Early Interstitial Lung Disease in Familial Pulmonary Fibrosis

Ivan O. Rosas[1], Ping Ren[1], Nilo A. Avila[2], Catherine K. Chow[2], Teri J. Franks[3], William D. Travis[3], J. Philip McCoy, Jr.[4], Rose M. May[1], Hai-Ping Wu[1], Dao M. Nguyen[5], Mauricio Arcos-Burgos[6], Sandra D. MacDonald[1], and Bernadette R. Gochuico[1]

[1]Pulmonary–Critical Care Medicine Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland; [2]Diagnostic Radiology Department, Clinical Center, National Institutes of Health, Bethesda, Maryland; [3]Department of Pulmonary and Mediastinal Pathology, Armed Forces Institute of Pathology, Washington, DC; [4]Flow Cytometry Core Facility, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland; [5]Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; and [6]Medical Genetics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland

Rationale: **Identification of early, asymptomatic interstitial lung disease (ILD) in populations at risk of developing idiopathic pulmonary fibrosis (IPF) may improve the understanding of the natural history of IPF.**

Objectives: **To determine clinical, radiographic, physiologic, and pathologic features of asymptomatic ILD in family members of patients with familial IPF.**

Methods: **One hundred sixty-four subjects from 18 kindreds affected with familial IPF were evaluated for ILD. Bronchoalveolar lavage fluid cells were analyzed using flow cytometry. Lung biopsies were performed in six subjects with asymptomatic ILD.**

Measurements and Main Results: **High-resolution computed tomography abnormalities suggesting ILD were identified in 31 (22%) of 143 asymptomatic subjects. Subjects with asymptomatic ILD were significantly younger than subjects with known familial IPF ($P < 0.001$) and significantly older than related subjects without lung disease ($P < 0.001$). A history of smoking was identified in 45% of subjects with asymptomatic ILD and in 67% of subjects with familial IPF; these percentages were significantly higher than that of related subjects without lung disease (23%) ($P = 0.02$ and $P < 0.001$, respectively). Percentages of activated $CD4^+$ lymphocytes were significantly higher in bronchoalveolar lavage fluid cells from subjects with asymptomatic ILD compared with related subjects without lung disease ($P < 0.001$). Lung biopsies performed in subjects with asymptomatic ILD revealed diverse histologic subtypes.**

Conclusions: **Asymptomatic ILD in individuals at risk of developing familial IPF can be identified using high-resolution computed tomography scan of the chest, especially in those with a history of smoking. Lung biopsies from individuals in this cohort with early asymptomatic lung disease demonstrate various histologic subtypes of ILD.**

Keywords: idiopathic pulmonary fibrosis; interstitial lung disease; high-resolution computed tomography; bronchoalveolar lavage; asymptomatic interstitial lung disease

Idiopathic pulmonary fibrosis (IPF), a chronic progressive interstitial lung disease (ILD), is largely unresponsive to medical treatment and is associated with an estimated survival of 20 to 50% at 5 years (1–4). Despite significant progress in the understanding of mechanisms involved in lung fibrosis, the natural history of the disease is poorly understood, in part because the majority of patients present with advanced disease stages. In familial IPF, a rare condition accounting for less that 5% of total IPF cases, an early stage of asymptomatic lung disease has been previously reported by Marshall and coworkers (5), Hodgson and colleagues (6), and Bitterman and colleagues (7). The authors showed that changes in alveolar protein and cellular content could be detected in asymptomatic first-degree relatives of subjects affected with familial IPF, and changes in the alveolar microenvironment were present in the absence of detectable abnormalities in chest radiographs or pulmonary function tests (PFTs). More recently, Steele and associates reported clinical features of the largest collection of families affected with interstitial pneumonia in the United States (8). The authors found that approximately 11% of subjects without symptoms of pulmonary fibrosis had high-resolution computed tomography (HRCT) scan findings consistent with probable or definite interstitial pneumonia. These studies strongly suggest that a period of asymptomatic disease precedes the clinical diagnosis of IPF.

In our study, we evaluated family members of patients affected with familial IPF to identify asymptomatic subjects with ILD. We characterized the clinical, radiographic, physiologic, and pathologic features in patients with findings suggestive of early asymptomatic ILD. Some of the results of these studies have been previously reported in the form of an abstract (9).

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Despite significant progress in understanding the mechanisms involved in lung fibrosis, the natural history of the disease is still not well understood. There is evidence that a period of asymptomatic disease may precede the clinical diagnosis of interstitial lung disease (ILD).

What This Study Adds to the Field

A study on familial interstitial pneumonia demonstrated that some subjects without symptoms of lung disease had high-resolution computed tomography scan findings of ILD. Lung biopsies performed in subjects with familial interstitial pneumonia within the same family showed heterogeneous types of ILD.

METHODS

Subjects

One hundred sixty-four subjects from 18 kindreds affected with familial IPF were evaluated at the Clinical Center, National Institutes of

(*Received in original form February 14, 2007; accepted in final form July 17, 2007*)

Supported by the Division of Intramural Research, NHLBI, National Institutes of Health.

Correspondence and requests for reprints should be addressed to Bernadette R. Gochuico, M.D., Pulmonary–Critical Care Medicine Branch, 10 Center Drive, MSC 1590, Bethesda, MD 20892-1590. E-mail: gochuicb@mail.nih.gov

Am J Respir Crit Care Med Vol 176. pp 698–705, 2007
Originally Published in Press as DOI: 10.1164/rccm.200702-254OC on July 19, 2007
Internet address: www.atsjournals.org

Health, in Bethesda, Maryland. Subjects, who were at least 18 years old, were recruited by advertisements, and were enrolled in protocols 99-H-0068 and/or 04-H-0211, which were approved by the National Heart, Lung, and Blood Institute Institutional Review Board. Subjects were eligible if they were diagnosed with familial IPF (*see below*); subjects without familial IPF were eligible if their family had two or more first-degree relatives with either an open-lung biopsy demonstrating usual interstitial pneumonia (UIP) and/or HRCT scan findings consistent with IPF as outlined by the American Thoracic Society/European Respiratory Society guidelines (10, 11). Written, informed consent was obtained from subjects.

Subject classification was based on three elements: history of chronic cough or dyspnea, self-reported history of ILD or pulmonary fibrosis, and HRCT scan findings consistent with ILD or pulmonary fibrosis. Using these criteria, subjects were divided into one of the following four groups:

1. Normal subjects: No history of chronic cough or dyspnea, no self-reported history of ILD or pulmonary fibrosis, and no HRCT scan findings consistent with ILD or pulmonary fibrosis. Subjects had at least two first-degree relatives with pulmonary fibrosis.
2. Subjects with nonspecific changes: No history of chronic cough or dyspnea, no self-reported history of ILD or pulmonary fibrosis, and HRCT scan findings that were probably artifacts. Subjects had at least two first-degree relatives with pulmonary fibrosis.
3. Subjects with asymptomatic ILD: No history of chronic cough or dyspnea, no self-reported history of ILD or pulmonary fibrosis, and HRCT scan findings consistent with early ILD. Subjects had at least two first-degree relatives with pulmonary fibrosis.
4. Subjects with familial IPF: History of chronic cough or dyspnea and/or self-reported history of ILD or pulmonary fibrosis and HRCT scan findings consistent with pulmonary fibrosis. Subjects had either a lung biopsy with UIP and/or HRCT scan findings typical of pulmonary fibrosis. Subjects had at least one first-degree relative with pulmonary fibrosis.

Pulmonary Function Testing

Measurements were made using standard equipment according to American Thoracic Society recommendations (SensorMedics, Yorba Linda, CA) (12). FEV_1, FVC, TLC, and diffusion capacity of carbon monoxide (D_{LCO}) were expressed as percentages of predicted values.

Cardiopulmonary Exercise Testing

Exercise tests using a computerized metabolic cart (Sensormedics) and a cycle ergometer were performed according to published guidelines (13). Measurements of work, oxygen consumption, heart rate, oxygen pulse, breathing reserve, dead space ventilation, and alveolar–arterial oxygen gradient at peak exercise were expressed as percentages of predicted values.

HRCT

Conventional and HRCT scans of the chest were performed without intravenous contrast during end-inspiration in the prone position. Baseline and follow-up HRCT scans were read independently by two chest radiologists using a previously described quantitative scale (14, 15). Discrepant readings were re-reviewed by both radiologists to determine consensus readings.

Fiberoptic Bronchoscopy with Bronchoalveolar Lavage

Bronchoscopy with bronchoalveolar lavage (BAL) was performed as previously described (16). Differential cell counts of BAL fluid cells on slides stained with Diffquik were performed.

Flow Cytometry

Mononuclear BAL fluid cells were separated by density gradient centrifugation and washed twice in phosphate-buffered saline (PBS). The number of cells suspended in PBS was adjusted to 1×10^6 cells/ml. Cells were incubated with the following antibody combinations:

Tube 1: Fluorescein isothiocyanate (FITC) CD3, phycoerythrin (PE) CD16 and 56, PE-Texas Red (TR) CD8, PE-Cyanine (Cy)5 CD4, PE-Cy7 CD25, Allophycocyanin (APC) CD14, APC-Cy7 CD195

Tube 2: FITC CD25, PE CD183, PE-TR CD8, PE-Cy5 CD3, PE-Cy7 CD4, APC CD38, APC-Cy7 CD195

Tube 3: FITC CD69, PE CD178, PE-TR CD8, PE-Cy5 CD3, PE-Cy7 CD4, APC HLA-DR, APC-Cy7 CD195

Monoclonal antibodies (10 μl) (BD Bioscience, San Jose, CA) were added to each tube containing 100-μl cell suspensions. Cells were stained on ice, for 30 minutes, washed twice, and resuspended in PBS, and $1–5 \times 10^4$ cells were analyzed using a nine-color CYAN flow cytometer (DakoCytomation, Fort Collins, CO). Forward scatter was the triggering parameter. Data acquisition, analysis, and compensation were performed using SUMMIT software by a single scientist blinded to subject group (DakoCytomation).

Lung Biopsy

Slides from surgical lung biopsies performed at the Clinical Center were independently reviewed by two pulmonary pathologists. Consensus readings were determined for discrepant pathology reports.

Statistical Analysis

Fisher's exact test was used to determine significant differences ($P < 0.05$) between the mean values of age and smoking history. Wilcoxon rank sum and Kruskal-Wallis nonparametric tests were used to determine significant ($P < 0.05$) differences between mean values of PFT, cardiopulmonary exercise testing, BAL fluid cell counts, and flow cytometry measurements.

RESULTS

HRCT Screening Detects Early ILD in Asymptomatic Relatives

A total of 164 subjects from 18 kindreds with familial IPF were evaluated (Table 1). Twenty-one (13%) of 164 subjects had chronic cough and/or dyspnea, and were previously diagnosed with IPF by an open-lung biopsy, classic HRCT changes, or both. One hundred forty-three (87%) of 164 subjects denied having cough, dyspnea, or lung disease, and therefore were defined as asymptomatic.

To identify subjects with asymptomatic ILD, we performed HRCT scans of the chest in unaffected relatives. We found that 31 (22%) of 143 asymptomatic family members had HRCT changes consistent with asymptomatic ILD (Table 1). The most common HRCT findings in these subjects were increased septal lines, peribronchovascular thickening, reticulation, and ground glass opacities (Figure 1A). Subpleural cysts and honeycombing

TABLE 1. SUBJECT CHARACTERISTICS

	HRCT Findings			
	Normal HRCT (*n = 53*)	Nonspecific Changes (*n = 59*)	Asymptomatic ILD (*n = 31*)	Familial IPF (*n = 21*)
Smoking history, % (n)	23 (12)	25 (15)	45 (14)*	67 (14)†
Age, yr, mean (SE)	35 (±1.7)	40 (±1.6)	46 (±2.1)†	67 (±2.7)†
Race, % (n)				
White	100 (53)	91 (54)	97 (30)	100 (21)
Hispanic	0 (0)	9 (5)	3 (1)	0 (0)
Female sex, % (n)	48 (25)	59 (35)	48 (15)	52 (11)

Definition of abbreviations: HRCT = high-resolution computed tomography; ILD = interstitial lung disease; IPF = idiopathic pulmonary fibrosis.

* $P = 0.02$.

† $P < 0.001$ estimated using Fisher's exact test.

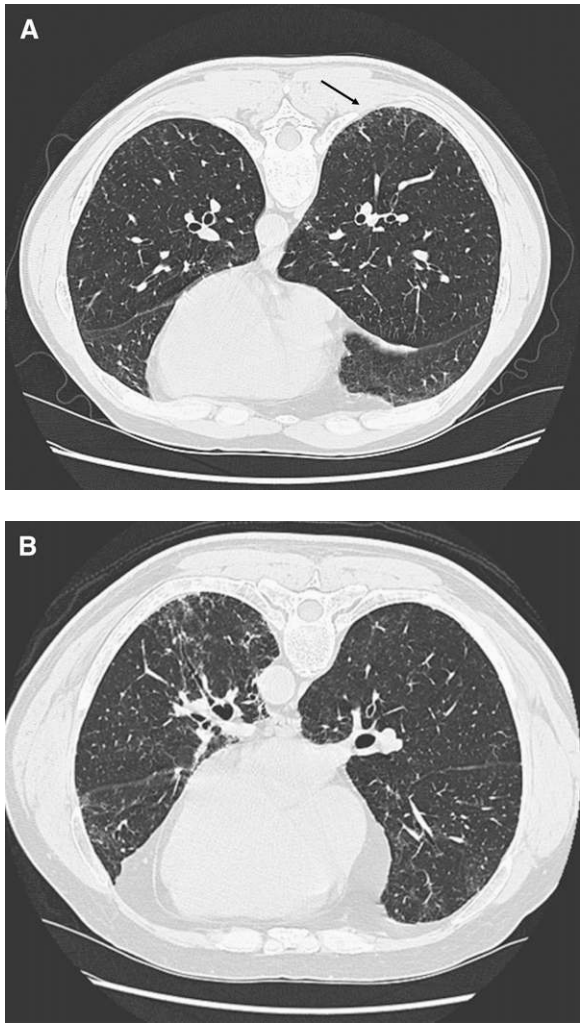

Figure 1. Representative abnormal high-resolution computed tomography (HRCT) scan images from two first-degree relatives without a prior diagnosis of idiopathic pulmonary fibrosis. (*A*) shows mild subpleural opacities (*arrow*) in an asymptomatic 50-year-old man. (*B*) An HRCT image from his brother, who was a 62-year-old man with progressive exertional dyspnea, shows moderate subpleural fibrosis and honeycombing.

were observed in some subjects. Subjects with known familial pulmonary fibrosis had classic HRCT findings, including bibasal subpleural fibrosis and honeycombing (Figure 1B). Fifty-nine (41%) and 53 (37%) of 143 asymptomatic subjects had nonspecific or normal findings on HRCT scan, respectively. Subjects with normal HRCT scans were used for a control population for the different comparisons in this study.

As shown in Table 1, significantly greater percentages of subjects with familial IPF (67%) and subjects with asymptomatic ILD (45%) were active or former smokers compared with subjects with normal HRCT scans (23%) ($P < 0.001$ and $P = 0.02$, respectively). Subjects with asymptomatic ILD (mean age, 46 ± 2.1 yr) were significantly younger than those with known familial IPF (mean age, 67 ± 2.7 yr) and significantly older than those with normal HRCT scans (mean age, 35 ± 1.7 yr) ($P < 0.001$ and $P < 0.001$, respectively). The mean age of the familial IPF group was consistent with prior reports (17). No significant differences in age or smoking history were found in subjects with nonspecific HRCT findings when compared with subjects with normal HRCT scans. One hundred fifty-eight (96%) and six (4%) of 164 subjects were white and Hispanic, respectively. There was no significant difference in sex among subgroups.

Pulmonary Function Testing Is Normal in Asymptomatic Familial ILD

As shown in Figure 2A, subjects with asymptomatic ILD had normal diffusion capacity, but their percentage of predicted D_{LCO} (97 ± 3% predicted) was significantly lower than that of subjects with normal HRCT scans (105 ± 2% predicted) ($P = 0.02$). Four subjects with asymptomatic ILD had abnormal D_{LCO} measurements; the extent of ILD on their HRCT scans was more than that observed in other subjects with asymptomatic ILD. Mean percentages of predicted D_{LCO} and TLC (data not shown) were significantly lower in subjects with familial IPF ($P < 0.001$). Percentages of predicted values of FEV_1, FVC, and TLC were normal in subjects with asymptomatic ILD (data not shown).

Because smoking can affect PFT results, we compared lung physiology measurements in subjects with a history of ever smoking with nonsmokers. We found that the percentage of predicted D_{LCO} in subjects with asymptomatic ILD was significantly lower in those with a history of ever smoking compared with nonsmokers ($P < 0.003$). However, there were no significant differences between nonsmokers and ever-smokers in percentages of predicted FEV_1, FVC, or TLC among the groups (data not shown).

Cardiopulmonary exercise testing revealed that percentages of reduction in dead space ventilation at peak exercise were significantly lower in subjects with asymptomatic ILD and subjects with familial IPF compared with those values from subjects with normal HRCT scans (Figure 2C) ($P < 0.001$ and $P < 0.001$, respectively). Percentages of predicted work, oxygen consumption, heart rate, oxygen pulse, breathing reserve, and alveolar–arterial oxygen gradient at peak exercise were not significantly different in subjects with asymptomatic ILD compared with subjects with normal HRCT scans (data not shown). In addition, there were no significant differences among the groups in cardiopulmonary exercise test measurements between nonsmokers and those with a history of ever smoking (data not shown).

Alveolar Lymphocyte Activation Is Present in Subjects with Early Asymptomatic ILD

Differential cell counts of BAL fluid cells were performed in 152 (93%) of 164 subjects. Percentages of BAL lymphocytes in subjects with asymptomatic ILD (13 ± 2%) tended to be higher than that of subjects with normal HRCT scans (8 ± 2%) ($P = 0.07$) (Figure 3A). Notably, however, percentage of BAL lymphocytes in subjects with asymptomatic ILD was significantly higher in nonsmokers (12.9 ± 11.2%, n = 15) compared with those with a history of ever smoking (5.25 ± 7.15%, n = 13) ($P < 0.008$) (Figure 3B). No significant difference in percentages of BAL neutrophils was found between subjects with asymptomatic ILD and subjects with normal HRCT scans (Figure 3C). However, the percentage of alveolar neutrophils was significantly higher in subjects with familial IPF (4 ± 0.5%) compared with subjects with normal HRCT scans (2 ± 0.3%) ($P < 0.001$). There were no significant differences between nonsmokers and ever-smokers in percentages of BAL alveolar macrophages, neutrophils, or eosinophils among the groups (data not shown).

To determine whether or not lymphocytes were activated, flow cytometry of cells from BAL fluid and peripheral blood was performed from a subset of subjects (n = 61). Subpopulations of T cells were identified, and four early and late activation markers (i.e., CD25, CD38, CD69, HLA-DR) were analyzed. Percentages of CD4 cells bearing CD38 or HLA-DR antigens were significantly higher in subjects with asymptomatic ILD

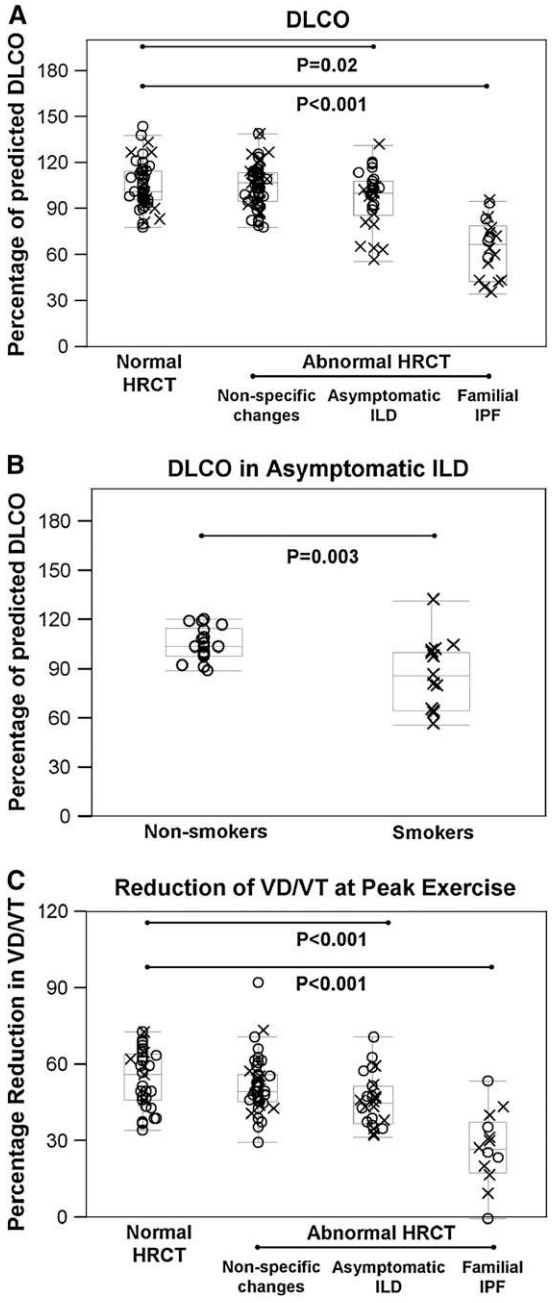

Figure 2. Pulmonary function and cardiopulmonary exercise tests indicate mild abnormalities in gas exchange. (*A*) Although the diffusion capacity of carbon monoxide (D_{LCO}) percentage of predicted was normal for all but three subjects with asymptomatic interstitial lung disease (ILD), pulmonary function tests showed significantly lower D_{LCO} percentage of predicted in subjects with asymptomatic ILD and familial idiopathic pulmonary fibrosis (IPF) compared with subjects with normal high-resolution computed tomography (HRCT) scans ($P < 0.02$ and $P < 0.001$, respectively). (*B*) D_{LCO} percentage of predicted for subjects with asymptomatic ILD was significantly lower in those with a history of ever smoking (n = 14) compared with nonsmokers (n = 15) ($P < 0.003$). (*C*) A significant difference in the percent reduction of dead space ventilation (VD/VT) during peak exercise was found in subjects with asymptomatic ILD compared with subjects with normal HRCT scans ($P < 0.001$). Nonsmoker = o, ever-smoker = x.

(7 ± 1% and 20 ± 2%, respectively) compared with subjects with normal HRCT scans (1 ± 1% and 1 ± 2%, respectively) ($P = 0.006$ and $P < 0.001$, respectively) (Figures 3D and 3E). There were no significant differences among the groups in percentages of CD4 cells bearing activation markers between nonsmokers and those with a history of ever smoking (data not shown); however, a limitation of these analyses is the small sample size of ever-smokers (n = 12). No significant differences in ratio of CD4/CD8 cells or percentage of CD8 T-cell activation were found among the groups (data not shown). In addition, no significant differences were found in peripheral blood lymphocytes among the groups (data not shown).

Open-Lung Biopsies of Subjects with Early Asymptomatic ILD Demonstrate Diverse Histologic Subtypes of ILD

We performed diagnostic open-lung biopsies in six subjects who had asymptomatic ILD and who belonged to three unrelated kindreds. All subjects denied any history of lung disease at the time of study recruitment; however, three subjects (one from each kindred) had mild PFT abnormalities (D_{LCO}, 74 ± 8% predicted) and HRCT findings consistent with a diagnosis of IPF. Open-lung biopsies in these three subjects showed a pattern of UIP confirming a diagnosis of IPF (Figures 4B, 4D, and 4F). The remaining three subjects (one in each kindred) had normal PFTs (D_{LCO}, 98 ± 8% predicted) and early HRCT changes suggestive of ILD, but not necessarily IPF. Open-lung biopsies of these three subjects showed different histologic subtypes, including hypersensitivity pneumonia, nonspecific interstitial pneumonia, and cellular interstitial and organizing pneumonia (Figures 4A, 4C, and 4E).

DISCUSSION

A major objective of this study was to identify clinical features of early, asymptomatic ILD in a population with high susceptibility to development of pulmonary fibrosis. We chose to study families affected with pulmonary fibrosis because of the high prevalence of IPF within these kindreds and the remarkable similarities in presentations, clinical outcomes, and gene expression patterns of familial and sporadic IPF (10, 17–20). We found radiographic evidence of ILD in 22% of asymptomatic subjects screened with HRCT scan. Our data showed that measurements of pulmonary function and cardiopulmonary exercise test parameters were not as sensitive as HRCT scan in identifying subjects with asymptomatic ILD who had normal airflow, lung volumes, diffusion capacity, oxygen consumption, oxygen saturation, and breathing reserve.

A significant age difference was found between subjects affected with asymptomatic ILD and familial IPF; their mean ages were 46 and 67 years, respectively. These data suggest that progression of early asymptomatic ILD to symptomatic IPF may occur over a period of decades. A prior report found that the estimated time from onset of symptoms to initial medical visit leading to a diagnosis of IPF was 3.8 years (21). Taken together with our findings, these results appear to indicate that the natural history of early and mild disease is relatively indolent compared with that of moderate to severe IPF (22).

In this cohort, 96% of subjects were white and 4% were Hispanic. Subjects who enrolled in these clinical research protocols were recruited by advertisements, and it is possible that our data regarding ethnic background of subjects reflect an ascertainment bias of the study.

In this study, significantly higher percentages of subjects with asymptomatic ILD and familial IPF were current or former smokers compared with control subjects. Only four former smokers with asymptomatic ILD had an abnormal D_{LCO}; they

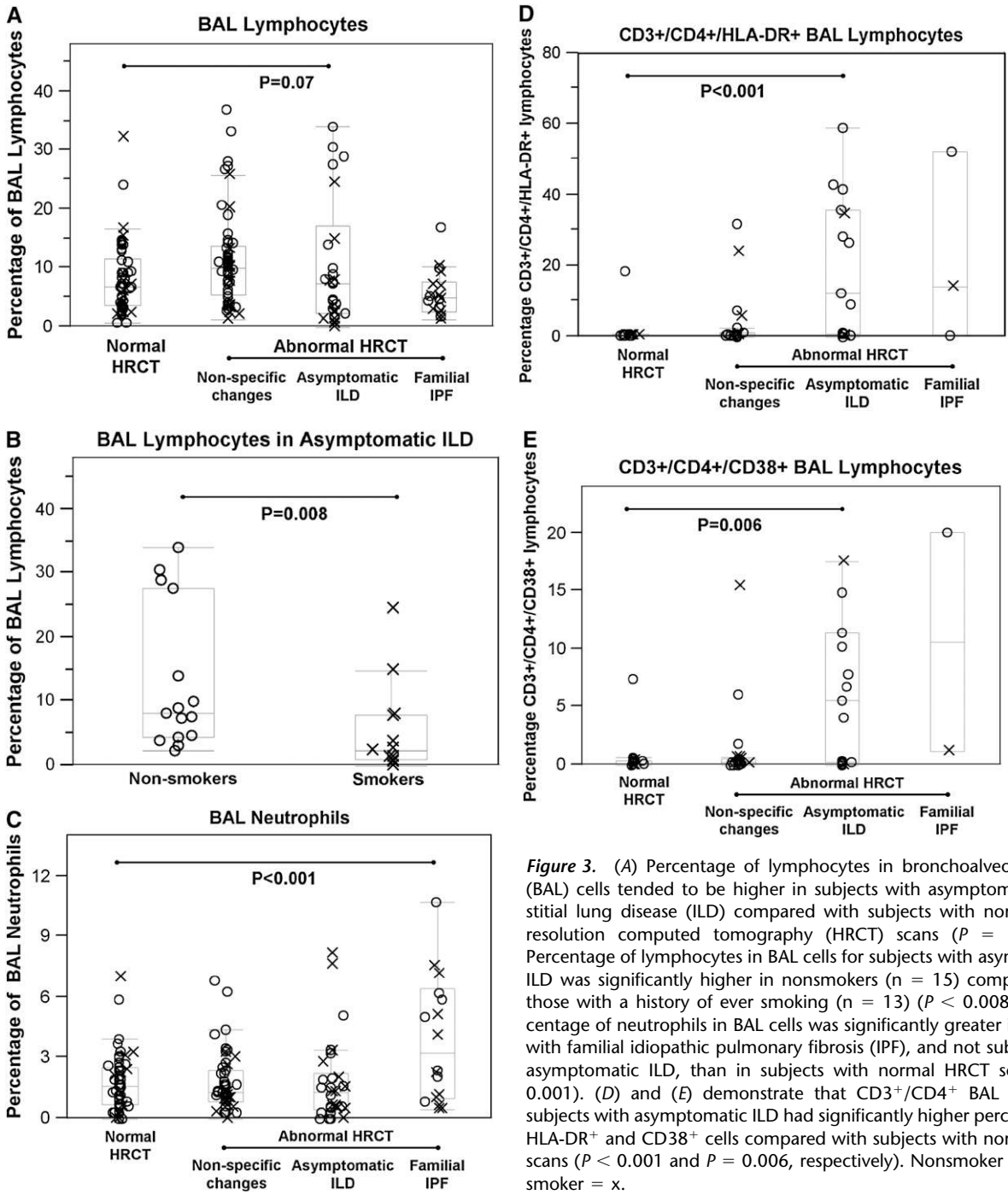

Figure 3. (*A*) Percentage of lymphocytes in bronchoalveolar lavage (BAL) cells tended to be higher in subjects with asymptomatic interstitial lung disease (ILD) compared with subjects with normal high-resolution computed tomography (HRCT) scans ($P = 0.07$). (*B*) Percentage of lymphocytes in BAL cells for subjects with asymptomatic ILD was significantly higher in nonsmokers (n = 15) compared with those with a history of ever smoking (n = 13) ($P < 0.008$). (*C*) Percentage of neutrophils in BAL cells was significantly greater in subjects with familial idiopathic pulmonary fibrosis (IPF), and not subjects with asymptomatic ILD, than in subjects with normal HRCT scans ($P < 0.001$). (*D*) and (*E*) demonstrate that $CD3^+/CD4^+$ BAL cells from subjects with asymptomatic ILD had significantly higher percentages of $HLA\text{-}DR^+$ and $CD38^+$ cells compared with subjects with normal HRCT scans ($P < 0.001$ and $P = 0.006$, respectively). Nonsmoker = o, ever-smoker = x.

differed from the other members of this cohort because their HRCT scans showed both interstitial and emphysematous changes. One of these subjects underwent an open-lung biopsy, which showed small airway injury and peribronchial fibrosis that were probably due to injury related to smoking. These data are consistent with prior reports demonstrating that smoking may be associated with development of early ILD in kindreds with familial interstitial pneumonia and progression of pulmonary fibrosis (8, 23). Although it is possible that some of the radiographic abnormalities in subjects with asymptomatic ILD could be attributed to smoking, these data seem to suggest that smoking further increases the risk of developing ILD in genetically susceptible hosts. Given this information, subjects who were actively smoking were counseled and referred to a smoking cessation program.

Analyses of BAL fluid cells revealed differences between subject groups. In agreement with reports focusing on sporadic IPF, we found significantly higher percentages of BAL neutrophils in subjects with familial IPF, and not asymptomatic ILD, compared with subjects with normal HRCT scans (24–27). Nonsmoking subjects with asymptomatic ILD had significantly higher percentages of BAL lymphocytes compared with those with a history of ever smoking, and subjects with asymptomatic ILD tended to have higher percentages of BAL lymphocytes compared with subjects with normal HRCT scans. Significantly higher percentages of $CD4^+$ T cells expressing early and late activation

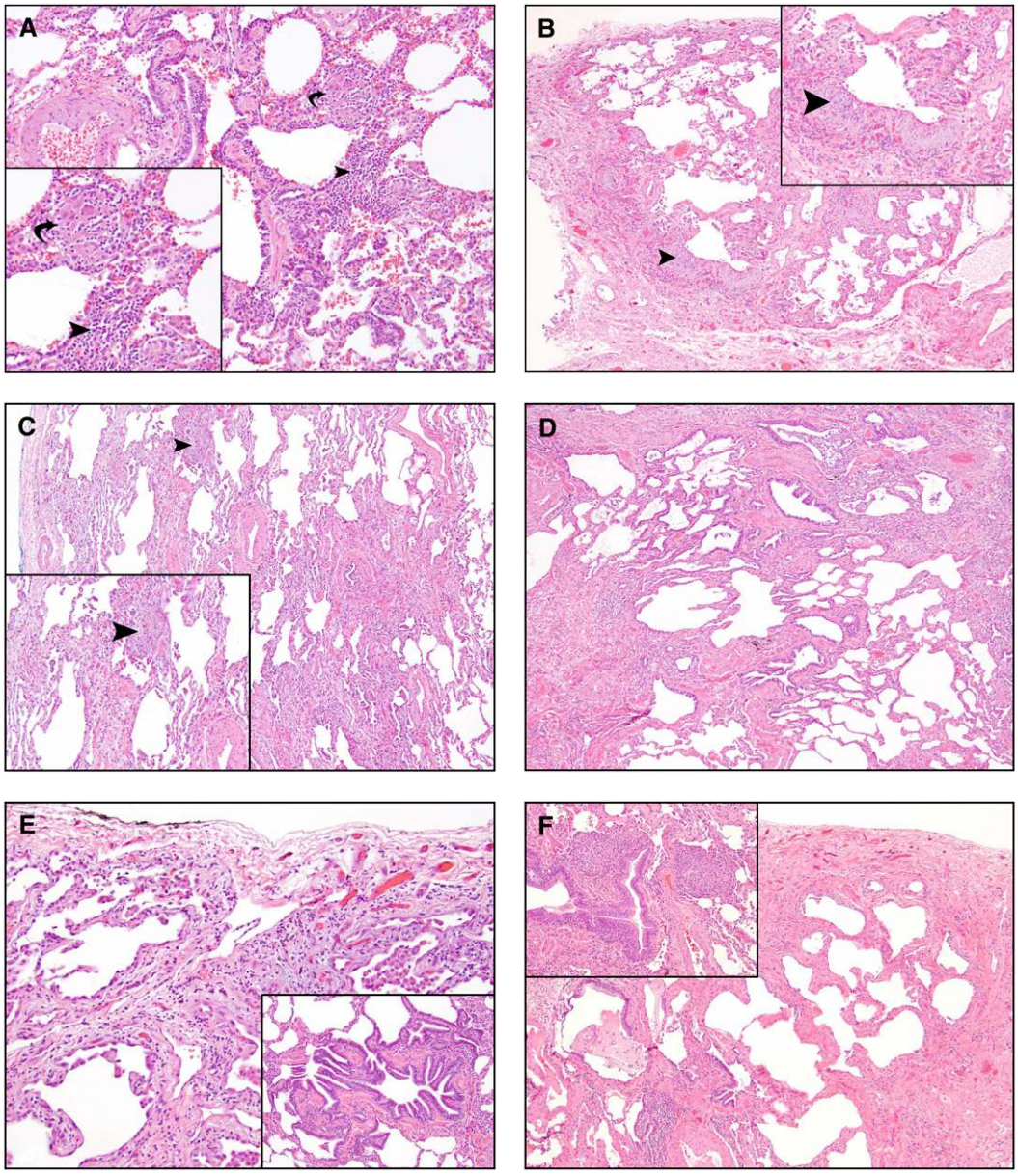

Figure 4. Representative fields from lung biopsies of subjects with asymptomatic interstitial lung disease (*A*, *C*, *E*) and mild pulmonary fibrosis (*B*, *D*, *F*) are shown. A photomicrograph demonstrating hypersensitivity pneumonia-like changes is shown in (*A*); *inset* shows an area of mild chronic interstitial inflammation (*arrowhead*) and a poorly formed granuloma (*curved arrow*) adjacent to a bronchiole (original magnification, ×10; hematoxylin-and-eosin [H&E] stain). (*B*) Photomicrograph demonstrating the geographic heterogeneity of usual interstitial pneumonia (UIP); *inset* shows fibroblast foci (*arrowhead*) (original magnification, ×4; H&E stain). (*C*) A photomicrograph demonstrating patchy, temporally uniform interstitial fibrosis is shown; *inset* shows an area with focal organizing pneumonia in detail (*arrowhead*) (original magnification, ×4; H&E stain). (*D*) A photomicrograph demonstrating areas of involved parenchyma alternating with less involved parenchyma that is characteristic of UIP (original magnification, ×4; H&E stain). (*E*) A photomicrograph demonstrating mild subpleural interstitial fibrosis and organizing pneumonia is shown (original magnification, ×10; H&E stain); *inset* shows peribronchiolar metaplasia resulting from injury to the small airways (original magnification, ×10; H&E stain). (*F*) A photomicrograph demonstrating honeycombing in a background of dense fibrous tissue seen in UIP (original magnification, ×4; H&E stain); *inset* shows nonnecrotizing granulomas adjacent to a bronchiole occasionally observed in the biopsy (original magnification, ×10; H&E stain).

markers were found in subjects with asymptomatic ILD compared with subjects with normal HRCT scans, and our findings of lymphocyte activation did not appear to be related to a history of active or former smoking. These findings are interesting in light of the experimental evidence suggesting that innate and adaptive immunity have a role in repair of injured lung and the development of pulmonary fibrosis (28–30). In addition, intrapulmonary infiltrations of activated CD4 T cells in early ILD are consistent with other reports that show the T-cell antigen receptor (TCR) repertoires of lymphocytes within BAL fluid of patients with IPF are highly biased, suggesting an antigen-driven process (31, 32). Influx of $CD4^+$ T cells directed against lung-specific antigens may follow alveolar epithelial disruption as well as exposure of basement membrane, vascular cells, and extracellular matrix proteins. Further studies are needed to determine whether alveolar lymphocyte recruitment contributes to pathogenesis of pulmonary fibrosis or is an epiphenomenon associated with early ILD.

We performed open-lung biopsies in six subjects with asymptomatic ILD from three different kindreds. Of this subset of patients, subjects with minor PFT abnormalities had mild to moderate HRCT changes and open-lung biopsies consistent with UIP, confirming a diagnosis of familial IPF. On the other hand, those with no PFT abnormalities had atypical HRCT changes and non-UIP pathologic findings, including hypersensitivity pneumonia, nonspecific interstitial pneumonia, and organizing pneumonia. It is interesting that in these three different kindreds, the subjects with mild PFT changes had UIP, whereas those

with normal lung function had non-UIP ILD. Diverse histopathologic findings in kindreds with familial IPF are consistent with another study that reported more than one histologic subtype within relatives in approximately 40% of kindreds affected with familial interstitial pneumonia (8). Although the number of subjects who underwent lung biopsies was small and is a limitation of our study, it is interesting that those subjects with UIP were generally older than those with non-UIP histopathologic findings. Taken together, these data indicate that the histopathology of early and late stages of familial interstitial pneumonia is different. The findings also suggest that UIP may be a final common histopathologic pathway for some subtypes of ILD in genetically susceptible individuals. Longitudinal evaluation of subjects with non-UIP histopathology is required to determine whether they develop UIP in the future.

There is a paucity of information related to genes associated with familial IPF. Previous reports indicate that familial IPF is an autosomal dominant disease with variable penetrance (5, 6). This mode of genetic transmission is also supported by our pedigrees, which demonstrate IPF in relatives from consecutive generations, approximately 50% of affected progeny descended from an affected individual, an approximately equal percentage of affected males and females, and evidence of male-to-male transmission (data not shown). Fifty-two (32%) of 164 subjects had radiographic evidence of asymptomatic ILD or familial IPF. Because many of the subjects with nonspecific or normal HRCT scan findings were younger than 40 years, it is possible that a subset of these subjects may develop asymptomatic ILD or familial IPF when they are older. Thus, it seems that the prevalence of asymptomatic ILD or familial IPF in our cohort is in agreement with an autosomal dominant inheritance pattern. Discovery of genetic mutations associated with disease and/or sensitive bioassays of early disease will facilitate the identification of individuals at high risk of development of asymptomatic ILD and will improve the ability to estimate the sensitivity and specificity of using HRCT scans for identification of early ILD.

In summary, we found that early, asymptomatic ILD can be identified using HRCT scan in a subset of individuals belonging to families affected with IPF. Progression of asymptomatic ILD to symptomatic IPF may occur over a period of decades, and smoking may be a risk factor for development of disease. We found different histopathologic findings in early and late stages of familial interstitial pneumonia within individuals belonging to the same kindreds. Overall, early identification of ILD in high-risk populations may improve clinical outcome by providing treatment to individuals with non-UIP subtypes (e.g., hypersensitivity pneumonitis), by counseling patients regarding smoking cessation, and by possibly referring individuals with UIP to medical centers involved with experimental clinical trials.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors thank Drs. Joel Moss, Martha Vaughan, Augustine Choi, Naftali Kaminski, James Dauber, and Steve Duncan for their insightful and critical review of the manuscript. They also thank their patients, the Pulmonary–Critical Care Medicine Branch nurse practitioners, and the Clinical Center bronchoscopy nurses for their contributions to this research.

References

1. Douglas WW, Ryu JH, Schroeder DR. Idiopathic pulmonary fibrosis: impact of oxygen and colchicine, prednisone, or no therapy on survival. *Am J Respir Crit Care Med* 2000;161:1172–1178.
2. Collard HR, Ryu JH, Douglas WW, Schwarz MI, Curran-Everett D, King TE Jr, Brown KK. Combined corticosteroid and cyclophosphamide therapy does not alter survival in idiopathic pulmonary fibrosis. *Chest* 2004;125:2169–2174.
3. Raghu G, Brown KK, Bradford WZ, Starko K, Noble PW, Schwartz DA, King TE Jr. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. *N Engl J Med* 2004;350: 125–133.
4. Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. *N Engl J Med* 2001;345:517–525.
5. Marshall RP, Puddicombe A, Cookson WO, Laurent GJ. Adult familial cryptogenic fibrosing alveolitis in the UK. *Thorax* 2000;55:143–146.
6. Hodgson U, Laitinen T, Tukiainen P. Nationwide prevalence of sporadic and familial idiopathic pulmonary fibrosis: evidence of founder effect among multiplex families in Finland. *Thorax* 2002;57:338–342.
7. Bitterman PB, Rennard SI, Keogh BA, Wewers MD, Adelberg S, Crystal RG. Familial idiopathic pulmonary fibrosis: evidence of lung inflammation in unaffected family members. *N Engl J Med* 1986;314:1343–1347.
8. Steele MP, Speer MC, Loyd JE, Brown KK, Herron A, Slifer SH, Burch LH, Wahidi MM, Phillips JA III, Sporn TA, *et al*. Clinical and pathologic features of familial interstitial pneumonia. *Am J Respir Crit Care Med* 2005;172:1146–1152.
9. Rosas IO, Ren P, Billings E, Sharma Y, Avila N, Chow C, Novero LJ, McGuire M, Wu HP, MacDonald SD, *et al*. Subclinical pulmonary fibrosis in families affected with idiopathic pulmonary fibrosis [abstract]. *Proc Am Thorac Soc* 2005;2:A242.
10. Wahidi MM, Speer MC, Steele MP, Brown KK, Schwarz MI, Schwartz DA. Familial pulmonary fibrosis in the United States. *Chest* 2002;121:30S.
11. American Thoracic Society; European Respiratory Society. American Thoracic Society/European Respiratory Society International multidisciplinary consensus classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2002;165:277–304.
12. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis* 1991;144:1202–1218.
13. American Thoracic Society; American College of Chest Physicians. ATS/ACCP statement on cardiopulmonary exercise testing. *Am J Respir Crit Care Med* 2003;167:211–277.
14. Avila NA, Brantly M, Premkumar A, Huizing M, Dwyer A, Gahl WA. Hermansky-Pudlak syndrome: radiography and CT of the chest compared with pulmonary function tests and genetic studies. *AJR Am J Roentgenol* 2002;179:887–892.
15. Brantly M, Avila NA, Shotelersuk V, Lucero C, Huizing M, Gahl WA. Pulmonary function and high-resolution CT findings in patients with an inherited form of pulmonary fibrosis, Hermansky-Pudlak syndrome, due to mutations in HPS-1. *Chest* 2000;117:129–136.
16. Ren P, Rosas IO, MacDonald SD, Wu HP, Billings EM, Gochuico BR. Impairment of alveolar macrophage transcription in idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 2007;175:1151–1157.
17. Lee HL, Ryu JH, Wittmer MH, Hartman TE, Lymp JF, Tazelaar HD, Limper AH. Familial idiopathic pulmonary fibrosis: clinical features and outcome. *Chest* 2005;127:2034–2041.
18. Garcia CK, Raghu G. Inherited interstitial lung disease. *Clin Chest Med* 2004;25:421–433.
19. Yang IV, Burch LH, Steele MP, Savov JD, Hollingsworth JW, McElvania-Tekippe E, Berman KG, Speer MC, Sporn TA, Brown KK, *et al*. Gene expression profiling of familial and sporadic interstitial pneumonia. *Am J Respir Crit Care Med* 2007;175:45–54.
20. Rosas IO, Kaminski N. When it comes to genes—IPF or NSIP, familial or sporadic—they're all the same [editorial]. *Am J Respir Crit Care Med* 2007;175:5–6.
21. King TE Jr, Tooze JA, Schwarz MI, Brown KR, Cherniack RM. Predicting survival in idiopathic pulmonary fibrosis: scoring system and survival model. *Am J Respir Crit Care Med* 2001;164:1171–1181.
22. Martinez FJ, Safrin S, Weycker D, Starko KM, Bradford WZ, King TE Jr, Flaherty KR, Schwartz DA, Noble PW, Raghu G, *et al*. The clinical course of patients with idiopathic pulmonary fibrosis. *Ann Intern Med* 2005;142:963–967.
23. King TE Jr, Schwarz MI, Brown K, Tooze JA, Colby TV, Waldron JA Jr, Flint A, Thurlbeck W, Cherniack RM. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. *Am J Respir Crit Care Med* 2001;164:1025–1032.
24. Reynolds HY, Fulmer JD, Kazmierowski JA, Roberts WC, Frank MM, Crystal RG. Analysis of cellular and protein content of bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *J Clin Invest* 1977;59:165–175.
25. Watters LC, Schwarz MI, Cherniack RM, Waldron JA, Dunn TL, Stanford RE, King TE Jr. Idiopathic pulmonary fibrosis: pretreatment bronchoalveolar lavage cellular constituents and their relationships with lung histopathology and clinical response to therapy. *Am Rev Respir Dis* 1987;135:696–704.

26. Boomars KA, Wagenaar SS, Mulder PG, van Velzen-Blad H, van den Bosch JM. Relationship between cells obtained by bronchoalveolar lavage and survival in idiopathic pulmonary fibrosis. *Thorax* 1995;50:1087–1092.
27. Schwartz DA, Helmers RA, Dayton CS, Merchant RK, Hunninghake GW. Determinants of bronchoalveolar lavage cellularity in idiopathic pulmonary fibrosis. *J Appl Physiol* 1991;71:1688–1693.
28. Fontenot AP, Canavera SJ, Gharavi L, Newman LS, Kotzin BL. Target organ localization of memory CD4(+) T cells in patients with chronic beryllium disease. *J Clin Invest* 2002;110:1473–1482.
29. Rosen D, Lee JH, Cuttitta F, Rafiqi F, Degan S, Sunday ME. Accelerated thymic maturation and autoreactive T cells in bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2006;174:75–83.
30. Bruder D, Westendorf AM, Geffers R, Gruber AD, Gereke M, Enelow RI, Buer J. CD4 T lymphocyte–mediated lung disease: steady state between pathological and tolerogenic immune reactions. *Am J Respir Crit Care Med* 2004;170:1145–1152.
31. Lympany PA, Southcott AM, Welsh KI, Black CM, Boylston AW, du Bois RM. T-cell receptor gene usage in patients with fibrosing alveolitis and control subjects. *Eur J Clin Invest* 1999;29:173–181.
32. Shimizudani N, Murata H, Keino H, Kojo S, Nakamura H, Morishima Y, Sakamoto T, Ohtsuka M, Sekisawa K, Sumida M, *et al*. Conserved CDR 3 region of T cell receptor BV gene in lymphocytes from bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis. *Clin Exp Immunol* 2002;129:140–149.