Interleukin-6, Interferon-Gamma, and Phospholipid Levels in the Alveolar Lining Fluid of Human Lungs*  
Profiles in Coal Worker’s Pneumoconiosis and Idiopathic Pulmonary Fibrosis  

Olivier J. Lesur, M.D., Ph.D, F.C.C.P.; Nicola M. Mancini, M.D.; Jean C. Humbert, M.D.; François Chabot, M.D.; and Jean-Marie Polu, M.D., F.C.C.P.

Cytokines are widely involved in physiologic as well as immunoinflammatory and fibrosing processes of the lung. The aim of this work was to study, by bronchoalveolar lavage, two groups of human interstitial lung diseases (ILD) with fibrosing propensity (ie, idiopathic pulmonary fibrosis [IPF], n=10; and coal worker’s pneumoconiosis [CWP], n=15). Patients were compared with nonsmoker control subjects (n=20). Cellularity, proteins, and phospholipids were determined in the alveolar fluids. In addition, two cytokines (interleukin-6 [IL-6] and interferon-gamma [IFN-γ]), which are presumed to possess respective antifibrotic and profibrotic activities, were measured in the respiratory tract. Compared with control subjects, IPF and simple CWP showed alveolar hypercellularity (p<0.05) and relative lymphocytosis (p<0.05). Both exhibited increased alveolar permeability (ie, increased albumin/urea ratio, p<0.05), with enhanced IL-6 and decreased IFN-γ in the alveolar spaces (p<0.05). On the other hand, IFP displayed an associated polymorphonuclear alveolitis, enhanced alveolar epithelial lining fluid (AELF) volume and low surfactant phospholipid levels (p<0.05 vs control), whereas simple CWP shared an exclusive lymphocytosis, normal AELF volume, and a surfactant lipid overflow (p<0.05 vs control). Relationships among all of these parameters were found only between alveolar cellularity, neutrophils and IL-6 levels in the AELF of IPF (respectively, r=0.85, p=0.0009, and r=0.89, p=0.0006). In summary, common alterations of cellular and cytokine turnover were observed in IPF and simple CWP and may reflect activity of the antifibrotic fight in these diseased lungs. Surfactant phospholipid levels are likely to represent a specific disturbance among IPF and CWP, but no clear relationship with respect to the other parameters could be established for explaining the difference in time course outcome.

I nflammatory interstitial lung diseases (ILDs) are initiated by known or unknown antigen challenges. Subsequent mechanisms of injury are relieved by complex immunoinflammatory cell events leading to very opposite outcomes, from ad integrum lung restoration to varying levels of fibrosing scar.1 Cell to cell interactions are crucial in immunoinflammatory or repair processes. The cytokine network is a main component inside this interconnection system leading to total or partial resolution.2 Unsurprisingly, there are more and more reports that most lung cells are spontaneously or, after activation, capable of synthesizing and secreting most known cytokines.2

Idiopathic pulmonary fibrosis (IPF) constitutes a model of diffuse and progressive cryptogenic lung fibrosis with a life-threatening outcome. Coal worker’s pneumoconiosis (CWP), which is an occupational pulmonary disease due to the inhalation of mixed dusts, can produce confluent fibrosing patterns called progressive massive fibrosis (PMF) and lead to variable outcomes.3

Current concepts on the mechanisms leading to alterations of connective lung tissue in IPF suggest that fibroblast activation/proliferation may play an important role in this disease. Many cells such as macrophages, lymphocytes, endothelial cells, and epithelial cells are capable of releasing regulatory growth factors for fibroblasts.1,2,4 Several lines of evidence suggest that the alveolar macrophage is a major component for directing fibrotic processes within the interstitium by a repetitive replication and

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*AFrom the Service des Insuffisants Respiratoires et Réanimation Respiratoire, Tour Drolet 1er, CHRU Nancy-Brabois (Drs. Lesur, Mancini, Chabot, and Polu); Unité 14 INSERM, Plateau de Brabois (Dr. Lesur); and Laboratoire de Cytologie, CRTC, Plateau de Brabois (Dr. Humbert), Vandoeuvre les Nancy, France. Supported by l’Association Pour la Promotion de la Recherche Respiratoire.

Manuscript received November 4, 1992; revision accepted November 17, 1993.

Reprint requests: Dr. Lesur, Unité de Recherche Repronnaire, CHUS Sherbrooke, Quebec, Canada J1H 5N4

CHEST / 106 / 2 / AUGUST, 1994 407
expansion in the lung,5-7 and by the release of factors that are chemotactic or proliferative promoters of fibroblasts and smooth muscle cells (eg, platelet-derived growth factor [PDGF], fibronectin, alveolar macrophage-derived growth factor [AMDG])8,9 On the other hand, type II epithelial cells such as stem cells and surfactant producers of the alveolar spaces and lymphocytes are also in close association with interstitial fibroblasts and are capable of producing several molecules that may regulate fibroblast activation/proliferation (eg, interleukin-6 [IL-6], interferon-gamma [IFN-γ], prostaglandin E2).1,2,10-12

In view of the above evidence, the present study was undertaken to evaluate (1) IL-6, IFN-γ, and surfactant phospholipid levels in the extracellular milieu of the lower respiratory tract of those with IPF and coal workers with or without pneumoconiosis, and (2) their relationship with alveolar cell profiles. Our hypothesis is that major molecules other than those described (PDGF, fibronectin, AMDGF) may be released from nonmacrophagic cells in altered amounts in the alveolar milieu and may also participate in the fibrotic processes of these diseases.

METHODS

Subject Groups

Bronchoalveolar lavage fluids (BALFs) were obtained from 45 adults recruited in the Service des Insuffisants Respiratoires et Réanimation Respiratoire (CHR Nancy-Brabois, France) from 1991 to 1992. Three groups were studied.

Group 1: A group (n=20, 15 men/5 women, mean age 52 ± 2 years) of normal healthy volunteers (normal) accepted the bronchoalveolar lavage (BAL) procedure. Official ethical approval (Comité d’éthique de Lorraine) and informed written consents were obtained for all. They had never smoked and lived in a non-tobacco environment; all had normal results of physical examinations, chest radiographs, and lung function tests. None were receiving medication or other drugs. Details about specific alveolar parameters of these nonsmoking subjects are published elsewhere.13

Group 2: This was a group of patients with IPF (n=10). All had consulted for progressive dyspnea and/or dry cough. No connective tissue disease, drug-induced pneumonitis, occupational exposure, mycobacterial infection, or other granulomatous pulmonary diseases were found. Diagnosis was confirmed by pathologic analysis of several abundant and distally derived transbronchial biopsy specimens (average, five per patient; n=8) performed for each subject undergoing fluoroscopy, in different segmental locations of the more altered parenchyma, or by open lung biopsy (n=2). Patterns consistent with usual interstitial pneumonia criteria with patchy sparing areas often emanating from septa and variable degrees of inflammation were observed. None of the patients received systemic or aerosol steroid therapy. All were nonsmokers. All showed typical clinical and respiratory function patterns. In addition, chest radiographs demonstrated typical reticular infiltrates predominant in basal areas, while computed tomographic (CT) scans showed areas of ground-glass density and diffuse honeycombing with thick alveolar walls and retraction bronchiectasis.

Group 3: Group 3 was a group of coal-workers from the Lorraine collieries (n=15). Mines of this region induce CWP following exposure to coal, iron, and quartz. Among the French mines, the Lorraine dust has the highest content in quartz (about 15 percent) and kaolin (about 10 percent), and is the richest in mineral matter (>75 percent).14 Clinical and radiologic patterns, as well as disease outcomes, are most likely more similar to silicosis than to anthracosis. The BALF samples from these miners show variable cellularity with lymphocytosis.15 The group was divided into three subgroups according to the ILO classification:16 currently exposed workers without obvious disease (n=4); exworkers with simple pneumoconiosis (n=7), subtracted from dust exposure since variable periods of 6 months to several years; and exworkers with confluent pneumoconiosis or PMF (n=4), removed from dust exposure for several years.

Several typical radiologic patterns were observed from the milky lung fields of simple CWP to the presence of massive lesions or nodules of more than 1 cm of diameter in confluent CWP. These lesions consisted of inflammatory conglomerations with occasional necrosis and often anthracosis but without pathologic evidence of tuberculosis or neoplasm as assessed by transbronchial biopsy specimens.

All of the workers removed from dust exposure had a recognized and compensated pneumoconiosis according to the French law on occupational diseases. None were current smokers, and two were exsmokers, having quit several years previously. None of these subjects had any acute viral or infectious respiratory disease for at least 1 month prior to the time of investigation.

Pulmonary Function Tests

Patients with diseased lungs underwent pulmonary function tests, including determination of lung volume (flow-volume curve and plethysmographic methods), carbon monoxide diffusion capacity (single-breath method), and arterial blood gases, as previously described.17 Normal subjects underwent a flow-volume curve only.

CT Scan Procedure

High-resolution CT was performed (using a Tomoscan 310, Massiot Philips, 256X256 calculator matrices, 512X512 picture matrices) using 1- and 8-mm sections at 10-mm spacing. Acquisition of each slice was performed in apnea at lung volume close to functional residual capacity.

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed by the same operator, using 5X50-ml aliquots of sterile 0.9 percent saline solution, at room temperature (pH 7.30). For each subject, the right middle lobe or the left lingula counterpart was chosen as the site of lavage, in conjunction with radiologic disease predominancy. Neuroleptanalgesia was done with midazolam and alfentanil after premedication and topical anesthesia (2 percent lidocaine [Xylocaine]). Lavages were performed under oxygen supplementation (3 L/min) and the SaO2 was monitored by continuous pulse oximetry (Ohmeda Biox 3700). Following wedging, all lavage procedures were completed within 5 min in order to reduce passive diffusion of molecules. The first aliquot reflecting a bronchial sample was discarded, while the others were pooled for study design. The BALF was then filtered through several layers of sterile nonsynthetic gauze to remove mucus, and centrifuged at 100g for 10 min. Supernatants were aliquoted and stored frozen for up to 6 months prior to assay. Overall BALF was processed within 60 min of sample collection. Unprocessed aliquots were routinely controlled and cultured for aerobic and anaerobic bacterial contamination.

Alveolar Cell Populations

Cellular pellets were counted in a hemacytometer. Wright-Giemsa stain smears served to identify differential profiles after cytopsin preparation.15 Optical morphologic assessment was obtained for the macrophage and lymphocyte subsets.
Table 1—Total Cellularity and Differential Profile*

<table>
<thead>
<tr>
<th></th>
<th>Total Cellularity</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
<th>Eosinophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=20)</td>
<td>13.2 ± 2</td>
<td>12.1 ± 1.8</td>
<td>0.78 ± 0.1</td>
<td>0.15 ± 0.04</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>(90.2)</td>
<td>(6.6)</td>
<td>(1.2)</td>
<td>(2)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>Idiopathic pulmonary fibrosis (n=10)</td>
<td>36.7 ± 9.1†</td>
<td>18.9 ± 6.5</td>
<td>6.8 ± 3†</td>
<td>12.1 ± 6.6</td>
<td>1.2 ± 0.3§</td>
</tr>
<tr>
<td>(58.4)</td>
<td>(10.4)</td>
<td>(26.8)</td>
<td>(3.8)</td>
<td>(0.5)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>Coal workers (n=15)</td>
<td>20.8 ± 5.7</td>
<td>16.1 ± 3.2</td>
<td>4 ± 1.8</td>
<td>0.4 ± 0.2</td>
<td>0.06 ± 0.04</td>
</tr>
<tr>
<td>(81)</td>
<td>(16)</td>
<td>(2)</td>
<td>(1)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>Exposed miners without lung disease (n=4)</td>
<td>18.7 ± 4.4</td>
<td>16.8 ± 3.9</td>
<td>1.5 ± 0.7†</td>
<td>0.15 ± 0.05</td>
<td>0</td>
</tr>
<tr>
<td>(91)</td>
<td>(6)</td>
<td>(0.75)</td>
<td>(0)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Simple pneumoconiosis (n=7)</td>
<td>23.7 ± 7†</td>
<td>16.6 ± 6.3</td>
<td>6.3 ± 3.5†</td>
<td>0.5 ± 0.4</td>
<td>0.12 ± 0.07</td>
</tr>
<tr>
<td>(74.1)</td>
<td>(21.7)</td>
<td>(1.8)</td>
<td>(1.9)</td>
<td>(1.9)</td>
<td>(1.9)</td>
</tr>
<tr>
<td>Confluent pneumoconiosis (n=4)</td>
<td>16.7 ± 4.5</td>
<td>13.7 ± 3.3</td>
<td>2.5 ± 1.1†</td>
<td>0.2 ± 0.1</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>(83)</td>
<td>(12.7)</td>
<td>(4)</td>
<td>(2)</td>
<td>(2)</td>
<td>(2)</td>
</tr>
</tbody>
</table>

*All data are presented as means ± SD. Total and differential cellularity are expressed in ×10⁶ cells/BALF and the percentage of each cell type is presented in parentheses.

†p<0.005.

‡p<0.05.

§p=0.0001 vs normal.

Biochemical Analysis of BALF

Lipids were extracted from the surfactant fraction of BALF by a chloroform:methanol preparation. Total amounts of phospholipids were first evaluated by phosphorus assay and expressed in micrograms per milliliter following a correction coefficient. Ten to 20 mL of BALF was needed for this measurement. Proteins were measured in lavage fluids by the technique of Pesce and Strande and expressed in micrograms per milliliter. Urea content was measured using the urease Berthelot reaction (Sigma, La Verpilliere, France) and allowed for the estimation of the alveolar epithelial lining fluid (AELF). Two ratios were established within alveolar biochemical parameters: total phospholipids/proteins and albumin/urea.

Cytokine Radioimmunoassays

All measurements were performed with unconcentrated BALF aliquots. Interleukin-6 and IFN-γ immunoassay kits were purchased (Medgenix Diagnostics, Bruxelles, Belgium) and used according to the recommendations of the manufacturer. Briefly, standards and samples (200 µl) were added to coated tubes in which monoclonal antibodies (MoAb) directed against distinct epitopes of selected cytokine (MoAb capture antibodies) were attached to the lower and inner surfaces and tubes were left for 20 h at room temperature. After washing the excess antigen, MoAb2(125I-labeled-antibody) were added for 2 h at room temperature. After incubation and washing, the remaining radioactivity bound to the tubes reflected the cytokine concentration. No significant cross-reactivity was observed for IFN-γ and IL-6 measurements with IL-1α, IL-1β, IL-6, IL-8, TNF-α, IL-10, tumor necrosis factor (TNF)-α, TNF-β, and with IL-1α, IL-1β, IL-10, IL-6, TNF-α, TNF-β, and IL-6. All samples were assayed in duplicate for each experiment. In addition, some samples in each group were tested several times in separate experiments. Sensitivity of IFN-γ was 0.2 IU/mL and 0.3 fmol/mL for IL-6. Several human serum samples were regularly evaluated and provided very low or nondetectable levels of IFN-γ and IL-6, as expected. The IL-6 levels were first expressed in picograms per milliliter of BALF and converted to femtomoles per milliliter of BALF, the most widely used unit in the literature. Twelve nonsmoker control subjects (normal) were randomly tested for cytokine assays, whereas all patients from selected lung disease groups were explored.

Correlations Between Parameters

As described in the initial hypothesis, cytokine levels were evaluated for relationships with respect to alveolar cellularity and surfactant phospholipids.

Statistical Analysis

All data were expressed as mean values ± standard deviation. Comparisons among diseased lung groups, smoker, and non-smoker control subjects were studied by analysis of variance with a factorial analysis of variance for parametric data, and a Mann-Whitney U test for nonparametric data. A Spearman test was used for correlation studies, and p<0.05 was chosen as the threshold of significance for statistical comparisons.

RESULTS

Pulmonary Function Tests

Patients with IPF demonstrated alterations in their pulmonary function tests that consisted of decreased lung volume (TLC: 58 ± 2 percent predicted), decreased diffusion capacity (DCO: 37.5 ± 10 predicted) and decreased PaO2 at rest, and 21 percent FIO2 (52 ± 4 mm Hg). The FEV1/VC ratio was normal (92 ± 7 percent predicted). Exposed miners with and without lung disease demonstrated only a slightly decreased PaO2 at rest and 21 percent FIO2 (73 ± 3 mm Hg), whereas lung volume (TLC: 85 ± 1 percent predicted), FEV1/VC ratio (86.5 ± 7 percent predicted), and diffusion capacity (DCO: 94 ± 7 percent predicted) were normal. However, patients with CWP with confluent pattern had decreased lung volume (TLC: 72 ± 3 percent predicted) and greater decreased PaO2 at rest and 21 percent FIO2 (65 ± 4 mm Hg). Normal subjects had a FEV1/VC ratio of 118 ± 5 percent predicted.

Alveolar Cell Profiles

Total alveolar cells in BALF were higher in those with IPF and in workers with simple CWP (IPF: 36.7 ± 4.1X10^-6/BALF, simple CWP: 23.7 ± 7X10^-6/
BALF, p<0.01) compared with normal control subjects (Table 1). All patients had a slight increase in number and percentage of lymphocytes, especially in several patients with simple CWP (eightfold increase vs normal, p<0.05). Lymphocytosis was generally less pronounced but less variable in advanced pneumoconioses (confluent CWP: threefold increase, p<0.005 vs normal). In addition, those with IFP had 80 times more neutrophils and 6 times more eosinophils (p=0.0001 vs normal).

Biochemical Measurements

Patients with IFP and CWP had much more protein in their respiratory tract (IPF: 245.8 ± 57 μg/ml; CWP: 227 ± 93 μg/ml BALF, p<0.05 vs normal) (Table 2). Patients with simple CWP showed the highest total protein and albumin contents (p<0.05 vs normal). Albumin to urea ratio was enhanced in all subgroups except for exposed coal workers without lung disease (normal values), and in patients with IFP in whom it was decreased (p<0.05 vs normal). The AELF was highly increased in patients with IFP (3.16 ± 0.75 percent, p=0.0001 vs normal).

Extracellular phospholipids were less abundant in patients with IFP and exposed coal workers without lung disease (respectively, 14.2 ± 9 and 16.7 ± 7.2 μg/ml BALF, p=0.0001 vs normal) but enhanced in patients with simple and confluent CWP (respectively, 70.7 ± 18 and 61.3 ± 35 μg/ml BALF, p<0.05 vs normal). Total phospholipids to protein ratio, however, was not different in these subgroups compared with normal.

The BALF returns were similar in the three groups (normal: 62 ± 4 percent; IPF: 60.8 ± 6 percent; CWP: 58.7 ± 5 percent; NS).

Cytokines in BALF

The within-assay precision for all cytokines tested was similar to that given by the manufacturer (IFN-γ: 0.3 ± 0.03 IU per tube, n=6; IL-6: 0.5 ± 0.08 fmol per tube, n=8). The between-assay variation was 0.5 ± 0.1 IU per tube for IFN-γ (n=10) and 3.4 ± 0.9 fmol per tube for IL-6 (n=8). Expressed in milliliters of BALF, the results showed elevated IL-6 and decreased IFN-γ levels in the respiratory tract of patients with IFP (p<0.05 vs normal) (Table 3).

Cytokine levels were standardized according to the epithelial lining fluid volume. The profile of IL-6 and IFN-γ levels remained similar in patients with IPF (p<0.05 vs normal). On the other hand, IL-6 levels were elevated in the AELF of patients with CWP (p<0.05 vs normal). Interferon gamma levels were enhanced in the AELF of exposed coal workers without lung disease and with confluent pneumoconiosis (p<0.05 vs normal) but decreased in workers with simple pneumoconiosis (p<0.05 vs normal) (Table 3).

### Table 2—Biochemical Parameters of BALF*

<table>
<thead>
<tr>
<th></th>
<th>Total Proteins</th>
<th>Albumin</th>
<th>AELF</th>
<th>Albumin/Alveolar Urea</th>
<th>Phospholipids</th>
<th>Phospholipids/Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=20)</td>
<td>87 ± 8</td>
<td>37.7 ± 3.4</td>
<td>0.95 ± 0.07</td>
<td>138 ± 0.3</td>
<td>32.8 ± 2.8</td>
<td>416.2 ± 45</td>
</tr>
<tr>
<td>Idiopathic pulmonary fibrosis (n=10)</td>
<td>245.8 ± 57†</td>
<td>54.9 ± 14</td>
<td>3.16 ± 0.75†</td>
<td>87.3 ± 231</td>
<td>14.2 ± 91</td>
<td>67.9 ± 81</td>
</tr>
<tr>
<td>Coal workers (n=15)</td>
<td>227 ± 93</td>
<td>95 ± 46.3</td>
<td>0.81 ± 0.11</td>
<td>338 ± 961</td>
<td>55.7 ± 13</td>
<td>382.8 ± 112</td>
</tr>
<tr>
<td>Exposed miners without lung disease (n=4)</td>
<td>83.5 ± 25</td>
<td>33.6 ± 10</td>
<td>0.48 ± 0.11</td>
<td>206 ± 160</td>
<td>16.7 ± 72†</td>
<td>312 ± 156†</td>
</tr>
<tr>
<td>Simple pneumoconiosis (n=7)</td>
<td>380.3 ± 179†</td>
<td>147.2 ± 91†</td>
<td>1.1 ± 0.15</td>
<td>460 ± 161†</td>
<td>70.7 ± 18§</td>
<td>340.5 ± 130</td>
</tr>
<tr>
<td>Confluent pneumoconiosis (n=4)</td>
<td>198.7 ± 59</td>
<td>55 ± 17</td>
<td>0.68 ± 0.3</td>
<td>296 ± 561</td>
<td>61.3 ± 35</td>
<td>375.8 ± 441</td>
</tr>
</tbody>
</table>

*p<0.05.
†p=0.0001 vs normal.
§p<0.005.

*All data are presented as mean ± SD. Total proteins, albumin, and phospholipids are expressed in micrograms per milliliter of BALF. The volume of AELF is expressed in milliliters.

### Table 3—Cytokine Levels in the Human Respiratory Tract*

<table>
<thead>
<tr>
<th></th>
<th>IL-6</th>
<th>IFN-γ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=12)</td>
<td>0.8 ± 0.1 (84.4 ± 18)</td>
<td>5.1 ± 0.3 (723.6 ± 78)</td>
</tr>
<tr>
<td>Idiopathic pulmonary fibrosis (n=10)</td>
<td>13.3 ± 5.6 (287.2 ± 102)†</td>
<td>3.7 ± 0.4† (199.5 ± 52)‡</td>
</tr>
<tr>
<td>Coal workers (n=15)</td>
<td>0.92 ± 0.18 (175.5 ± 54)</td>
<td>4.6 ± 0.3 (938.4 ± 176.0)‡</td>
</tr>
<tr>
<td>Exposed miners without lung disease (n=4)</td>
<td>0.42 ± 0.4 (157.7 ± 150)</td>
<td>4.6 ± 0.7 (1536.2 ± 241)†</td>
</tr>
<tr>
<td>Simple pneumoconiosis (n=7)</td>
<td>1.11 ± 0.3 (118.1 ± 20)†</td>
<td>4.1 ± 0.3 (418.5 ± 59)‡</td>
</tr>
<tr>
<td>Confluent pneumoconiosis (n=4)</td>
<td>0.96 ± 0.2 (335.9 ± 69)†</td>
<td>4.8 ± 0.6 (1331.8 ± 391)†</td>
</tr>
</tbody>
</table>

*All data are presented as means ± SD. Interleukin-6 and interferon-gamma levels are either expressed as femtomole per milliliter and international unit per milliliter of BALF or in femtomole per milliliter and international unit per milliliter of AELF, respectively (in parentheses).
†p<0.05.
‡p<0.005. §p=0.0001 vs normal.
Correlation Among Cytokines, Cellularity, and Surfactant Phospholipids

A clear and strong correlation was demonstrated between alveolar cellularity and the number of alveolar neutrophils, and IL-6 levels in the AELF of patients with IPF (respectively, r=0.85, p=0.0009, and r=0.89, p=0.0006, n=10). No additional informative relationships were found among the other main alveolar parameters evaluated in the study.

Discussion

This study assessed the alterations of biologic parameters in the lungs of patients with IPF and miners with and without obvious CWP compared with normal subjects. Our patients with IPF and simple pneumoconioses showed active inflammatory processes in their lungs and shared several common particularities: (1) an alveolar hypercellularity with lymphocytosis; (2) an increased alveolar permeability (ie, increased total proteins an albumin/urea ratio); and (3) enhanced IL-6 and decreased IFN-γ levels in the alveolar spaces. However, patients with IPF had associated polymorphonuclear alveolitis, high AELF volume increase, and low phospholipids, whereas patients with simple CWP had normal AELF volume and a surfactant phospholipid overflow. On the other hand, advanced CWP with confluent patterns had a cellularity within the range of normal volunteers but exhibited a threefold increase of IFN-γ levels when compared with simple CWP.

Alveolar cell differential profiles showed expected values according to the type of disease present. High lymphocytosis in IPF is associated with responsiveness to corticosteroids and good prognosis, while increased neutrophils and eosinophils lead to worsened outcome. In our hands, clinical, radiologic, and respiratory function observations suggested that these patients had an advanced disease at the time of diagnosis. However, hypercellularity with lymphocytosis associated with highly increased AELF volume and albumin/urea ratio confirmed that most of these patients still had an active inflammatory stage of disease and suggested that fibrosing processes might be influenced by an anti-inflammatory and/or immunosuppressive therapy.

Patients with CWP, and especially workers with simple pneumoconiosis, also had increased amounts of alveolar lymphocytes, whereas hypercellularity was restricted to the active inflammatory stage (ie, simple CWP), apart from any sign of associated lung disease. This has been reported in several animal models of mineral dust-induced pneumonitis. Compared with IPF, CWP-AELF volumes were not enhanced and albumin/urea ratio was either increased in the early inflammatory stage (ie, simple CWP) or in the advanced fibrosing stage (ie, confluent CWP) of the disease.

Surfactant phospholipid amounts were low in the respiratory tract of patients with IPF as reported elsewhere and high in patients with CWP as in most cases of pneumoconioses. Interestingly, unaffected workers still exposed to mineral dusts had decreased amounts of alveolar surfactant while no arguments for any immunoinflammatory processes were noted except for an increased number of lymphocytes in their respiratory tract. Such decreased surfactant phospholipid levels have recently been reported in miners from the Québec eastern townships. Most of the data relating to lipid overflow as the hallmark of pneumoconioses has come from experimental animal models in which dust exposure produces acute or subacute forms of disease that may differ from that observed in humans. However, the meaning of such different profiles remains unclear and needs further investigation.

Although sharing several and probably unspecific alterations, IPF and CWP show, nevertheless, very distinct lung processes that may explain their different outcomes. In this regard, our data on cytokines are clearly original and provide several insights into the lymphomonokine network in IPF and CWP.

Interleukin-6 is a molecule sharing a broad range of activities on many target cells and is involved in immunoinflammatory processes. Most resident and migratory lung cells are capable of producing IL-6. Originally described as IFN-β2, IL-6 shares, along with interleukin-1 (IL-1) and tumor necrosis factor (TNF), a multifunctional capacity, including regulatory control of B-lymphocyte, T-lymphocyte, granulocyte, macrophage, and endothelial cell function, and has also been suggested to possess a chemotactic activity on lymphocytes. Several studies have demonstrated IL-6 to share both pro-inflammatory and anti-inflammatory properties. Interleukin-6 may also exhibit a negative feedback on fibrosing processes.

Interferon-gamma is a product of immune effector cells in response to many stimuli. It induces the expression of HLA DR II antigen, IL-2 receptor expression on T cells, enhances NK cell activity, and inhibits suppressor T-cell function. The ability of IFN-γ to directly influence fibrosis has been subject to conflicting reports but is generally recognized to have a growth-supporting effect on activated fibroblasts.

Interleukin-6 is greatly increased in patients with IPF and to a lesser degree in the AELF of patients with CWP. Similar increased levels or activity of IL-6 or related molecules have been reported in IPF and bleomycin-induced fibrosis and CWP. These high IL-6 levels are correlated with alveolar hypercellularity and neutrophil counts in IPF, but not with...
other cell types. Taking into account that alveolar hypercellularity and neutrophilia suggest an active inflammatory and fibrosing stage of disease, this clear increase may be related to intense counterbalanced anti-inflammatory and antifibrotic activities. Indeed, recent evidence suggests that pretreatment of mice with anti-IL-6 antibodies significantly suppresses the fibrosing outcome in hypersensitivity pneumonitis.\(^{36}\) In this regard, CWP could be considered as a different model of fibrosing mechanics in which areas of massive fibrosis are more patchy and centralized by the conglomeration of nodules, whereas neighboring alveolar spaces are essentially normal. This could provide at least a partial explanation for the lower values of IL-6 in patients with CWP depending on the choice of the lobule where the lavage was done.

The impact of IFN-\(\gamma\) on fibroblast activities, although controversial,\(^{11-38}\) is generally considered as a positively regulated process. Interestingly, in accordance with our observation of decreased levels in the alveolar milieu, Prior and Haslam\(^{43}\) recently reported a defect in IFN-\(\gamma\) production by lymphocytes from patients with fibrosing alveolitis. To our knowledge, no similar data are available in CWP.

Taken together, IL-6 and IFN-\(\gamma\) cytokine levels showed similar profiles in lung diseases with active inflammation such as in our patients with IPF and miners with simple pneumoconiosis. Hence, enhanced availability of an “antifibrotic” IL-6 and decreased presence or production of a “profibrotic” IFN-\(\gamma\) may reflect the intensity of the fight for saving the lung from irreversible scarring.

The choice of radioimmunoassay (RIA) for testing cytokine levels in biologic fluids was based on its high sensitivity and specificity. Although bioassays assess biologic activity, this may be the result of the action of agonistic and antagonistic factors not necessarily related to the tested lymphokines or monokines. Ideally, coupling an ELISA or a RIA technique with a bioassay may be the most adequate assessment. On the other hand, if such measurements do not allow for the discrimination of cell types responsible for cytokine release, the lack of relationship between tested cytokines and lymphomonocyte number may suggest that non-BAL-recoverable resident cells (eg, epithelial and endothelial cells) are active producers of these molecules.

In summary, several original insights are highlighted in this work comparing two distinct fibrosing interstitial lung diseases. Enhanced alveolar cellularity and lymphocytosis with lung hyperpermeability, in addition to increased amounts of IL-6 and decreased levels of IFN-\(\gamma\) in the alveolar milieu, delineate a stage of disease common to our patients with IPF and miners with simple pneumoconiosis. These alterations might reflect the presence of intensive and active inflammatory processes where profibrotic and antifibrotic trends are confronted. Phospholipid amounts are the only clear discriminating factor among biologic parameters tested in IPF and simple CWP.

ACKNOWLEDGMENT: Isabelle Brunot provided technical assistance, and Dr. Pierre Cervantes rendered helpful collaboration.

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