RESPIRATORY MEDICINE (1998) **92**, 338–344

Interleukin-8 expression in bronchoalveolar lavage cells in the evaluation of alveolitis in idiopathic pulmonary fibrosis

A. XAUBET*, C. AGUSTÍ*, P. LUBURICH†, J. A. BARBERÁ*, M. CARRIÓN*, M. C. AYUSO†, J. ROCA* AND R. RODRIGUEZ-ROISIN*

*Servei de Pneumologia i Allèrgia Respiratòria, Departament de Medicina, and †Servei de Radiodiagnòstic, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain

Interleukin-8 (IL-8) is a neutrophilic chemotactic factor which may have a prominent role in the attraction of neutrophils to the lung in idiopathic pulmonary fibrosis (IPF). The objective of this study was to investigate the usefulness of IL-8 expression in bronchoalveolar lavage (BAL) cells in the evaluation of alveolitis in IPF. We analysed the BAL cell expression of IL-8 by immunocytochemistry in 19 patients with IPF (six smokers, three ex-smokers and ten non-smokers) and in a control group composed of 14 individuals (six smokers, eight non-smokers). In IPF, BAL was performed on both the pulmonary lobe with the most extensive involvement and the one less extensively involved on high-resolution computed tomography (HRCT) scans. The percentages and absolute numbers of BAL IL-8$^+$ macrophages from lobes with the most extensive HRCT scan involvement ($36 \pm 6\%$ and $(6 \pm 2 \times 10^4\ ml^{-1})$ (SE) and from those less extensively involved [$26\% \pm 4\%$ and $(6 \pm 1) \times 10^4\ ml^{-1}$] were significantly higher with respect to both those from healthy smokers [$17\% \pm 6\%$ and $(7 \pm 4) \times 10^4\ ml^{-1}$] and those from non-smokers [$2\% \pm 1\%$ and $(1 \pm 0{\cdot}3) \times 10^4\ ml^{-1}$] ($P=0{\cdot}005$ and $P=0{\cdot}001$, respectively), without differences between the two lobes. In contrast, both the proportions and the absolute numbers of BAL neutrophils in IPF were significantly higher in lobes with the most extensively involved HRCT scan in comparison with lobes with the least extensive involvement [$13\% \pm 3\%$, $(3 \pm 1) \times 10^4\ ml^{-1}$ *vs.* $8\% \pm 2\%$, $(1 \pm 0{\cdot}3) \times 10^4\ ml^{-1}$, $P=0{\cdot}05$]. Moreover, the numbers of BAL neutrophils, but not those of IL-8$^+$ macrophages, correlated with the extent of total pulmonary HRCT scan abnormalities in the most involved lobe ($r=0{\cdot}64$, $P=0{\cdot}04$). A correlation between neutrophils and IL-8$^+$ cells was not observed. The results of this study suggest that, in IPF, BAL neutrophilia offers a better description of the disease inflammatory process than the expression of IL-8 in BAL cells.

RESPIR. MED. (1998) **92**, 338–344

Introduction

Idiopathic pulmonary fibrosis (IPF) is an interstitial lung disease of unknown aetiology characterized by a chronic inflammatory process and progressive fibrosis of the alveolar structures and interstitium. Bronchoalveolar lavage (BAL) from patients with idiopathic pulmonary fibrosis shows an increased number of neutrophils (1) and the chronic presence of these cells in the alveolar structures probably plays a prominent role in the parenchymal injury seen in this disorder. It has been shown that several neutrophil chemoattractants may be responsible for the accumulation of neutrophils in the lung (2). Interleukin-8 (IL-8) is a peptide of the C-X-C chemokine family with a significant neutrophil chemotactic activity. It also has been suggested that this cytokine may have an important role in the attraction of neutrophils to the lung in IPF (3,4). Several studies have demonstrated that the constitutive expression of IL-8 mRNA and cell-associated protein in alveolar macrophages is significantly elevated in patients with IPF and that alveolar macrophages are the major cell source of IL-8 in this condition (5–9). Although some studies (5–7) have shown that the level of IL-8 expression correlates with the proportion of BAL neutrophils in IPF, the role of IL-8 as a clinical marker of disease inflammatory activity has not been completely defined as yet. In a previous study, we have demonstrated that in patients with IPF the BAL cell populations in the different pulmonary lobes are not uniform and appear to be related to the extent of the high-resolution computed tomography (HRCT) scan abnormalities present in the lavaged lobe (10). In order to determine further the usefulness of IL-8 in the evaluation of the presence of alveolitis in IPF, we have investigated the relationship

Received 14 April 1997 and accepted in revised form 3 December 1997.
Correspondence should be addressed to: A. Xaubet, Servei de Pneumologia, Hospital Clínic, Villarroel 170, Barcelona 08036, Spain.

0954-6111/98/020338+07 $12.00/0

between IL-8 expression on BAL cells and BAL cell analysis and HRCT findings.

Methods

POPULATION STUDIED

Control Group

The control group was composed of 14 individuals with no evidence of diffuse respiratory disease, normal pulmonary function tests and absence of respiratory infections in the preceding 3 months. Fibre-optic bronchoscopy was performed in view of a single episode of haemoptysis with normal chest X-ray, isolated pulmonary node or suspicion of upper airway disease. In all patients, bronchoscopy was macroscopically normal with negative microbiological and cytological analysis of bronchial washings. The control group was divided into two subpopulations: the non-smoking group was composed of eight subjects (five men, age 45 ± 5·8 years) (SE) and the smoking group consisted of six subjects (four men, age 45 ± 6 years) who had been smoking 39 ± 9 standard packs yr^{-1} (range, 5–70 packs yr^{-1}).

Idiopathic Pulmonary Fibrosis

The population studied was composed of 19 untreated patients (15 men, age 67 ± 2 years) with IPF. Six of them were smokers (39 ± 8 packs yr^{-1}, range 15–70 packs yr^{-1}), three stopped smoking at least 5 yr before entry into the study and the remaining ten patients had never smoked. All patients had cough and/or dyspnoea with a duration of symptoms of 10 ± 3 months (range, 1–36 months). Eight patients had finger clubbing and 16 bilateral widespread crackles. Chest X-ray showed persistent bilateral interstitial infiltrates in all the patients. The diagnosis of IPF was established by open lung biopsy in seven patients. In the remaining 12 patients, two conditions were required to establish the diagnosis: (1) to fulfil the clinical criteria described by Turner-Warwick *et al.* as we previously used (11–13), and (2) to have findings compatible with IPF on HRCT scan (14,15). Pulmonary function tests were performed as has been previously described, using the reference values from our own laboratory (16,17). None of the patients had associated collagen vascular disease. The study was performed as part of the current diagnostic assessment and outpatient monitoring for interstitial lung diseases in our department (12,13). BAL differential cell counts and HRCT scan findings of some of the patients included in this study have been previously reported (10). All subjects were informed of the characteristics and nature of the procedures to be performed and all gave full informed written consent. The study was approved by the Ethics Committee of the Hospital Clinic.

HIGH-RESOLUTION COMPUTED TOMOGRAPHY SCAN

Computed tomography scans were performed in either a Somaton HiQ or a Somaton Plus scanner (Siemens, Erlangem, Germany), as has been previously described (10). HRCT scans were obtained at six predetermined levels: the great vessels, the aortic arch, the tracheal carina, the pulmonary hilae, the pulmonary venous confluence and 1 cm above the right diaphragm. The scans were performed with a 1–2 mm section thickness and 1–2 s scanning time during breath-holding at end-inspiration.

Two radiologists examined the HRCTs without knowledge of clinical, functional and radiographic findings. The overall extent of lobar abnormalities was scored in the six levels in which HRCT scans were obtained, the percentage of lung parenchyma affected being calculated. Then, a semiquantitative analysis of the relative proportion (to within 10%) of both the ground glass and the reticular patterns was performed. By consensus agreement, two pulmonary lobes were selected: the one with the most extensive involvement and that with the least extensive involvement (10).

BRONCHOALVEOLAR LAVAGE

BAL was performed at random on both the pulmonary lobe with the most extensive involvement and the one less extensively involved on HRCT scan. In the control group, BAL was performed only on the middle lobe or the lingula. Total cell count, cell viability and differential cell counts were determined as previously described (10,18). Immunocytochemical analysis of IL-8 on BAL cells was performed using the immunoalkaline phosphatase method (19). Cytocentrifuge smears were fixed in acetone and incubated in foetal calf serum at 25% to block the non-specific binding sites. Cell preparations were sequentially incubated with rabbit anti-human IL-8 antibodies (ICN Biomedicals, CA, USA) at a dilution of 1/100 for 16 h at 4°C, mouse anti-rabbit immunoglobulins (Dakopatts, Denmark), rabbit anti-mouse immunoglobulins (Dakopatts, Denmark) and alkaline phosphatase anti-alkaline phosphatase complexes (Dakopatts, Denmark). The development of the reaction was performed using an alkaline phosphatase substrate. Slides were counterstained with Mayer's haematoxylin and mounted in an aqueous medium. The negative control of the immunocytochemical reaction was carried out by incubating the cell smears with all reagents except the anti-IL-8 antibody and replacing the anti-human IL-8 antibody with normal rabbit serum. The IL-8^+ cells were red stained and easily distinguished from the negative cells. All quantifications of stained cells were done by two independent investigators using light microscopy (1000 ×) to determine the number of stained cells.

Pulmonary function tests, HRCTs and BAL were performed at the time of the clinical diagnosis. The time taken for the three procedures was within less than 1 week.

STATISTICAL ANALYSIS

Results are expressed as mean ± SE. Wilcoxon's test was used for comparison of paired data and Mann–Whitney's

TABLE 1. Pulmonary function tests of patients with idiopathic pulmonary fibrosis

	Actual values	Predicted (%)
FEV_1 (l)	1·9 ± 0·1 (0·0–3·4)	73 ± 4·0 (46–97)
FVC (l)	2·4 ± 0·2 (1·1–4·4)	63 ± 3·5 (39–93)
FEV_1/FVC		84 ± 1 (70–99)
TLC (l)	4·5 ± 0·3 (3·3–6·9)	74 ± 5 (44–107)
*D*LCO (ml min^{-1} $mmHg^{-1}$)	13 ± 1·2 (6–19)	54 ± 5 (26–82)
*K*CO (ml min^{-1} $mmHg^{-1}$ ml^{-1})	4 ± 0·3 (2–6)	77 ± 7 (51–131)
PaO_2 (kPa)	9·6 ± 0·5 (4·1–12)	
$AaPO_2$ (kPa)	4·5 ± 5 (2·3–10)	

Ranges of values in parentheses.

TABLE 2. High-resolution computed tomography scan scores in lobes with maximal and minimal involvement

	Maximal involvement	Minimal involvement	*P* values
Ground glass	29 ± 6·5 (0–90)	5 ± 2·2 (0–30)	0·001
Reticular	37 ± 1·1 (0–70)	22 ± 3 (0–50)	0·0001
Total	66 ± 6 (20–99)	27 ± 4 (10–60)	0·0001

Ranges of values in parentheses.

test for unpaired data. Spearman's correlation coefficients were used when appropriate for correlation analysis. The influence of smoking in IL-8 expression was tested by a two-way analysis of variance (ANOVA). Statistical significance was established when the *P* value was equal to or lower than 0·05.

Results

PULMONARY FUNCTION TESTS (TABLE 1)

One patient was not able to undergo the pulmonary function tests. *D*LCO was not performed in six patients because of severe reduction of lung volumes and/or lack of co-operation. All patients had a moderate to severe restrictive ventilatory impairment and eight patients a TLC below 80% predicted. *D*LCO was below the lower limit of reference (80% predicted) and 11 out of 12 patients in whom it was measured and *K*CO was below 80% predicted in seven cases. Arterial blood gases were measured in all but one patient. Arterial hypoxaemia (PaO_2 <10·7 kPa) was present in 15 patients and $AaPO_2$ was increased (>2·7 kPa) in 15.

HIGH-RESOLUTION COMPUTED TOMOGRAPHY SCAN SCORING ANALYSIS

In Table 2, the scoring analysis of HRCT scans in the lobes with both maximal and minimal involvement is represented. There was a significant difference in the ground glass and reticular scores (P=0·001 and P=0·0001, respectively) as well as in the total score between both lobes (P=0·0001).

BRONCHOALVEOLAR LAVAGE FLUID ANALYSIS

In the control group, the total cell counts and the absolute numbers of macrophages were higher in smokers with respect to non-smokers, although there were no differences in differential cell counts (Table 3). In patients with IPF (Table 4), the percentages and absolute numbers of neutrophils and eosinophils were significantly elevated in BAL from both the lobe with the most extensive involvement and that with the least extensive involvement on HRCT scans, with respect to the control group. The comparisons of the characteristics of BAL in the two lavaged lobes showed that both the percentages and absolute numbers of neutrophils and those of eosinophils were significantly higher in BAL performed on areas with the most extensive HRCT scan involvement (P=0·05 and P=0·02, respectively) (Table 4).

In the control group, immunocytochemical analysis of cytocentrifuge BAL cell smears showed that both the percentages and the absolute numbers of IL-8-positive alveolar macrophages had a tendency to be higher in healthy smokers [17% ± 6% and $(7 \pm 4) \times 10^4$ ml^{-1}] in comparison with non-smokers [2% ± 1% and

TABLE 3. Bronchoalveolar lavage characteristics of the control group

	Smokers (n=6)	Non-smokers (n=8)	P values
Volume recovered (ml)	58 ± 7 (35–83)	45 ± 7 (24–81)	NS
Total cells (× 10^4 ml^{-1})	54 ± 11 (5–81)	24 ± 4 (6–45)	0·05
Cell viability (%)	84 ± 4 (71–95)	75 ± 5 (50–95)	NS
Macrophages (%)	96 ± 1 (94–98)	94 ± 1 (91–99)	NS
Macrophages (× 10^4 ml^{-1})	52 ± 10 (14–76)	23 ± 4 (6–41)	0·02
Lymphocytes (%)	3 ± 1 (2–6)	4 ± 1 (1–8)	NS
Lymphocytes (× 10^4 ml^{-1})	2 ± 1 (0·3–5)	1 ± 0·5 (0·1–4)	NS
Neutrophils (%)	0·3 ± 0·1 (0–1)	1 ± 1 (0–3)	NS
Neutrophils (× 10^4 ml^{-1})	0·1 ± 0·1 (0–0·3)	0·3 ± 0·1 (0–0·9)	NS
Macrophages IL-8$^+$ (%)	17 ± 6 (0–36)	2 ± 1 (0–6)	0·06
Macrophages IL-8$^+$ (× 10^4 ml^{-1})	7 ± 4 (0–21)	1 ± 0·3 (0–2)	0·06

Ranges of values in parentheses.

TABLE 4. Characteristics of bronchoalveolar lavage performed on both the pulmonary lobe with the most extensive involvement and the one least extensively involved on high-resolution computed tomography scan in patients with idiopathic pulmonary fibrosis

	Maximal involvement	Minimal involvement	P values
Volume recovered (ml)	31 ± 2 (17–45)	32 ± 2 (16–48)	NS
Total cells (× 10^4 ml^{-1})	22 ± 3 (5–51)	23 ± 5 (5–77)	NS
Cell viability (%)	73 ± 3 (44–99)	71 ± 3 (47–96)	NS
Macrophages (%)	77 ± 3 (56–96)	83 ± 3 (43–96)	NS
Macrophages (× 10^4 ml^{-1})	16 ± 2 (4–39)	19 ± 4 (4–73)	NS
Lymphocytes (%)	5 ± 1 (1–14)	6 ± 2 (0–32)	NS
Lymphocytes (× 10^4 ml^{-1})	1 ± 0·5 (0·1–7)	1 ± 0·5 (0–7)	NS
Neutrophils (%)	13 ± 3 (0–34)	8 ± 2 (0–26)	0·05
Neutrophils (× 10^4 ml^{-1})	3 ± 1 (0–15)	1 ± 0·3 (0–5)	0·05
Eosinophils (%)	5 ± 1 (0–19)	2 ± 1 (0–12)	0·02
Eosinophils (× 10^4 ml^{-1})	1 ± 0·3 (0–4)	0·5 ± 0·2 (0–5)	0·02
Macrophages IL-8$^+$ (%)	36 ± 6 (2–74)	26 ± 4 (0–60)	NS
Macrophages IL-8$^+$ (× 10^4 ml^{-1})	6 ± 2 (0·2–28)	6 ± 1 (0–20)	NS

Ranges of values in parentheses.

(1 ± 0·3) × 10^4 ml^{-1}) (P=0·06) (Fig. 1). While in non-smokers only a few macrophages (0–6%) expressed IL-8, three smokers had an elevated percentage of IL-8-positive macrophages (28%, 29% and 36%, respectively). No correlation was shown between the amount of pack-years smoked and the percentage of IL-8$^+$ macrophages. Moreover, a correlation between neutrophils and IL-8$^+$ macrophages was not found. In patients with IPF, the percentages and absolute numbers of BAL IL-8$^+$ macrophages from lobes with the maximal and minimal involvement according to HRCT scans [36% ± 6% and (6 ± 2) × 10^4 ml^{-1} *vs.* 26% ± 4% and (6 ± 1) × 10^4 ml^{-1}, respectively] were significantly greater compared with both healthy smokers and non-smokers (Table 4, Fig. 1). However, there were no differences between the numbers of IL-8$^+$ macrophages in the BAL performed on areas with maximal HRCT involvement and the BAL from areas with minimal involvement (Table 4, Fig. 1). In IPF patients, the numbers of macrophages expressing IL-8 were not different in smokers in comparison with ex-smokers and non-smokers. Differences in IL-8 expression between the control group and IPF patients were not influenced by smoking (P=0·68). A correlation between BAL neutrophils and IL-8$^+$ macrophages was not observed. We found a correlation between both the percentages and absolute numbers of BAL neutrophils and the extent of total pulmonary HRCT scan abnormalities in the most involved lobe (r=0·64, P=0·01, and r=0·6, P=0·005, respectively), not present with the other cell types. In contrast, the numbers of IL-8$^+$ macrophages did not correlate with HRCT findings.

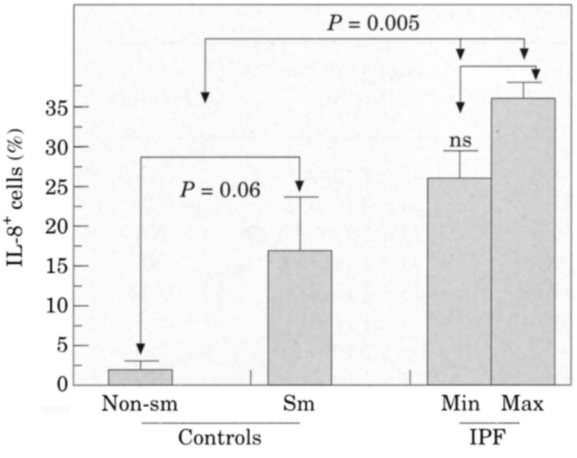

FIG. 1. Percentage of BAL IL-8$^+$ cells in the subpopulations of the control group (nonsm, non-smokers; sm, smokers) and in patients with idiopathic pulmonary fibrosis (IPF) (min, pulmonary lobes with the least extensive involvement on HRCT scan; max, pulmonary lobes most extensively involved on HRCT scan).

Discussion

The novel findings of our study is that we investigated the usefulness of IL-8 expression in the evaluation of alveolitis in IPF, by comparing the results of IL-8 expression with both BAL cellular analysis and HRCT scan findings. Different studies have demonstrated that the appearance of HRCT scans predicts histological patterns made evident in open lung biopsy. Both reticular and honeycombing patterns correlate with the presence of fibrosis whereas a ground glass pattern identifies zones of alveolar and interstitial inflammation (20). In a previous study, we have demonstrated that the percentage of both neutrophils and eosinophils in BAL fluid is significantly higher in the most involved areas of the lung parenchyma determined by HRCT scan and that the percentage of neutrophils correlates with the extent of both whole HRCT scan abnormalities and ground glass pattern in the lavaged lobe (10). The findings of the present study confirm and further extend our previous results, suggesting that computed-tomography-guided BAL may improve the assessment of alveolitis in IPF. However, we did not find differences between the percentage of IL-8$^+$ macrophages in the BAL performed on areas with the most extensive involvement and that from areas less extensively involved on HRCT scan. In addition, a correlation between the number of IL-8$^+$ macrophages and both the percentage of BAL neutrophils and HRCT findings was not shown. Our results are therefore at variance with those of other studies (5–7) in which the level of BAL cell IL-8 expression correlated with the proportion of neutrophils. These differences and the lack of correlation between BAL IL-8 expression and HRCT score may be explained by the different methods used to determine the expression of IL-8. Carré *et al.* (5) and Southcott *et al.* (7) used the polymerase chain reaction and ELISA to assess the presence of IL-8, methods probably more quantitative than immunocytochemistry. However, the sensitivity of immunocytochemistry may be higher than that of other antibody-based techniques (e.g. ELISA) because the antigen may be highly concentrated in a localized area, such as within a cell or within a cell membrane (21). On the other hand, there is evidence that in IPF other chemotactic factors may contribute to the accumulation of neutrophils in the lung parenchyma, as shown by Car and coworkers (22). Moreover, it has been shown that several neutrophil chemotactic factors, such as leukotriene B_4 and C5-derived neutrophil chemotactic factor, are present in BAL from patients with IPF (2,23).

This study has confirmed that the expression of IL-8 in BAL cells is increased in patients with IPF in comparison with healthy smokers and non-smokers. Several former studies have demonstrated that both the expression of IL-8 in BAL cells and IL-8 levels in BAL supernatants are increased in patients with IPF (5–8,22). In the present study, the immunocytochemical analysis of BAL cells showed that IL-8 was expressed by alveolar macrophages but not the other cell types. Although other cells, such as bronchial epithelial cells, fibroblasts and neutrophils, are also able to synthesize IL-8, Strieter *et al.* (8) demonstrated that airspace macrophages are the main source of this cytokine in IPF. Similar results have been obtained by Southcott *et al.* (7) using *in situ* hybridization studies in lung biopsies from patients with IPF. All in all, these findings suggest that elevated levels of IL-8 are compartmentalized to the alveolar space and not to the interstitium of the lung in IPF.

It is interesting to note that the immunocytochemical analysis showed that the number of BAL IL-8$^+$ macrophages had a tendency to be higher in healthy smokers in comparison with non-smokers. Our findings are at variance with those of other studies (5,7) which did not find any significant difference in IL-8 expression between healthy smokers and non-smokers. In contrast, other authors have demonstrated that an elevated concentration of IL-8 in BAL fluid as well as an increased expression of IL-8 in BAL macrophages may be found in healthy smokers with normal pulmonary function tests (24). These findings can be explained because particulates present in cigarette smoke may stimulate alveolar macrophages both *in vivo* and *in vitro* to exhibit a neutrophil chemotactic activity probably related to IL-8 (25). However, the results of the present study show that smoking does not play a role in influencing the differences observed in IL-8 expression between controls and IPF patients. The role of smoking in the pathogenesis of IPF has been widely studied. It has been shown that a history of smoking is associated with an increased risk for the development of IPF (26). Moreover, Schwartz *et al.* (27) have suggested that smoking may be a determinant of the increased numbers of BAL neutrophils in these patients. Probably, the lack of influence of smoking on IL-8$^+$ macrophages in IPF found in our study may be explained by the small number of current smokers in the IPF group (six patients).

In conclusion, the results of our study confirm that alveolar macrophages of patients with IPF constitutively express IL-8. On the other hand, the lack of correlation

between IL-8 expression in BAL cells and both HRCT findings and BAL differential cell counts suggests that the presence of neutrophilia offers a better description of the disease inflammatory process than the expression of IL-8 in BAL cells.

Acknowledgements

This work was supported by Grants 94/998 from the FIS (Fondo de Investigación Sanitaria), GRQ, 94-9103 from the Comissionat per a Universitats i Recerca (Generalitat de Catalunya), Fundació Catalana de Pneumologia (FUCAP/94) and Sociedad Española de Neumología y Cirugía Torácica (SEPAR/95).

References

1. BAL Cooperative Group. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis and selected comparison groups. *Am Rev Respir Dis* 1990; **141:** S188–S192.
2. Ozaki T, Hayashi H, Tani K, Ogushi F, Yasouka S, Ogura T. Neutrophil chemotactic factors in the respiratory tract of patients with chronic airway diseases or idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1922; **145:** 85–91.
3. Kunkel SL, Standiford T, Kasahara K, Strieter RM. Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung. *Exp Lung Res* 1991; **17:** 17–23.
4. Baggiolini M, Walz A, Kunkel S. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J Clin Invest* 1989; **84:** 1045–1049.
5. Carré PC, Mortenson RL, King TE, Noble PW, Sable CL, Riches DW. Increased expression of the interleukin-8 gene by alveolar macrophages in idiopathic pulmonary fibrosis. *J Clin Invest* 1991; **88:** 1802–1810.
6. Lynch JP III, Standiford TJ, Rolfe MW, Kunkel SL, Strieter RM. Neutrophilic alveolitis in idiopathic pulmonary fibrosis. The role of interleukin-8. *Am Rev Respir Dis* 1992; **145:** 1433–1439.
7. Southcott AM, Jones KP, Li D, Majumdar S, Cambrey AD, Pandelidis P *et al.* Interleukin-8. Differential expression in lone fibrosing alveolitis and systemic sclerosis. *Am J Respir Crit Care Med* 1995; **151:** 1604–1612.
8. Strieter RM, Koch AE, Antony VB, Fick RB, Standiford TJ, Kunkel SL. The immunopathology of chemotactic cytokines: the role of interleukin-8 and monocyte chemoattractant protein-1. *J Lab Clin Med* 1994; **123:** 183–197.
9. Nakamura H, Fujishima S, Waki Y, Urano T, Sayama K, Sakamaki F *et al.* Priming of alveolar macrophages for interleukin-8 production in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1995; **152:** 1579–1586.
10. Agustí C, Xaubet A, Luburich P, Ayuso MC, Roca J, Rodriguez-Roisin R. Computed tomography guided bronchoalveolar lavage in idiopathic pulmonary fibrosis. *Thorax* 1996; **51:** 841–845.
11. Turner-Warwick M, Burrows B, Johnson A. Cryptogenic fibrosing alveolitis: clinical features and their influence on survival. *Thorax* 1980; **35:** 171–180.
12. Agustí C, Xaubet A, Agustí AGN, Roca J, Ramirez J, Rodriguez-Roisin R. Clinical and functional assessment of patients with idiopathic pulmonary fibrosis: results of a 3 year follow-up. Eur Respir J 1994; **7:** 643–650.
13. Agustí C, Xaubet A, Roca J, Agusii AGN, Rodriguez-Roisin R. Interstitial pulmonary fibrosis with and without associated collagen vascular disease: results of a two year follow-up. *Thorax* 1992; **47:** 1035–1040.
14. Hansel DM, Kerr IH. The role of high resolution computed tomography in the diagnosis of interstitial lung disease. *Thorax* 1991; **46:** 77–84.
15. Leung AN, Miller RR, Müller NL. Parenchymal opacification in chronic infiltrative lung disease: CT–pathologic correlation. *Radiology* 1993; **188:** 209–214.
16. Roca J, Sanchis J, Agustí-Vidal A, Segarra F, Navajas D, Rodriguez-Roisin R, Casán P, Sans S. Spirometric reference values from a Mediterranean population. *Bull Eur Physiopathol Respir* 1986; **22:** 217–224.
17. Roca J, Rodriguez-Roisin R, Cobo E, Burgos F, Pérez J, Clausen JL. Single-breath carbon monoxide diffusing capacity (DL_{co}) prediction equations from a Mediterranean population. *Am Rev Respir Dis* 1990; **140:** 1026–1032.
18. Xaubet A, Agustí C, Roca J Picado C, Rodriguez-Roisin R. BAL lymphocyte activation antigens and diffusing capacity are related in mild to moderate pulmonary sarcoidosis. *Eur Respir J* 1993; **6:** 715–718.
19. Xaubet A, Agustí C, Picado C, Urbano-Ispizua A, Carrión M, Agustí-Vidal A. Demonstration of a surface antigens on bronchoalveolar lavage cells using the immunoalkaline phosphatase method. *Respiration* 1989; **56:** 43–47.
20. Nishimura K, Litaichi M, Izumi T, Nagay S, Kanaoka M, Itoh H. Usual intersitital pneumonia: histologic correlation with high resolution CT. *Radiology* 1992; **182:** 337–342.
21. Williams MC, Dobbs LG. Expression of cell-specific markers for alveolar epithelium in fetal rat lung. *Am J Respir Cell Mol Biol* 1990; **2:** 533–542.
22. Car BC, Meloni F, Luisetti M, Semenzato G, Gialdroni-Grassi G, Walz A. Elevated IL-8 and MCP-1 in the bronchoalveolar lavage fluid of patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. *Am J Respir Crit Care Med* 1994; **149:** 655–659.
23. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJ. Leukotien B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 1980; **286:** 264–265.
24. McCrea KA, Ensor JE, Nall K, Bleecker ER, Hasday JD. Altered cytokine regulation in the lungs of cigarette smokers. *Am J Respir Crit Care Med* 1994; **150:** 696–703.

25. Gregory H, Young J, Schroeder JM, Mrowietz U, Christofers E. Structure determination of a human lymphocyte derived neutrophil activating peptide. *Biochem Biophys Res Commun* 1988; **151:** 883–890.
26. Baumgartner KB, Samet JM, Stidley CA, Colby TV, Waldrom JA. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* 1997; **155:** 242–248.
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.