonary Physiology

In patients with IPF, a progressive restrictive pulmonary physiology develops. It is not surprising then that in the absence of concomitant chronic obstructive lung disease, the severity of this restriction correlates with prognosis. The extent of the decrease in total lung capacity (TLC) and FVC, as well as an increase in the ratio of forced expiratory volume in 1 s to FVC, have been associated with a poorer prognosis (9,27,28). Changes in these physiological indicators over time correlate with outcome with progressive restriction portending a poorer prognosis and an increased FVC suggesting improved survival (9,29). Measures of gas exchange reveal that a decreased diffusing capacity of carbon monoxide in the lung (D_{LCO}) (9,27) and a progressive rise in the A-a gradient similarly predict a poor outcome (13). The presence of a relationship between survival and the severity of resting hypoxemia at baseline has been found in some (8,30) but not all studies (28).

H. Response to Initial Therapy

Although unusual, a response to therapy with corticosteroids either alone or in combination with cytotoxic therapy, as measured by an improvement in pulmonary function test abnormalities (FVC) or the standardized composite CRP score (31) after 3–6 months, is associated with improved survival (16,22,26) in IPF.

IV. Rationale for Conventional Management

The rationale for the conventional approach to therapy of IPF has evolved by incorporating the results of historical clinical studies as well as scientific investigation of both clinical samples and animal models. The operative hypothesis proposes that the progressive fibrosis that characterizes IPF results from chronic persistent inflammation, and that this chronic inflammation precedes and promotes the development of lung fibrosis. Moreover, the hypothesis proposes that aggressive suppression of this inflammation will block subsequent scar formation. The following provides the background for this approach.

By the early 1950s, the response of sarcoidosis to the use of corticosteroids had been described (32). In 1957, Rubin and Lubliner reported on 63 patients with what was then called the Hamman-Rich syndrome and certainly

represented a heterogeneous group of patients with fibrotic interstitial lung disease (ILD) (33). Nineteen patients were treated with corticosteroids, three (16%) of whom noted a sustained ''improvement'' for at least 1 year. Although these results are unimpressive, and the associated side effects were significant, corticosteroids became and remain a standard therapy, reflecting the lack of an effective alternative. A subsequent study by Turner-Warwick supported this approach. Evaluation of 143 patients who received corticosteroids indicated that 57% had substantial subjective improvement after 4–6 weeks of therapy and 17% had objective physiological improvement. The median survival of the ''responders'' was greater than 7 years. The improved survival correlated with the amount of cellularity on biopsy (22). Identification of younger, less symptomatic patients as those most likely to respond to therapy ushered in an era that encouraged early diagnosis and therapy in all patients with IPF.

Cyclophosphamide was originally added to the therapeutic regimen of Wegener's granulomatosis to minimize the significant morbidity associated with the use of corticosteroids alone. By the 1960s, this cytotoxic therapy was considered to be essential for the treatment of vasculitits given its primary effect on the disease as well as its ability to allow for a reduction of the corticosteroid dose. Cytotoxic therapy was subsequently applied in IPF with a similar rationale: to minimize corticosteroid complications, for those patients at high risk for these complications, and for patients nonresponsive to corticosteroids alone. Results of a study conducted by Johnson and coworkers (34) suggested that the addition of cyclophosphamide to modest doses of corticosteroids as first-line therapy offered benefit beyond that noted with corticosteroids alone. They performed a randomized controlled study for the treatment of IPF. Forty-three previously untreated patients were enrolled in either the prednisolone alone or prednisolone plus cylclophosphamide arms. After 3 years of follow-up, 10 of 22 patients in the prednisolone-alone arm had died compared with 3 of 21 in the prednisolone plus cyclophophamide group, a trend although not a statistically significant difference. When evaluated for either death or disease progression on initial therapy (defined as worsening of dyspnea, radiology, and restrictive physiology), combination therapy was significantly better with 53% of the patients allocated to the cyclophosphamide and prednisolone group still receiving their assigned treatment or no treatment at 3 years versus 24% of the patients in the prednisolone-alone arm. However, the patients were not matched by disease severity at entry, and the prednisolone-alone arm contained 9 of the 12 patients with the most severe restrictive disease total lung capacity (TLC) (<60% of predicted). As all 12 of these patients died or showed progressive disease within 2 years on their initially assigned therapy, any benefit attributable to cyclophosphamide must viewed with caution.

Interpretation of these and other older reports requires additional care. Until recently, studies of patients with IPF did not have the benefit of our

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current strict histological criteria and assuredly included other idiopathic interstitial pneumonias that are known to respond to anti-inflammatory therapy. Moreover, they lacked a clear definition of the natural history, there were no placebo controls, the study duration was variable, and inconsistent and nonvalidated criteria were used for assessment of response to therapy.

Subsequent investigations have addressed a number of the limitations of these previous studies and have shed additional light on the benefits and risks of an aggressive anti-inflammatory approach. Although a number of retrospective studies have failed to show a benefit of immunosuppressive therapy (9,15,27), a more recent study prospectively evaluated the response to therapy and complications of corticosteroid treatment in 41 patients with previously untreated, biopsy-proven IPF (16). Eleven (27%) patients responded to therapy as defined by a 10-point drop in the standardized CRP score after 3 months of therapy, whereas 46% remained stable. Those patients who showed progression of disease as defined by a rise in their CRP score had a significantly shortened survival. The benefit achieved was accompanied by a substantial risk of clinically significant side effects. All patients experienced at least one side effect, and although it is not clear what percentage of patients had to have therapy stopped or modified secondary to side effects, the treatment protocol was changed after the first 24 patients to lower doses of prednisone.

The overall benefit of cytotoxic therapy is also questionable. Raghu and colleagues published the first prospective, double-blind, randomized, placebo-controlled trial in IPF in 1991, evaluating the benefits of prednisone with and without the addition of azathioprine (35). All patients had the diagnosis confirmed by surgical or transbronchial lung biopsy, were untreated, and had shown clinical evidence of disease progression at the time of enrollment. During the 9-year follow-up period, 6 of 14 patients in the azathioprine group had died compared to 10 of 13 in the prednisone alone group. This difference, although not significantly different, suggested a potential survival advantage for the combination therapy. Others have suggested a similar benefit with the use of cyclophosphamide (36), although the data are decidedly mixed, with others showing no obvious benefit (21,37,38). In a recent prospective evaluation of the addition of cyclophosphamide after the failure of initial steroid therapy, only 1 of 17 patients showed sustained improvement at 1 year, whereas 11 of 17 patients deteriorated while on the drug. Toxicity was significant with two of three of patients developing clinically significant side effects and nearly half stopping the drug (20).

After reviewing the relevant world literature on IPF, in 1999, a panel of experts came to the following conclusions: (1) no data adequately document that any of the current treatment approaches improves survival or the quality of life for patients with IPF and (2) there is insufficient clinical evidence to conclude that any treatment improves survival or the quality of life for patients with IPF (1). However, in order to give some guidance to clinicians and

acknowledging that the supporting data on which therapeutic decisions could be made were limited, this international consensus conference did provide therapeutic recommendations for those IPF patients in whom treatment is considered appropriate. These are summarized in (Table 3). A combination of corticosteroids with a cytotoxic agent, either cyclophosphamide or azathioprine, is used for a minimum of 6 months if no intolerable side effects occur. Six months is generally considered the earliest time point a significant response to therapy can be expected. Describing a response to therapy requires a complete evaluation with a combination of clinical, physiological, and radiographic features being proposed as a means of determining improvement or deterioration after initiation of therapy (Table 4).

So although 10–30% of patients may show an objective improvement in response to treatment, this benefit is accompanied by a significant risk of clinically important adverse events. Overall, the results of an aggressive anti-inflammatory approach to treatment remains unsatisfactory for the majority of IPF patients.

V. Pathogenesis and Rationale for Novel Treatment Approaches

The progressive fibrosis that characterizes IPF is a result of tissue injury and aberrant wound healing. Current hypotheses point to the excessive deposition of extracellular matrix, failure of the normal remodeling mechanisms, and

Table 3 Current International Consensus Conference Treatment Recommendations for IPF

Corticosteroid (prednisone or equivalent)
0.5 mg/kg LBW/day orally for 4 weeks
0.25 mg/kg/day for 8 weeks
Taper to 0.125 mg/kg/day or 0.25 mg/kg on alternate days
Plus
Azathioprine
2–3 mg/kg LBW/day
Maximum dose 150 mg daily
Or
Cyclophosphamide
2 mg/kg LBW/day
Maximum dose 150 mg/day
Therapy should be continued for a minimum of 6 months
Response is determined by symptoms, radiological findings, and physiological findings

LBW, lean body weight.

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Table 4 Assessing Response to Therapy

- Clinical improvement
  Two or more of the following on two consecutive visits over a 3- to 6-month period:
  Symptoms: Decreased level of dyspnea or severity of cough
  Radiology: Reduced parenchymal abnormalities on chest radiograph or HRCT scan
  Physiology: ≥10% increase in TLC or FVC (minimum 200 mL)
  ≥15% increase in DLCO (minimum 3 mL/min/mm Hg)
  Significant improvement in O_2 saturation or PaO_2
- Clinical Stability
  Two or more of the following on two consecutive visits over a 3- to 6-month period:
  Physiology: <10% change in TLC or FVC
  <15% change in DLCO
  No significant change in O_2 saturation or PaO_2
- Clinical Failure (after 6 months of therapy)
  Symptoms: Increased dyspnea or severity of cough
  Radiology: Increased parenchymal abnormalities or development of honeycombing or pulmonary hypertension on chest radiograph or HRCT scan
  Physiology: ≥10% decrease in TLC or FVC
  ≥15% increase in DLCO
  Significant worsening in O_2 saturation or PaO_2

DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; HRCT, high-resolution computed tomography; PaO_2, partial pressure of oxygen in arterial blood; TLC, total lung capacity.

abnormal angiogenesis as central features of the disease. However, the absence of a clear understanding of the initiating injury or what causes the fibrosis to progress unabated has been a limiting factor in designing novel therapies.

Although the central importance of fibrosis is evident, a current debate concerns what role, if any, does chronic persistent inflammation play in the progression of disease (39). In the absence of an animal model that truly mimics IPF (40), only inferential data are available. Since the original definition, it has been presumed that inflammation plays a critical role in the progression of IPF, hence the therapeutic approach of aggressive immunosuppression. New experimental evidence combined with the relative inefficacy of aggressive immunosuppression call this into question. Data that now span 30 years confirm that only a fraction of those patients treated with anti-inflammatory drugs manifest any response. Once the alternative histopathological patterns such as NSIP or granulomatous inflammation are excluded, close evaluation of histopathological usual interstitial pneumonia (UIP) reveals no interstitial neutrophilic and little lymphoplasmacytic infiltration. And when UIP is stratified by the amount of lymphocytic

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inflammation, there is no difference in outcome (15). Experimentally, the ability to produce progressive fibrosis in the mouse with little or no inflammation by overexpressing transforming growth factor-β (TGF-β) in the lung (41) and the development of fibrosis in an in vivo interleukin-1β (IL-1β) overexpression system long after IL-1β expression has ceased (42) makes a strong argument against the importance of inflammation in the pathogenesis or progression of the disease.

However, other recent data also provide some support for the role of chronic inflammation. The radiographic features of IPF recapitulate the histological features, showing evidence of temporal and spatial heterogeneity. The disease is patchy throughout the lung with a predilection for the peripheral lower lobes to show end-stage fibrosis or honeycombing. Radiographic progression of the disease shows changes consistent with progression of fibrosis in previously abnormal regions, as well as the development of abnormalities in previously normal areas. By obtaining biopsies from more than one lobe, a hypothesis about the pathological progression of disease can be suggested. Data indicate that there is both interlobar and even intralobar variability in the pathological appearance. Up to a third of patients with UIP also show at least some areas of NSIP; a histological pattern characterized by significant amounts of lymphoplasmacytic inflammation (43). This implies that not only can chronic inflammation and a UIP pattern of fibrosis coexist in the same patient but also raises the possibility that NSIP may precede the development of UIP or that both patterns can result from the same injury, representing different reparative responses. The results of studies that determine the outcome of the inflammation/fibrosis debate will dictate the next generation of therapies for IPF.

An emphasis on antifibrotic agents is currently in vogue. Given that a general "antifibrotic" approach using colchicine (44) or D-penicillamine (45) failed to show clinical or survival benefit in prospective treatment trials, a targeted approach utilizing our current understanding of the pathobiology of the disease is reasonable. The search for potential therapeutic targets based on the biology of the disease provides a number of profibrotic targets. In order for a profibrotic mediator to be considered as being pathogenic, several criteria should be met: It must be capable of producing the disease; it must be differentially expressed during the course of the disease; and modifying its level should effect the development and/or course of the disease. Characteristics of a useful drug would include an ability to decrease lung fibroblast proliferation and increase apoptosis, decrease extracellular matrix (ECM) synthesis and deposition, promote ECM breakdown and remodeling, and protect against ongoing tissue injury, thereby promoting restoration of the normal tissue architecture. The following sections review current and potential therapeutic targets based on our current understanding of the biology of IPF.

VI. Therapeutic Targets

A. TGF-β

There is evidence, both animal and human, that TGF-β plays a central role in the development and persistence of fibrosis in multiple organ systems. In rodents, pulmonary fibrosis induced by bleomycin, radiation, asbestos, and silica result in upregulation of TGF-β gene expression and protein production (46–48). When spontaneously active TGF-β attached to a adenoviral vector is delivered into the airway of a rat, induction of myofibroblasts with rapid and pathologically significant fibrogenesis occurs within the pulmonary interstitium with little or no associated inflammation (41). Animal models of intraperitoneal fibrosis (adhesions) are TGF-β dependent; neutralizing antibodies and antisense approaches can both reduce the development of adhesions, whereas delivery of exogenous TGF-β increases their formation (49). Bleomycin-induced lung fibrosis in the rodent can be blocked by TGF-β signaling inhibitors, blocking antibodies, or chimeric TGF-β receptors (50–52). In human lung, large amounts of TGF-β are present in the healthy state; however, increases in both message and protein have been described in a number of clinical conditions associated with fibrosis including radiation-induced disease, asbestosis, and pneumoconiosis as well as IPF (53–55). Besides the lung, excessive TGF-β levels or activity have been found in other organs that develop pathological fibrosis including the skin, kidney, pancreas, and liver. Taken together, these data strongly suggest TGF-β as a potent, and likely key, mediator of tissue fibrogenesis.

Mammalian TGF-β has three isoforms (1, 2, and 3) that are generally secreted as large complexes that include TGF-β latency–associated peptide and latent TGF-β–binding proteins (56). The effect of these complexes is to prevent TGF-β from binding to its receptors. "Activation" or release of the TGF-β molecule from this complex is incompletely understood, although binding to thrombospondin-1 (57), the epithelial intergrin $\alpha v\beta 6$ (58), and macrophage-mediated activation by plasmin (59) have all been described. When activated, all bind to the same receptors TGF-β type I receptor (Alk5) and the TGF-β type II receptor. Engagement of these receptors activates cytoplasmic Smads which ultimately translocate to the nucleus and bind to response elements in the regulatory regions of target genes (60). Different cell types employ a variety of mechanisms that modulate the TGF-β pathway, including the extracellular control of activation and intracellular inhibitors of Smad activation. These modifiers lead to significant differences in gene expression, which suggest a number of potential therapeutic targets. For example, connective tissue growth factor (CTGF) is known to mediate a number of TGF-β's fibrogenic effects and is responsible for the induction of collagen synthesis in cultured fibroblasts (61). It is highly expressed in the lungs of patients with IPF (62), and is produced in a number of cell types, including fibroblasts. CTGF is induced

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primarily, but not exclusively, through a TGF-β response element in the CTGF promoter, and failure to activate CTGF gene expression appears to offer relative protection against TGF-β overexpression–induced pulmonary fibrosis (63).

Interferons

Although full exploitation of the molecular targets associated with TGF-β signaling will remain a fertile area for the future, there are therapeutic agents currently available that modify TGF-β activity. The interferons are cytokines that as a class have many of the characteristics of a prototypical antifibrotic agent, including the ability to cause fibroblast proliferation (64), reduce collagen gene expression and synthesis (65), and collagen contraction (66). There are two types of interferons (INFs). INF-alpha, INF-beta, INF-omega, and INF-tau are type I, with IFN-gamma being the sole type II INF. The primary differences between types I and II lie primarily in the cells of origin type I INFs are produced in almost all cell types, whereas IFN-gamma is produced in T lymphocytes and natural killer (NK) cells (67). There are also different receptor specificities and signal transduction mechanisms, and a difference in their ability to stimulate major histocompatibility complex (MHC) class II antigens (68).

The ability of type I INFs to modulate pulmonary fibrosis has been explored in both animal models and humans. The capacity of IFN-alpha2a to inhibit bleomycin-induced lung fibrosis in the mouse was recently evaluated (69). Treatment with IFN-alpha2a for 14 days enhanced bleomycin-induced injury. Combined with other evidence of a proinflammatory effect in the lung, there appears to be no role for IFN-alpha in the treatment of IPF. IFN-beta, another type I INF, was recently studied as a potential treatment for IPF in a large multicentered, randomized, double-blind, placebo-controlled trial. Unpublished results of this trial were presented at the annual International Conference of the American Thoracic Society in 2000, and revealed no evidence of therapeutic benefit in the treated subjects.

The type II INF, IFN-gamma has been shown to limit fibroblast proliferation (64), inhibit the transcription of collagen mRNA and the synthesis of protein in both normal and isolated IPF fibroblasts (70–72), abrogate the increased collagen synthesis associated with TGF-β stimulation (73), and reduce tissue myofibroblast numbers (74) as well as increase the expression of matrix metallo-proteinase-1 (MMP-1) message (75). Immuno-histochemical studies of lung tissue from patients with IPF show a deficiency of IFN-gamma, and a high helper T cell type 1/helper T cell type 2 (Th1/Th2) cytokine ratio (76). A recent prospective, randomized study of INF-gamma for the treatment of IPF suggests it may have therapeutic benefit (77). In this small study, 18 patients were prospectively enrolled in a treatment trial comparing

subcutaneously administered IFN-gamma to placebo. All patients had failed to respond to corticosteroids prior to enrollment. The patients who received subcutaneously administered IFN-gamma for 1 year responded with a significant improvement in lung function and gas exchange, whereas those receiving placebo remained unchanged or had physiological deterioration. Subsequent data (Ziesche, personal communication) revealed that all of the patients enrolled in this trial were deficient in IFN-gamma message measured in transbronchial biopsies. However, not all patients with IPF will respond to this therapy. Two subsequent studies evaluated the benefits of this approach in an open-label, nonrandomized fashion. One reviewed the off-label experience in 17 patients with IPF. No improvements in symptoms or consistent improvements in pulmonary physiology were noted (78). Another study enrolled 33 consecutive patients who had experienced progressive disease despite conventional immunosuppressive therapy in an open-label trial. Six patients died, five dying secondary to progressive fibrotic disease. No patient showed significant improvement in physiological parameters (79). However, compared to the positive study, in both of these, the patients had more advanced disease.

The hypothesis that IFN-gamma is useful in the treatment of IPF is being tested in a large multicentered, double-blind, placebo-controlled treatment trial. Although the complete results are eagerly awaited, some preliminary results are available. A total of 330 patients were randomized at 58 centers around the United States and Europe. Patients received either placebo or 200 μg of IFN-gamma injected subcutaneously three times per week. Patients were allowed to take up to 15 mg of prednisone daily. The trial was continued until the last patient received 48 weeks of therapy. Median treatment duration was 60 weeks. The primary endpoint was progression-free survival time, defined as any of the following: (1) a decrease in FVC of >10%, (2) an increase in the resting room air A-a gradient of 5 mm Hg or more, or (3) death. This endpoint did not reach statistical signifance, although there was a trend in favor of the actively treated patients, with an approximately 10% relative reduction in the rate of progression-free survival versus placebo. The data also suggest a mortality benefit in patients with mild to moderate disease. Of the 254 patients with an FVC >55% of predicted, 6 of 126 died in the treated group (4.8%), whereas 21 of 128 died in the placebo group (16.4%), representing a 70% decrease in mortality in favor of the treated group ($P = .004$) (80).

Pirfenidone

The investigational pyriodone molecule (5-methyl-1-phenyl-2[1H]-pyradone), or pirfenidone, has also been shown to have antifibrotic activity. It inhibits both the TGF-β–induced collagen synthesis in isolated IPF-derived lung fibroblasts as well as the mitogenic effects of other profibrotic cytokines.

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In bleomycin-induced lung fibrosis in rodents, it inhibited collagen production, reduced BAL inflammatory cell numbers, and decreased lung cell transcription of TGF-β and platelet-derived growth factor (PDGF) (81,82). A phase II, open-label study for the treatment of IPF with pirfenidone was recently published (83). In patients who had previously manifested progressive disease on conventional therapy, 29 of 54 showed evidence of stability or improvement of the FVC at 6 months. An effect on survival was difficult to interpret given the study design and a likely survivorship effect. Although a majority of the patients developed significant side effects while taking pirfenidone, only a handful had to discontinue therapy. Overall the results were encouraging and have resulted in a larger prospective multicentered phase II trial in Japan. This trial has recently been completed with results pending.

B. Tumor Necrosis Factor-α

Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine that has been variably described as being profibrotic, antifibrotic, and proinflammatory. It has been suggested that it stimulates fibroblast proliferation and collagen gene upregulation through a TGF-β and/or PDGF pathway (84), although it has also been shown to suppress collagen gene expression (85). TNF-α overexpressing mice have been shown spontaneously to develop lung fibrosis accompanied by a chronic lymphocytic infiltrate (86), although new data suggest that this same overexpression may protect against bleomycin-induced or TGF-β–induced fibrosis (87). TNF-α gene expression rises in the rodent lung after the administration of bleomycin (88), whereas animals missing TNF-α receptors (89) or treated with soluble TNF-α receptors are both relatively resistant to bleomycin-induced fibrosis (90).

In the human lung, alveolar macrophages from patients with IPF or asbestosis obtained by BAL produce increased amounts of TNF-α when compared to nondiseased controls (91). Hyperplastic type II cells of IPF patients contain significant amounts of the protein by immunohistochemistry (92). New data also suggest an association between TNF-α promoter polymorphisms and an increased risk of developing IPF (93). This and similar data led to a recent open-label pilot study that prospectively tested the use of the TNF-α blocker entanercept in the treatment of nine consecutive patients with UIP diagnosed by transthoracic needle biopsy (94). All patients had significant reductions in FVC (range 30–70% of predicted) and DLCO (range 10–54% of predicted) at the time of enrollment, and had shown worsening lung function despite conventional immunosuppressive therapy. After an average of 9 months of treatment with twice weekly entanercept and 10 mg of prednisone, one patient had died, whereas improvement (as measured by >15% increase from baseline) in FVC was noted in three patients, DLCO in four and A-a gradient in five patients. A prospective, multicentered,

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double-blind, placebo-controlled trial of entanercept for the treatment of IPF has been designed.

C. Th1/Th2 Cytokine Imbalance

Initially named after the groups of cytokines produced by subsets of CD4 helper T lymphocytes (95), it was found that other cell types, including fibroblasts, can produce these cytokines. Type I cytokines include IL-2, IL-12, IL-18, TNF-β, and IFN-gamma. Type II cytokines include IL-4, IL-5, IL-10, IL-13, and monocyte chemotactic protein-1 (MCP-1). Traditionally, type I responses are associated with cell-mediated and type II humoral immunity. However, information suggests that the pattern of cytokine expression plays an important role in the host response to tissue injury, with type I cytokines being responsible for repair and the restoration of normal architecture. A type II response, on the other hand, promotes fibrogenesis with exuberant ECM deposition. IL-4, the prototypic type II cytokine, promotes collagen gene expression and protein production (96), and the prototypic type I cytokine, IFN-gamma, has the opposite effect. In vivo, upregulation of IL-4, IL-5, and MCP-1 occur in the rodent model of bleomycin-induced lung injury (97,98).

Neutralizing antibodies to IL-5 or MCP-1 limit the fibrosis in this model (99). Murine IL-10 knockouts are protected from silica-induced pulmonary fibrosis, whereas wild-type mice show increased type II responses (100,101). Tight skin mice with the IL-4 and Stat 6 genes knocked out develop significantly less skin fibrosis (102). In humans, multiple isolated cell types from fibrotic lung disease patients produce an excess of IL-4, IL-5, IL-13, and MCP-1. Increases in some of the type II cytokines are also present in BAL and serum of IPF patients (103–106). Two groups have shown either an excess number of type II cytokine–producing mononuclear cells or the cytokines themselves in fibrotic lung disease (76,107). The weight of these data supports the hypothesis that there is an imbalance between type I and II cytokines in IPF, with an excess of type II cytokines being associated with the development of tissue fibrosis. Therapeutic approaches that decrease the level of these fibrogenic cytokines or increasing type I cytokines may prove fruitful.

D. Endothelins

Endothelins are a family of 21 amino acid peptides with vasoactive, mitogenic, bronchogenic, and immunomodulatory activity. There are three isoforms ET-1, ET-2, and ET-3, with ET-1 being the most abundant and best characterized. The lung contains the highest levels of ET-1, and it is secreted by cells of the airway (epithelial and smooth muscle cells), vasculature

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(endothelial and smooth muscle cells), and hematopoetic system (platelets and macrophages) (108). All three hormones are synthesized as prehormones necessitating posttranslational processing to become active. Two receptors, ETA and ETB, are known. ETA has a higher affinity for ET-1 and ET-2, whereas all three have equal affinity for ETB. ET-1 appears to be important in the initial response to lung injury by activating neutrophils, monocytes, and mast cells (109,110). Along with inducing the production of TGF-β (111), ET-1 stimulates fibroblast proliferation, migration, and their conversion to myofibroblasts (112). It stimulates collagen synthesis while decreasing collagen breakdown (113) and increases fibronectin production (114). In an animal model of bleomycin-induced lung fibrosis, increased ET-1 expression was seen in alveolar macrophages and the epithelium, with the increase preceding the development of the fibrosis (115). Mice engineered constitutively to overexpress human ET-1 develop age-dependent pulmonary fibrosis and glomerulosclerosis (116). Although blocking endothelin receptors has been shown to decrease fibroblast proliferation and collagen production in vitro, blocking them in vivo has produced mixed results (117,118). In IPF, both BAL and plasma levels of ET-1 are increased (119,120), and increased protein is noted by immunohistochemistry in airway epithelial cells and type II pneumocytes (121). Stimulated alveolar macrophages from patients with scleroderma-related lung disease secrete increased amounts of ET-1, and fibroblast proliferation induced by BAL from these patients is inhibited by ETA receptor blockers (122,123). The weight of this evidence supports a potential role for ET-1 in the development of progressive pulmonary fibrosis, and with the commercial availability of endothelin antagonists, suggests that a formal treatment trial should be undertaken.

E. Eicosanoids

Eicosanoids are lipid mediators that are derived from the cyclooxygenase and lipoxygenase metabolic pathways of arachadonic acid (124). Prostaglandins and thromboxane are the end result of the cyclooxygenase pathway, whereas the lipoxygenase pathway leads to hydroxyeicosatetraenoic acid (HETE) and leukotrienes (LTs) A4, B4, and the cysteinyl leukotrienes LTC4, D4, and E4. Present throughout the body, they are responsible for critical pleiotropic physiological functions. Almost all eicosanoids have been shown to have some physiological function in the lung. Prostaglandin E2 (PGE2) has potent bronchodilatory, immunomodulatory, and antifibrotic effects via its action on specific cell receptors. In mice engineered to be deficient in cyclooxygenase-1, reduced PGE2 in BAL, an increased acute inflammatory response, and enhanced bronchoconstriction in response to an inhaled allergen result (125). More interestingly, cyclooxygenase-2 knockout mice respond to bleomycin administration with an increased amount of lung fibrosis (126). In IPF

patients, isolated pulmonary fibroblasts have an intrinsic defect in their ability to upregulate cyclooxygenase-2 and biosynthesize PGE2 (127).

In contrast, the lipoxygenase pathway results in the generation of LTs that possess proinflammatory and bronchoconstrictive effects as well as possessing the ability to promote fibroblast migration, and proliferation and the production of ECM proteins (128,129). Mice engineered with absent 5-lipoxygenase (5-LO), the critical enzyme required for LT and HETE production, develop less airway inflammation and bronchoconstriction following allergen challenge (130). Hence, the therapeutic use of 5-LO blockers or cysteinyl LT receptor antagonists for patients with asthma. LTs also have a role in IPF. Increased levels of LTB4 and C4 are found in IPF patient's lungs (131). Recently, evidence has been presented that 5-LO–deficient mice have a decreased capacity to produce cysteinyl LTs and produce less inflammation and lung fibrosis in a bleomycin lung injury model (132). Interestingly, this decreased capacity for the production of LTs was associated with an increased production of PGE2 and IFN-gamma. Based on this, a clinical trial of the 5-LO inhibitor zileuton is currently underway at the University of Michigan.

F. Antioxidants

Recent evidence suggests that there is an imbalance between oxidation products and antioxidants in the lungs of patients with IPF (133). Large amounts of reactive oxygen species are identified in the lower respiratory tract of patients; at least partly due to the presence of activated inflammatory cells (134). These free radicals are associated with cell death and tissue injury by oxidizing substrates such as proteins (causing the inactivation of protease inhibitors or the activation of proteases) and lipids (activating mediators such as LTs). Lipid peroxidation products are increased in the blood of patients with IPF, particularly those with clinical evidence of progressive disease (135). Hydrogen peroxide is a stimulant for fibroblast proliferation, and oxidants effect extracellular matrix makeup, influencing collagen deposition by altering its gene activation and synthesis (136), and by altering MMP activation (137).

Glutathione is a well-characterized protein, functioning as an antioxidant in its reduced form and regulating the process of oxidant-induced cellular damage (138). Its levels are reduced in the epithelial lining fluid of patients with IPF. Interestingly, TGF-β has been shown to inhibit intrinsic glutathione biosynthesis by suppression of the rate-limiting enzyme. Glutathione is also capable of inhibiting oxidant-induced MMP activation and fibroblast growth (137,139).

Approaches to increasing glutathione levels and activity in the lung are available. N-acetyl-L-cysteine (NAC), a metabolic precursor of glutathione, has been shown to increase glutathione levels in the alveolar lining fluid in patients with IPF (133). In a rodent model of bleomycin-induced lung fibrosis,

aerosolized delivery of NAC reduced BAL and tissue infiltration by inflammatory cells as well as collagen deposition. Oral NAC has been tested in a proof-of-concept study. Twenty patients with IPF had oral NAC (600 mg tid) added to their conventional immunosuppressive regimen for 12 weeks. Neither symptoms nor examination findings changed; however, a significant improvement in DLCO was appreciated (133). There is currently a multicentered treatment trial in Europe, the Idiopathic Pulmonary Fibrosis International Group Exploring NAC-1 Annually (IFIGENIA), investigating the efficacy of prednisone and azathioprine with or without NAC. The results of this study may be available shortly.

G. Renin-Angiotensin-Aldosterone System

Systemic activation of the renin-angiotensin-aldosterone system (RAAS) is important for regulation of blood pressure and maintenance of the intravascular volume. There is also a local tissue RAAS that is normally quiescent after birth but can be reactivated in response to tissue injury (140). Locally elaborated angiotensin II appears to initiate tissue repair as a primary event and through stimulation of TGF-β (141). Both in vitro and in vivo studies have shown that angiotensin II stimulates TGF-β gene and protein expression, and it can directly induce both cell growth and matrix accumulation in the kidney (142) through angiotensin type 1 and 2 receptors (143). A marked increase in angiotensin II is found by immunohistochemistry in a number of lung cell types, including fibroblasts after radiation injury (144). The angiotensin-converting enzyme inhibitor (ACEI) captopril has been shown to inhibit radiation-induced lung fibrosis in a rodent model (145). It is also believed that angiotensin II can inhibit the degradation of matrix by creating an imbalance in the plasminogen/plasmin system. The primary fibrinolytic enzyme in the body is plasmin. Plasminogen activator inhibitor-1 (PAI-1) is the major physiological inhibitor of tissue-type plasminogen activator (t-PA), and urokinaselike plasminogen activator (u-PA), both of which activate plasminogen to plasmin, promoting fibrinolysis and proteolysis and activation of latent MMPs. PAI-1 also encourages the migration of matrix-producing cells into damaged tissue (146). PAI-1 is upregulated in bleomycin-induced pulmonary fibrosis, and the fibrosis generated by bleomycin is worse in PAI-1–overexpressing mice (147). Angiotensin is linked to induction of PAI-1; likely via both type 1 and 4 angiotensin receptors (148).

In the human, angiotensinogen is produced by isolated fibroblasts from fibrotic but not normal human lung and is capable of inducing alveolar epithelial cell death (149). Although PAI-1 levels in BAL fluid are considerably elevated in patients with IPF (150), unfortunately, treatment with captopril does not appear to provide a survival benefit in patients with biopsy-proven IPF (151) (M. Selman, personal communication). Nor does

the use of ACE inhibitors appear to protect against radiation-induced lung injury (152).

Aldosterone increases TGF-β expression in the kidney (153). It is also capable of causing myocardial fibrosis through a mechanism that appears to be independent of the associated systemic hypertension (154). Clinical evidence supports these data, as the mineralocorticoid receptor blocker spironolactone appears to be able to retard cardiac fibrosis and is beneficial in patients with advanced heart failure (155). A role in the lung for aldosterone has yet to be described, however, and the lung seems to lack the aldosterone receptor (156). Although through a separate mechanism, inhibition of aldosterone may decrease PAI-1 in vivo suggesting that targeting both angiotensin and aldosterone may be necessary for an optimal effect on PAI-1 (157).

H. ECM and Proteases

The initiation and progression factors responsible for the development of lung fibrosis may revolve around the ability of resident lung cells to interact with the surrounding ECM. Both epithelial cells and fibroblasts produce matrix proteins. The production and remodeling of this provisional matrix determines whether the locally injured lung returns to its normal architecture or is replaced by scar. Some of the factors involved in normal wound repair are known. In the mouse, a deficiency of plasminogen activator inhibitor-1 protects against the development of fibrosis in response to injury (147), whereas an excess of PAI-1 has been shown in IPF (158). Recent rodent studies have shown presenilin-1 and MMP-7 as being important proteolytic enzymes in the development of pulmonary fibrosis (159,160), whereas MMP-7 has also been shown to be differentially upregulated in human fibrotic lung (161). Unfortunately, how resident lung cells respond to provisional matrix proteins is unclear, and whether activation or suppression of epithelial or fibroblastic cells is required to instigate appropriate tissue remodeling is uncertain. Therapies based on modifying a set of wound repair or matrix-modifying proteins must await additional investigation.

I. Cellular Apoptosis/Proliferation

The interplay of apoptosis, the intrinsic pattern of programmed cell death built into nearly every cell, and the proliferative response of cells plays a critical role in wound healing. Both of these responses are active in the epithelium and mesenchymal cells of the lung after an injury. Fibroblast and myofibroblasts are required to undergo both processes in sequence in order for wound healing to proceed appropriately. Epithelial cells undergo apoptosis in response to Fas-Fas-ligand cross linking on their surface or upon exposure to bleomycin (162). In human lung, there is evidence of significant cellular apoptosis in

the fibromyxoid Masson bodies of subjects with bronchiolitis obliterans–organizing pneumonia (BOOP), an interstitial lung disease with an excellent clinical outcome while the fibroblastic foci of UIP contains few if any apoptotic cells (163). Fibroblasts from patients with some fibrotic lung diseases constitutively express growth-promoting genes (164). This suggests that the failure of appropriate apoptosis may be an explanation for the persistence and unopposed proliferation of fibroblasts in IPF. An approach that increases fibroblast or myofibroblast apoptosis in IPF is reasonable. Lovastatin has been shown to induce apoptosis in isolated normal lung and fibrotic lung fibroblasts, as well as reduce granulation tissue formation and induce fibroblast apoptosis in a rodent wound chamber model (165).

VII. Conclusions

IPF is an aggressive pulmonary fibrotic disease of unknown etiology with a dismal prognosis that differs little from that of lung cancer. Although the ultimate prognosis is almost always known, the practicing physician can use a number of patient- and disease-specific features to make some prediction about the rate of progression. In particular, changes in clinical features such as dyspnea and pulmonary physiology over time can serve as surrogate endpoints for predicting survival. To date, no data exist adequately to prove that any of the currently accepted treatment approaches improves survival or quality of life for patients with IPF. An immunosuppressive approach has become the conventional therapy because of the lack of any proven alternative. A number of new treatment trials are underway, with the results of some likely to be available by the time this text is published. These novel therapies are taking advantage of new insights made into the pathobiology of the disease; it is likely that newer therapies, more uniquely focused on defined molecular targets, will be forthcoming.

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Future Directions in Genetic Strategies for Understanding and Treating Idiopathic Pulmonary Fibrosis

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I. Introduction

Insights into the pathogenesis of idiopathic pulmonary fibrosis (IPF) and the strategies for treatment of this disorder have evolved over the past 30 years. In the pregenetic era, prior to the early 1970s, the pathogenesis and treatment of IPF was based on classic clinical and pathological assessments. In this period, the disease was defined on a clinical level, with the natural history, clinical presentation, blood chemistry, radiographic features, and morphology of the disorder understood (1–5). In what can be called the early genetic era, from the late 1970s to the present, as the newly developed technologies of mammalian molecular biology, cell biology, and culture were applied to lung disease, IPF was understood to be an inflammatory disorder with superimposed dysfunction of lung repair mechanisms, and new approaches to therapy were developed (6–10). In the current genetic era, with the sequencing of the human and murine genomes, the development of array-based strategies to evaluate gene expression, and the evolution of high-throughput sequencing and screening of drug libraries, the stage is set to make new insights into the pathogenesis of IPF and evolve new therapeutic approaches to the treatment of this disorder (11).

In this chapter, we will focus on future genetic-based strategies for understanding the pathogenesis of IPF by first reviewing what is known about the inherited susceptibility to IPF, and then discussing the strategies that can now be used to understand the pathogenesis of IPF at a genetic level, including

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the use of animal models, assessment of gene expression, and strategies to assess genetic susceptibility. We will then discuss the future genetic-based strategies for therapy of IPF by first reviewing the current therapies and then the new technologies to identify drug targets, the development of recombinant protein, monoclonal and gene therapy–based treatments for IPF, and the use of high-throughput screening with libraries of potential drugs.

II. Pathogenesis of IPF

As the name suggests, the etiology of IPF is unknown. There is a widely held, but unproven concept, that the disease develops in susceptible individuals following a lung insult of some sort, such as a respiratory viral infection (7). Whatever the trigger, it is clear that the early events are persistent inflammation of the lower respiratory tract, and that this inflammation plays a central role in the pathogenesis of the disease (7,12). The inflammatory cells in the alveolar space and alveolar interstitium are dominated by activated alveolar macrophages with smaller numbers of lymphocytes, neutrophils, and sometimes eosinophils. These cells release a variety of mediators that injure the alveoli and to a lesser extent the small airways (12). There is increasing evidence that IPF is also associated with dysfunction of lung repair mechanisms, particularly processes involved in the production and metabolism of connective tissue components (9–11).

A. What Is Known About Inherited Susceptibility to IPF?

In addition to the common sporadic form of IPF, there is a rare familial form that is identical but inherited as an autosomal dominant trait with incomplete penetrance (13–16). The existence of a familial form of the disease supports the concept that genetic differences are linked to the susceptibility to the sporadic form of IPF; for example, by modifying the host response to lung injury. Other arguments favoring a genetic basis for the sporadic form of IPF include the observation that there is marked variation in the response of humans to agents that induce pulmonary fibrosis even when the levels of exposure to such agents are similar. For example, only a small portion of individuals who receive the antineoplastic drug bleomycin or the antiarrythmic drug amidarone develop pulmonary fibrosis (17). In addition, different strains of mice have different susceptibilities to the development of interstitial lung disease caused by bleomycin or other agents (18–23), and knockout/transgenic mice that either do not express or that overexpress cytokines thought to be involved in fibrogenesis can either develop or be protected against lung fibrosis (24,25).

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Familial IPF

It has been known for some time that there are familial forms of IPF, with descriptions of chronic lung fibrosis, likely IPF, in one family with two affected individuals described in 1907 (3) and in twin sisters in 1950 (26). Since then, there have been several reports of IPF in twins, including that of Swaye et al. (27), Javaheri et al. (28), and Thomas (29). Familial IPF is rare, representing <3% of the diagnosed cases of IPF (13) and <100 family cases being reported in the literature (Table 1).

The pattern of inheritance of familial IPF is most likely autosomal dominant with variable penetrance (14–16). Complicating the picture, there may be different forms of familial IPF. For example, the age of onset seems to be an important factor. Familial IPF occurring in infancy has the most rapid progression and is the most responsive to immunosuppressive therapy, but is fatal for those who do not respond to this treatment (30). In contrast, the adult-onset form of familial IPF has a relatively less rapid progression, and it is not clear whether the early-onset and the adult-onset forms of the disease are distinct entities (13).

Supporting the concept that genetic factors relating to inflammation play a role in familial IPF, lung inflammation has been observed in unaffected family members of familial IPF patients (31). Seventeen individuals belonging to two generations of three families afflicted with familial IPF were evaluated for the presence of alveolar inflammation over a period of several years using gallium 67 scanning and bronchoalveolar lavage. Eight nonaffected individuals, all nonsmokers, showed evidence of alveolar inflammation in the lavage fluid, including increased numbers of neutrophils and activated macrophages that released neutrophil chemoattractants and growth factors for fibroblasts. Of these eight individuals, four had a positive gallium 67 scan, which is consistent with the presence of inflammation. Over a period of 3 years, two individuals who were initially negative for the presence of neutrophils in the lavage fluid developed an increase in neutrophil count on repeat assessment (31). These observations have not been extended to other families, and more studies are required to determine whether this represents a universal finding in families of individuals with the common sporadic form of IPF. It is also not clear whether the observation of neutrophils or activated macrophages in the lavage fluid is an early marker to predict progression of disease.

One early genetic study in familial IPF demonstrated a linkage to the immunoglobulin haplotype Gm1 (now known as IGHG3) (32). Although all the affected siblings in a family shared this marker, there was no linkage with human leukocyte antigen (HLA) haplotypes, as previously suggested by Evans (33) in a study of 20 cases of sporadic IPF. A linkage of familial IPF to chromosome 14 has been described (34). There is both IPF and

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Table 1 Reported Cases of Familial IPF

Reference	Affected individuals	Country	Proposed genetic loci/mode of transmission
Sandoz, 1907 (3)	1 family, 2 members	Germany	None
Peabody et al., 1950 (26)	Twins	USA	None
Hughes et al., 1964 (114)	1 family, 3 members	UK	None
Bonanni et al., 1965 (115)	Same family as Peabodyet al., 1950	USA	Autosomal dominance
Koch et al., 1965 (116)	1 family, 3 members	Canada	None
Adelman et al., 1966 (117)	Father and 5 children	Canada	None
Young et al., 1966 (118)	Father and daughter	Canada	None
Swaye et al., 1969 (27)	8 family members	USA	None
Rezek and Talbert, 1962 (119)	1 family	Germany	None
Solliday et al., 1973 (16)	1 family, 5 members, including twins	USA	None
Nezelof et al., 1974 (120)	2 siblings	France	None
Hoste et al., 1979 (121)	1 family, 2 members	Denmark	None
Javaheri et al., 1980 (28)	Monozygotic twins	USA	None
Beaumont et a1., 1981 (15)	1 family, 5 members	Netherlands	Autosomal dominance
Murphy and Sullivan, 1981(122)	1 family, 2 members	Ireland	None
Manigrand et al., 1982 (123)	2 siblings	France	None
McDonnell et al., 1982 (124)	2 siblings	Ireland	None
Tal et al., 1985 (30)	3 siblings	Israel	None
Auwerx et al., 1985 (125)	1 family	Belgium	Associated with hypocalciuric hypercalcemia
Sansonetti et al., 1985 (126)	1 family, 7 members	France	None

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Barzó, (127)	2 sisters	Hungary	None
Bitterman et a1., 1986 (31)	3 families	USA	None
Ostimelli et al., 1986 (128)	2 families	France	None
Barzó, 1985 (127)	2 sisters	Hungary	None
Musk et al., 1986 (32)	1 family, 12 members	Australia	Immunoglogulin haplotype -Gm
Farrell et al., 1986 (129)	1 family, 3 members	USA	None
Tran-Van-Nhieu et al., 1988 (130)	1 family	France	Associated with hypocalciuric hypercalcemia
Stinson and Tomkin, 1992 (131)	1 family, 3 members	Ireland	None
Thomas et al., 1996 (29)	Monozygotic twin sisters	Germany	Single dominant gene
Marshall et al., 2000 (132)	25 families	UK	None
Lane et al., 2001 (34)	2 male cousins	USA	Chromosome 14, linked to alpha 1 antitrypsin deficiency
Marney et al., 2001 (14)	Extension of the family as Peabody et al. (1950)	USA	Single dominant gene
Wahidi et al., 2002 (133)	38 families	USA	None
Thomas et al., 2002 (41)	11 individuals	USA	4 exhibited loss of heterozygosity by at least one marker

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alpha$_1$-antitrypsin deficiency in different members of the family in this study, with two members being affected by both IPF and the ZZ genotype of alpha$_1$-antitrypsin deficiency (34). This observation was consistent with a study of individuals with sporadic IPF that suggested a linkage to susceptibility to IPF of specific alpha$_1$-antitrypsin alleles, located on chromosome 14q32 (35). Interestingly, IGHG3, previously associated with an IPF family (32), is also located on chromosome 14q23, suggesting the possibility of IPF candidate genes in that locus.

One study identified a splicing mutation in the gene for surfactant protein C (SP-C) (36) in a mother and her infant, both of whom were affected by interstitial lung disease. Although these cases are clearly not pathological equivalents of IPF, they are interesting, as they pertain to evaluating genetic susceptibility to similar interstitial lung disorders. The mutation caused a substitution (G to A) at base 1728 at the junction of exon 4 and intron 4, resulting in exon 4 skipping, and decreased or absence of SP-C in the lungs. A subsequent study of infants with chronic interstitial lung diseases of unknown etiology identified de novo mutations, not present in the parents, in one allele of SP-C in 11 out of 34 cases investigated. Included in this group were two individuals with a G to T nucleotide substitution at the same base in the exon 4–intron 4 junction, resulting in exon 4 skipping and decreased SP-C size, as well as a decrease in the levels of normal-sized SP-C (37). Missense mutations in the codons of highly conserved residues in SP-C and a frameshift mutation were found in 10 other infants (38). These mutations were not found in 100 control chromosomes, suggesting that they do not represent frequent polymorphisms in the general population. These studies suggest that mutations in the SP-C gene can cause both familial and sporadic forms of interstitial lung disease.

Given the higher risk of lung cancer in patients with IPF compared to control subjects (39), and that lung cancer is frequently associated with microsatellite instability (MSI) and loss of heterozygosity (LOH), one study analyzing the incidence of MSI and LOH at 10 highly polymorphic microsatellite markers in 26 IPF patients without malignancies found that 50% of these patients exhibited genetic alterations, which was either LOH or MSI (40). The incidence of MSI and LOH at six highly polymorphic loci was also investigated in 11 IPF patients, and 36% of these patients exhibited LOH by at least one marker, with all of these markers being adjacent to putative tumor suppressor genes (41). This allelic imbalance may play a role in the pathogenesis of IPF and/or be helpful in the identification of candidate loci for genes conferring susceptibility to IPF.

Genetic Factors Linked to Susceptibility to the Sporadic Form of IPF

A number of studies have focused on genetic links to sporadic IPF, but the total number of studies is limited by the fact that this is an uncommon disease

Table 2 Genotypes/Polymorphisms Associated with Sporadic IPF

Gene	Genotype/Polymorphism	Reference
TGF-β	+915, codon 25/Arg	Awad et al., 1998 (66)
TNF-α	−308, G/A	Whyte et al., 2000 (51)
IL-1rα	+2018, C/T	Whyte et al., 2000 (51)
ACE	287-bp deletion in exon 16	Morrison et al., 2001 (61)
IL-6	Intron 4 G	Pantelidis et al., 2001 (44)
TNF-RII	1690, C	Pantelidis et al., 2001 (44)
IL-1α	−889, T	du Bois, 2002 (134)

TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; IL-1rα, interleukin-1 receptor antagonist; ACE, angiotensin-converting enzyme; IL-6, interleukin-6; TNF-RII, tumor necrosis factor receptor-type II; IL-1α, interleukin-1α.

with an annual incidence of 7 cases per 100,000 for women and 10 per 100,000 for men (42). Most studies have focused on the analysis of genotypes or polymorphisms in candidate pathogenic genes, such as genes for proinflammatory cytokines or their receptors (Table 2).

The early studies of the genetics of the sporadic form of IPF were centered on highly polymorphic genetic loci known to be involved in the inflammatory response, such as the HLA system. The linkage with given HLA types is conflicting, suggesting that HLA is unlikely to be the primary genetic determinant of susceptibility to this disorder. The associations between IPF and HLA types that have been described include HLA B12 (6,33), and HLA B8, which have been associated with females with IPF, with an age of onset $<$50 years old (43). A correlation between the immunoglobulin haplotype IGHG3 in 12 patients from one family with IPF was found, but there was no correlation between any HLA haplotype and IPF observed (32).

One study focused on known single nucleotide polymorphisms (SNPs) in four candidate genes in IPF: two genes in the tumor necrosis factor (TNF) cluster (TNF-α, lymphotoxin-α [T = α]), TNF receptor II (TNF-RII), and interleukin-6 (IL-6) (44). TNF-α was chosen since elevated expression of this gene has been observed in alveolar and interstitial macrophages and type II epithelial cells in individuals with IPF (45,46). The possible importance of the TNF-α gene in the susceptibility to IPF is supported by studies in animal models of lung fibrosis. Overexpression of TNF-α has been identified as a mediator of fibrosis in animal models of progressive pulmonary fibrosis (47), and knockout mice doubly deficient in TNF-α and LT-α are resistant to bleomycin-induced lung fibrosis (48). Elevated levels of IL-6 have been found in the lavage fluid of individuals with IPF (49), and in concert with TNF-α, IL-6 promotes fibrogenesis in bleomycin-induced lung injury (50). In an analysis of

these four genes in the TNF-α cluster in sporadic cases of IPF, Pantelidis et al. (44) observed no differences in genotype or allele frequencies for any of the four genes examined individually, but there was increased cosegregation of the IL-6 intron 4G allele with the TNF-RII 1690C allele in spite of the fact that these genes are located in different chromosomes. In addition, the presence of the IL-6 intron 4G allele was the only genotype found to be associated with a greater reduction in diffusing capacity adjusted for the duration of dyspnea as a marker of rapid disease progression.

Two polymorphisms previously associated with inflammatory diseases, the TNF-α promoter polymorphism at nucleotide position -308 and the IL-1 receptor antagonist (IL-1Ra) gene at position +2018, have been evaluated for their potential association with IPF (51). In two case cohorts, one Italian and one English, homozygotes and heterozygotes carrying the TNF-α -308 allele or the IL-1Ra +2018 allele were observed to be associated with an increased risk of development of IPF (51). The TNF-α -308 allele has been previously associated with asthma (52) and chronic bronchitis (53), and it has been shown to lead to increased transcriptional activity of the TNF-α promoter (54). The significance of the IL-1Ra +2018 in terms of either decreased or increased production of IL-1Ra is not known, but this allele has been associated with coronary artery disease, another disorder with an inflammatory component (55).

The association of IPF with polymorphisms in the three genes of the interleukin-1 cluster, IL-lα, IL-1β, and IL-1Ra has been assessed in a west Slavonic cohort in the Czech Republic (56). There was no association found between IPF and any of the polymorphisms investigated, but the IL-1α -889 polymorphism was found to be associated with sarcoidosis (56). Although the IL-1Ra polymorphism investigated in this study was the intron 2 variable number tandem repeat (VNTR) polymorphism and not the IL-1Ra +2018 that was reported to be associated with IPF (51), the IL-1Ra intron 2 VNTR polymorphism has been linked to the IL-1Ra +2018 in some populations (57).

There has been interest in the possible association of mutations in the angiotensin-converting enzyme (ACE) gene and susceptibility to IPF based on the observation that angiotensin II (AII) acts as a mitogen for lung fibroblasts by activating the angiotensin type I receptor pathway (58), and that elevated levels of ACE resulting from chronic hypoxia lead to increased angiotensin II levels (59). Elevated levels of ACE have been reported in IPF (60), and the association of an increased frequency of the ACE D allele has been observed in patients with IPF (61). The D allele corresponds to an ACE polymorphism at exon 16 that involves the deletion of a 287–base pair fragment. The D/D genotype is associated with higher levels of ACE and angiotensin II, whereas individuals heterozygous for the D allele show intermediate levels of both enzymes (62). In one study, the incidence of the

ACE D allele in 24 white American with IPF was 69% compared to 50–60% in the overall white American population (61).

Transforming growth factor-β (TGF-β) is a prosclerotic cytokine that is an important mediator in the pathogenesis of tissue fibrosis. The three mammalian TGF-β isoforms, TGF-β1, -2, and -3 are members of a superfamily that includes more than 40 members, all of which signal through heterodimeric transmembrane serine/threonine kinases (63,64). Polymorphisms in the TGF-β1 gene have been associated with lung allograft fibrosis in lung transplant patients (65). There are two polymorphisms in the sequence encoding the leader peptide located at codons 10 and 25. The codon 25 arginine allele is associated with higher levels of TGF-β production in activated cells in vitro (66). Homozygosity for arginine at codon 25 correlates with fibrotic lung pathology before lung transplantation as well as with the development of fibrosis in the transplanted lung, and in combination with the arginine allele for codon 10, serves as a marker for posttransplant prognosis (65).

III. Strategies that Can Be Used to Understand the Pathogenesis of IPF at a Genetic Level

Although the progress in understanding the genetic basis of IPF has been interesting, it has been slow, suggesting that single-candidate gene strategies based on current concepts of pathogenesis are unlikely to identify the genes responsible for susceptibility to IPF. In this section, we will review the new technologies that, in the context of the data regarding the sequence of the human and mouse genomes, should enable major advances in the identification of genes linked to the susceptibility of both the familial and sporadic forms of IPF and a further understanding of the pathogenesis of these disorders.

A. Animal Models Based on Genetics: Advantages and Limitations

Animal models of human genetic diseases are potentially useful tools in the identification of the genes and molecular and cellular mechanisms underlying the disease, as well as for evaluating new therapeutic approaches. Animal models of human mendelian genetic disorders are easier to generate, because such diseases involve only one gene, and knockout mice with a null phenotype or transgenic mice overexpressing the gene can be constructed once the gene is identified. However, even in the case of mendelian diseases, it may prove difficult to generate a mouse model reproducing exactly the phenotypic and clinical manifestations of the human disease. For example, mouse models of cystic fibrosis, where mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) genes have been unequivocally identified as causing the phenotypic manifestations of this disease, have not been successful

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in reproducing the pulmonary phenotype of the human disease (67). In the case of complex polygenic diseases in which many genes, each exerting a small effect, may be involved in the underlying pathogenesis, the generation of animal models is more difficult. The identification of the genes is far more complex and labor intensive, and even if all the genes were identified, the creation of multiple knockout or transgenic animals harboring many different genes is a daunting challenge.

There is no animal model for IPF, but there are several animal models in which genetics have been demonstrated to play a role in susceptibility to developing pulmonary fibrosis. An early model of interstitial lung disease was the *moth-eaten* mouse, which is based on the C56B1/6J strain (68). These animals inherit a single mutation in the *moth-eaten* gene, which causes death in homozygotes by respiratory insufficiency at 8–10 weeks (68). In these severely immunodeficient mice, spontaneous pulmonary disease is characterized by excessive macrophage accumulation in the lung accompanied by fibrosis. The development of lung injury in viable *moth-eaten* mice correlates with increased levels of TNF-α in the serum and lung, and the alveolar macrophages isolated from *moth-eaten* mice produce significantly greater amounts of TNF-α when stimulated with lipopolysaccharide compared with alveolar macrophages from control animals (69). Interestingly, administration of anti–TNF-α antibody to *moth-eaten* bone marrow recipient mice decreases the severity of acute lung injury.

The susceptibility to experimental interstitial lung disease (i.e., bleomycin-induced or secondary to inhalation of organic particles) in different strains of mice has been correlated to both immune-related (H-2 type) and non–immune-related genes (18). Differential susceptibility to asbestos-induced fibrosis has been observed in two different strains of mice, where the 129 strain is resistant, but C56B1/6 mice are highly susceptible. Intratracheal administration of the profibrotic mediator TGF-β via an adenoviral vector produces lung disease in both strains of mice, but there is a delay in the fibroproliferative response in the 129 strain, suggesting that a cluster of genes responding to TGF-β may be mediating the development of lung fibrosis (23). Based on the observation that administration of bleomycin has been shown to induce TGF-β overexpression in the lung, inhibitors of the TGF-β pathway have been tested in rodent models of lung injury. For example, the antifibrotic drug pirfenidone inhibits the overexpression of TGF-β at the transcriptional level in a hamster model of bleomycin-induced lung injury (70). In bleomycin-induced lung fibrosis in mice, IL-7, which downregulates the production of TGF-β in murine macrophages, inhibits both TGF-β production and TGF-β signaling by increasing the levels of the inhibitory regulator Smad7 (24). These results are consistent with the prevention of bleomycin-induced lung fibrosis in mice treated with an adenoviral vector carrying the *Smad7* gene (25).

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Many different transgenic and knockout animal models have been used in conjunction with bleomycin-induced lung injury to investigate the molecular pathways involved in pulmonary fibrosis. Mice overexpressing a TNF-α transgene in the lung develop fibrosing alveolitis that has some of the features of human IPF (47). Mice overexpressing endothelin-1 show an increase in perivascular extracellular matrix deposition and a chronic lymphocytic inflammatory infiltrate in the lung (71). Mice overexpressing the extracellular superoxide dismutase gene under the control of the lung-specific SP-C promoter are resistant to bleomycin-induced lung injury (72).

Knockout mice have also been proven to be useful to investigate the role of genes in bleomycin-induced pulmonary fibrosis. Transgenic mice with increased fibrinolytic activity due to a targeted deletion of the plasminogen activator inhibitor-l gene are protected from fibrosis after bleomycin-induced lung injury (73). Other genes that have been implicated in mediating fibrosis using knockout mice are those coding for the adhesion molecules L-selectin and intercellular adhesion molecule-1 (ICAM-1). These adhesion proteins are believed to mediate fibrosis via their role in facilitating the accumulation of leukocytes. Mice lacking both of these genes do not show any collagen deposition in the lung in response to bleomycin, and decreased expression of proinflammatory cytokines and TGF-β parallel the inhibition of collagen deposition in the lung (74). A double knockout mouse model lacking both TNF receptors (p55, p75) has been shown to be resistant to bleomycin-induced lung injury in spite of the fact that TNF-α expression is increased in these mice (75). IL-12 knockout mice show significantly decreased pulmonary mononuclear cell inflammation following bleomycin treatment compared to control wild-type mice (76).

Taken together, the study of these animal models has contributed to the list of genes that may play a role in the susceptibility to pulmonary fibrosis. However, these animal models do not necessarily reflect human IPF, and thus it is unclear whether any of these candidate genes have relevance to the human disease. In this context, although animal models of pulmonary fibrosis should continue to be investigated with the hope of developing insights into genetic susceptibility to IPF, this line of investigation has not been fruitful to date.

B. Assessment of Gene Expression in Individuals with IPF

Gene expression profiling of pathological states can shed light on the underlying molecular mechanisms of disease. Classically, gene expression studies have been based on methods of mRNA analysis that could assess the levels of expression of only a few genes at a time; for example, using in situ hybridization, Northern analysis, reverse transcript polymers chain reaction (RT-PCR), or real-time PCR. Using these methods, several genes have been

proposed to be of importance in various aspects of the fibrotic process, such as fibronectin and collagen, growth factors, cytokines, and protease inhibitors. Using in situ hybridization, 82% of the alveolar macrophages recovered from the lungs of patients IPF have been shown to express fibronectin mRNA compared with only 66% of the alveolar macrophages in normal individuals (77). The expression of collagen VI was shown to be elevated in lung fibrosis (78). Specific growth factors have been shown to be elevated in IPF. Alveolar macrophages recovered from IPF patients release elevated levels of platelet-derived growth factor (PDGF) (79), and this correlated with an upregulation of the rate of transcription of both PDGF-A and PDGF-B (*c-sis*) in these cells (80). Other studies have also shown upregulation of PDGF-B in alveolar macrophages of IPF patients (81,82). Another growth factor, connective tissue growth factor (CTGF), a growth and chemotactic factor for fibroblasts that is transcriptionally activated by TGF-β, is increased in the lung tissue of patients with IPF compared to normal lungs (83,84). RT-PCR analysis shows that CTGF mRNA is expressed at a higher level in fibrotic lungs than in normal lungs, with expression of CTGF being confined predominantly to proliferating type II alveolar epithelial cells and activated fibroblasts, suggesting that these cells may play a critical role in the fibrotic process (83). An increase in expression of the genes coding for αl (I) collagen, TGF-β, gelatinase B, and tissue inhibitor of metalloproteinase (TIMP)-1, -2, -3, and -4 has been observed in vitro in fibroblasts derived from IPF patients (85). Given the importance of inflammatory processes in IPF, cytokine gene expression in IPF individuals has also been evaluated by mRNA analysis. IL-8, IL-1β, IL-1 receptor antagonist (IL-1Ra), IL-10, and TNF-α have all been found to be elevated in alveolar macrophages of IPF patients (86–89). The expression level of RANTES, a member of the C-C chemokines, was observed to be elevated in cells recovered by bronchoalveolar lavage of individuals with IPF, suggesting that RANTES may be one of the chemokines that are involved in the accumulation of inflammatory cells in the IPF lung (90).

The advent of the genomics era, with most of the human and mouse genomes fully sequenced, has given rise to expression microarrays, a powerful new tool that allows the simultaneous assessment of the expression levels of thousands of genes with a minimal amount of biological material that is far less than that required for any of other method. There are basically two widely used methods for microarray analysis: one that employs a series of short oligonucleotide probes directly synthesized onto slides by masked photolithography (e.g., Affymetrix GeneChips, Santa Clara, CA) and the other that utilizes a series of individual cDNA probes on slides or filters that can be custom-made or available commercially (91). The first method has the advantage of being easy to use and commercially available in a format that provides broad coverage of human or murine genomes, but it has the disadvantage of high cost. The second method can be done less expensively, but usually covers a

more limited number of genes, and it lacks specificity of long cDNAs for distinguishing closely related family members and splice variants.

Microarray technology enables the evaluation of the expression profile of hundreds to thousands of genes, up to practically the entire genome, in patient samples from which only a few micrograms of high-quality RNA can be obtained. Consequently, there is great enthusiasm in applying these techniques to elucidate pathways involved in the pathogenesis of many diseases, as well as to discover new potential targets for therapeutic intervention in the form of drug targets or of targets for genetic therapies. However, microarray data analysis presents several challenges related to statistical analysis, sample number, number of replicates, and costs. In addition to intrinsic problems of methodology, microarray analysis can be used only to evaluate relative mRNA abundance, and therefore the issue of biological sample purity is critical.

Microarray expression analysis has already shed some light on potential genes and pathways involved in lung fibrosis, using the analysis of lung gene expression in bleomycin-induced lung injury in two strains of susceptible mice, 129 and C57BL/6. These data were compared with 129 mice carrying a null mutation of the epithelial-specific integrin $\beta 6$ subunit, mice known to develop inflammation, but not pulmonary fibrosis, in response to bleomycin (92). Several clusters of genes characterizing the response to bleomycin were identified, many of which were already known to regulate inflammation and fibrosis. Many genes known to be regulated by TGF-β were upregulated in the mice susceptible to fibrosis, providing further support for the central role of this cytokine in the pathogenesis of pulmonary fibrosis.

Pilot studies of IPF using oligonucleotide microarray technology have been carried out on samples from patients with histologically proven IPF using oligonucleotide microarrays (93,94). One limitation of the interpretation of these studies is that the specimens used for this analysis were whole tissue samples (not pure populations of cells) obtained by thoracotomy or thoracoscopy (94). The gene expression pattern in these samples was compared to normal samples obtained from noncancerous tissue of lung cancer patients or from commercially available total RNA from healthy human lung. As expected, in the fibrotic lungs there was an increase in the expression of genes encoding proteins associated with extracellular matrix formation and degradation as well as proteins expressed in smooth muscle. A coordinated induction of metalloprotease genes was also observed. Matrilysin, also known as matrix metalloproteinase-7, was highly increased in the fibrotic specimens. This metalloprotease had not been previously associated with pulmonary fibrosis, and its induction in fibrotic lung was confirmed by immunohistochemistry. Consistent with these observations, matrilysin knockout mice were dramatically protected from bleomycin-induced pulmonary fibrosis.

These preliminary results illustrate the potential usefulness of global gene expression analysis of human tissue samples to identify novel molecular pathways involved in IPF. However, future studies utilizing pure populations of specific groups of cells will be necessary in order to make major inroads using this technology to understand the cellular and molecular pathways related to the pathogenesis of IPF. Specific cell types for microarray analysis of human IPF samples can be obtained by fiberoptic bronchoscopy, including alveolar macrophages, a major source of mediators that stress lung parenchymal cells in the fibrotic disorders, and the airway epithelium which is the cellular origin of lung carcinoma, which affects 10–40% of individuals with IPF (39). Lung biopsy via video-assisted thoracoscopy, together with laser capture dissection of specific foci in the biopsy, can also provide a cell source of specific groups of cells within the lung parenchyma that play a central role in the pathogenesis of the fibrotic state, and that can be analyzed by microarray methods.

IV. Strategies to Assess Genetic Susceptibility to IPF

Although the rare familial form of IPF may be inherited as a single dominant trait with reduced penetrance (see Table 1), the most common form of IPF is a sporadic complex disease likely resulting from the interactions between environmental factors and the genetic background of the individual. This type of complex trait disorder is likely to involve an interplay of many low-penetrance genes and complex gene-environment interactions. Although mapping single genes for mendelian genetic disorders has been highly successful, the search for genes underlying complex trait, polygenic diseases has often been arduous and disappointing, because it involves finding multiple interacting genes, each with a rather small effect. In the case of sporadic IPF, most studies have focused on analyzing the genotype, in particular, single nucleotide polymorphisms (SNPs) of genes encoding inflammatory or profibrotic mediators that have been postulated to play a role in the etiology of the disease.

In the current genetic era, the search for disease susceptibility genes can be carried out by evaluating tens of thousands of SNP markers at a time. Linkage analyses and association mapping are the current major approaches for identifying candidate genes for susceptibility to disease. Both are powerful methods that have yielded important results, but each approach has limitations. Linkage analysis requires data from multiple family members, and it is not always possible to have access to biological material from several family members encompassing two or more generations. Association mapping is a common approach that is based on testing for differences in SNP allele frequencies between affected and unaffected individuals (case-control studies).

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The SNPs with significant association to disease are then assumed to be within or close to disease susceptibility genes, and the data are used to map disease genes of unknown location.

In spite of the promise of the current genetic era, most current approaches to IPF are based on the evaluation of a single SNP marker at a time. In the case of complex polygenic diseases such as IPF, evaluating one SNP at a time may involve focusing on a gene with a marginal effect on the disease, decreasing the chances of identifying bona fide candidate genes. In addition, each time a single SNP is tested for association with a given disease, it leads to a type I error, meaning a locus-specific probability of a false-positive result (95,96). Type I errors can be easily inflated when large numbers of SNPs are tested simultaneously but treated independently (96), and for genomewide linkage analysis, appropriate measures have been developed to keep the type I errors to a minimum (97). However, for genomewide association analysis, no general method exists, because the markers do not follow a known mendelian pattern. Moreover, the marker-by-marker approach, even if carried out on a genomewide scale, ignores the multigenic nature of complex trait diseases, because it does not take into account possible interactions between susceptibility genes. Given the nature of complex trait diseases involving many low-penetrance genes, it would be more appropriate to search for sets of marker loci in different genes and to analyze these markers jointly rather than testing each marker in isolation. Several statistical methods have been recently proposed to carry out the analysis in a joint manner while minimizing the number of false-positive results (95–97). In the case of sporadic IPF, the fact that it is a rare disease affecting only 7–10 individuals/100,000 annually (42) makes both the linkage and the association mapping approaches more difficult. However, we should expect that, in the next decade, the use of genomic databases, including public SNP databases, will facilitate the search for genes underlying complex trait diseases such as IPF.

In addition to association mapping or linkage studies involving the entire human genome, tasks that are extremely labor intensive and complicated because of the huge number of genes and SNPs that need to be analyzed, other tools that evolved from the boom in genomics can be applied to the preselection of prospective candidate genes. In particular, microarray analysis can provide a critical, biologically relevant tool for identifying large numbers of genes that are potentially relevant to disease susceptibility. Equally important is the fact that screening by microarray analysis provides a biologically based method for screening out large numbers of irrelevant genes, resulting in a more robust analysis. In the case of genetic susceptibility to IPF, inherited allelic differences in key genes involved in the pathogenesis of the disease may result in both substantial or small differences in the expression levels of multiple genes at many different loci. The particular combination of these effects, which is presumed to vary from individual to individual, may

result in a predisposition to IPF. Microarray analysis of inherent gene expression differences in the alveolar macrophages or airway epithelium of IPF patients compared to healthy airway epithelium of disease-free matched controls may help to focus the analysis by allowing the preselection of a limited number of prospective candidate genes to be genotyped in addition to genes that have been identified as being potential candidates by more traditional cell biology or gene expression studies.

Although microarray analysis of gene expression differences focuses on gene expression patterns, the ultimate result of these patterns of expression is a combination of proteins, or protein profile, that are expressed by a cell or tissue. Proteomics is the systemwide study of proteins in a given biological sample (98). This methodology was born from the merging of two-dimensional gel electrophoresis and mass spectrometry and has, as its scope, the identification of protein signature profiles. One of the applications of proteomics in human disease is discovery-oriented proteomics, with an emphasis on the identification of proteins with altered abundance relative to a reference sample. An integrated proteomics analysis will eventually yield information regarding the abundance, modifications, activity, localization, and interaction of all the proteins present in a given sample. This strategy could be readily applied to IPF as a new approach to identify targets for therapeutic intervention, as well as to aid in the identification of key proteins and alterations in the pattern of protein expression that may lead to fibrosis. One approach that may be particularly useful would be the unbiased analysis of proteins in the bronchoalveolar lavage fluid or in isolated alveolar macrophages of individuals with IPF compared to healthy individuals. This type of experiment has the potential to result in the identification of previously unidentified proteins and known proteins with an altered expression pattern in IPF, as well as to aid in the determination of how these proteins interact to produce the IPF phenotype.

V. Genetic-Based Therapies for Treatment of IPF

A. Current Therapies for IPF

There are no clinical trials demonstrating that any currently available medical therapy improves the survival or quality of life for patients with IPF (99). Conventional treatment options for this disease include corticosteroids, either alone or, as recent American Thoracic Society (ATS) guidelines suggest (99), corticosteroids in combination with a cytotoxic agent, such as cyclophosphamide or azathioprine.

Corticosteroids are considered to be the mainstay of therapy for IPF, with the rationale that this class of drugs may suppress chronic alveolitis, hence, either stabilizing or preventing disease progression (8,12,100). There are

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no prospective, randomized, double-blind, placebo-controlled trials evaluating the efficacy of corticosteroids in the treatment of IPF (101). Ten to 30% of IPF patients will show clinical improvement with corticosteroid therapy, although cures are rare, and most responses to therapy are either partial or transient (99). Most clinicians initiate therapy with high-dose corticosteroids, ranging from 40–100 mg of prednisone daily, for 2–4 months, with gradual taper to a lower maintenance dose thereafter. There have been no studies comparing different dosages or length of corticosteroid treatment in appropriately matched or randomized patients, nor is it clear what is the ideal duration of treatment. The drug dose is sometimes increased with relapses, and high-dose intravenous ''pulse'' methylprednisolone (1–2 g once weekly or biweekly) has been used in patients with aggressive or severe disease in attempts to suppress the neutrophilic alveolitis (102,103). Corticosteroid therapy, although usually well tolerated by patients, has many common, potentially disabling, and sometimes life-threatening side effects, including peptic ulcer disease, cataracts, glaucoma, hypertension, and a variety of endocrine and metabolic consequences (i.e., increased appetite and weight gain, salt and water retention, redistribution of body fat with resultant truncal obesity, moon facies, menstrual irregularities, impotence, hyperglycemia, hypokalemia, metabolic alkalosis, and secondary adrenal insufficiency). Effects on the musculoskeletal system may include osteoporosis, vertebral compression fractures, avascular necrosis of femoral and humeral heads, and myopathy. Psychological effects including euphoria, depression, and psychosis may also occur. Immunosuppression predisposing patients to opportunistic infections may result in those who receive >15 mg of prednisone daily for over 3 weeks.

The use of cytotoxic agents (e.g., cyclophosphamide or azathioprine) is usually reserved for those patients with IPF who are classified as being steroid nonresponders or those who are at increased risk for complications of corticosteroids (e.g., age >70 years, poorly controlled diabetes mellitus or hypertension, severe osteoporosis or peptic ulcer disease) (99). Favorable responses have been noted in 15–50% of those with IPF treated with one of the above agents in small trials (99). Anecdotal favorable responses to azathioprine therapy or azathioprine plus corticosteroids have been described (104,105).

There are no studies comparing cyclophosphamide to other cytotoxic agents in the treatment of IPF, and there are no convincing data that the use of cyclophosphamide is either superior to or worse than corticosteroids in treating IPF. One small randomized controlled trial comparing prednisolone alone compared to cyclophosphamide/low-dose prednisolone combination therapy suggested a possible survival advantage for the group of IPF patients treated with combination therapy (106). When compared with prednisone, cyclophosphamide has been shown to reduce the neutrophil levels in lavage fluid (107).

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The major limitations of any of the cytotoxic drugs are the potentially dangerous side effects, which include myelosuppression with cytopenias, including leukopenia, and their resultant complications (azathioprine, cyclophosphamide); gastrointestinal symptoms, such as nausea and vomiting (azathioprine, cyclophosphamide); hepatitis (azathioprine); development of hematological malignancy (cyclophosphamide); hemorrhagic cystitis (cyclophosphamide); as well as interstitial pneumonitis (azathioprine, cyclophosphamide) and diffuse alveolar damage (azathioprine) (100). As a result, both the complexity and cost of care for those individuals receiving cytotoxic therapy with these agents is increased given the need for routine monitoring of blood cell counts, liver enzymes, and urine.

Other potential alternative treatments for IPF based on anecdotal use, but for which there are no data confirming efficacy, include such agents as cyclosporin A, methotrexate, chlorambucil, colchicine, and D-penicillamine.

Given the therapeutic limitations and marginal benefits of existing drug therapies for IPF, newer approaches to treatment have focused on exploring the potential use of agents involved in collagen synthesis or fibrosis. Of these, the most interesting has been interferon-gamma (INF-γ), a cytokine that downregulates the expression of profibrotic growth factors, such as TGF-β and interstitial collagens, and which has been shown to decrease bleomycin-induced pulmonary fibrosis in mouse models (108). In a randomized, double-blind but small trial of 18 patients with IPF who had experienced clinical deterioration despite previous therapy with corticosteroids, other immunosuppressive agents, or a combination of both for at least 6 months, treatment with IFN-γ plus low doses of prednisolone yielded a significant improvement in lung function, oxygenation, and symptoms, whereas the conditions of those treated with corticosteroids alone deteriorated (109). This clinical improvement correlated with a concomitant decrease in the level of transcription of several fibrogenic growth factors, such as TGF-β1 and CTGF, after 6 months of treatment. A subsequent investigation of this study by an independent panel, however, revealed that a subgroup of subjects had actually presented with nonspecific interstitial pneumonitis (NSIP), which has a better overall prognosis than IPF. Preliminary results of a recent large U.S. and European multicenter double-blind, placebo-controlled, multicenter phase III trial of IFN-γb (Actimmune) for IPF reveal a statistically significant survival benefit in patients with mild to moderate IPF in those who received the study drug compared to those who received placebo control (110). However, there was no correlative improvement in lung function, so the interpretation of these results is unclear.

In a small prospective clinical trial treating IPF with pirfenidone, an agent known to inhibit fibroblast proliferation and collagen synthesis, as well as diminish bleomycin- and cyclophosphamide-induced pulmonary fibrosis in animal models, IPF patients experiencing deterioration in their

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condition on conventional therapy had stabilization of both symptomatology and respiratory function (70,111,112).

B. Recombinant Protein, Monoclonal Antibody, Gene Therapy, and High-Throughput Screening

The technologies of recombinant protein, monoclonal antibodies, gene therapy, and high-throughput screening are all potential strategies to develop new drug therapies for IPF. For all of these drugs, IPF is an inviting target, because the drug can be delivered by aerosol and thus directly to the site of disease. The major challenge is not the drug-related technology but rather identification of drug targets. The hope is that gene expression arrays, coupled with genomics, will identify specific phenotypes that can be evaluated in the context of new drugs.

In this regard, the application to IPF of novel technologies from the current genetics era, such as microarrays, SNP analysis, and proteomics, will result in the identification of genes and proteins involved in the etiology and pathogenesis of this disease, and thus will represent new targets for therapeutic intervention. Some of these potential targets may be amenable to drug intervention using inhibitory or stimulatory molecules that can block, reverse, or prevent the fibrosis. One approach to the discovery of these molecules is the high-throughput screening of random libraries of small molecular weight compounds or other agents (such as natural products, peptides, recombinant proteins, monoclonal antibodies, and gene therapy). This approach has been used, for example, to find activators of the cystic fibrosis transmembrane regulator (113). However, before such an approach can be applied to IPF, not only must bona fide targets be identified, but assays to assess a clear phenotype relevant to pathogenesis of the disease (e.g., the function of a profibrotic mediator protein or a gene with a protective effect) must be developed. These assays must be simple, robust, and quantitative in order to be amenable to the level of automation required for high-throughput screening. Once a suitable chemical structure is identified, combinatorial chemistry can be used to increase potency and decrease toxicity. When active compounds are identified, they can also be tested in animal models of lung fibrosis. Ultimately, these compounds will need to be tested in clinical trials for safety and efficacy.

Acknowledgments

We thank N. Mohamed and T. Virgin-Bryan for help in preparing this manuscript. These studies were supported, in part, by P01 HL59312 and the Will Rogers Memorial Fund, Los Angeles, CA.

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