Idiopathic pulmonary fibrosis: can cell mediated immunity markers predict clinical outcome?

R Meliconi, E Lalli, R M Borzi, C Sturani, V Galavotti, G Gunella, R Miniero, A Facchini, G Gasbarrini

Abstract
Most of the cells found in lung parenchyma in patients with idiopathic pulmonary fibrosis are activated T lymphocytes and macrophages. The serum levels of these markers of cell mediated immunity were measured in 20 patients with idiopathic pulmonary fibrosis, in 20 normal subjects and in 12 patients with sarcoidosis to evaluate their clinical and prognostic significance in idiopathic pulmonary fibrosis. The three markers were: soluble CD8 (from activated suppressor-cytotoxic lymphocytes), soluble interleukin (IL)-2 receptors (from activated T cells and macrophages), and neopterin (from activated macrophages). Patients with idiopathic pulmonary fibrosis had higher levels of all three markers than the control subjects. Soluble IL-2 receptor and neopterin tended to be lower (though not significantly) in patients with idiopathic pulmonary fibrosis than in those with sarcoidosis, whereas soluble CD8 was similar in the two groups of patients. No correlation was found between soluble IL-2 receptors or soluble CD8 and the clinical, radiological, and physiological measures of disease activity or with clinical outcome (after a mean follow up of 23 months). Tumour necrosis factor levels were also determined. Only 30% of patients with idiopathic pulmonary fibrosis or sarcoidosis had detectable circulating tumour necrosis factor; these patients had a lower percentage of bronchoalveolar lavage fluid neutrophils in their lavage fluid. Tumour necrosis factor levels did not correlate with clinical measures of severity or outcome. Thus our data support the hypothesis that cell mediated alveolitis occurs in idiopathic pulmonary fibrosis. They do not, however, provide evidence to support the use of these markers of cell mediated immunity to monitor the clinical course in these patients.

Idiopathic pulmonary fibrosis or cryptogenic fibrosing alveolitis is a chronic inflammatory disease that affects the interalveolar walls and small airways of the lungs, eventually leading to severe fibrosis and respiratory insufficiency. The interstitial tissue damage is associated with infiltration by macrophages and lymphocytes, both of which are found in the alveolar spaces in addition to type II alveolar cells and neutrophils. Extensive fibroblastic proliferation and moderate collagen deposition may also be found.1

An immune mediated pathogenesis has been proposed because circulating immune complexes and antinuclear antibodies are frequently detected.5-9 Other investigators have suggested the occurrence of a T cell alveolitis, given the predominance in the lung of T cells showing the suppressor-cytotoxic phenotype (CD8) and expressing activation markers (HLA-DR, CD25).6-7

Recently Tomkinson and coworkers showed that changes in the levels of the soluble form of CD8 (a cell free 5 kD molecule) closely parallel the timing and magnitude of CD8+ T cell activation after stimulation, both in animal models and in human disease.8 Patients with chronic autoimmune inflammatory disease and those receiving exogenous antigenic stimulation often have high serum levels of soluble (s) IL-2 receptor (IL-2R, a cell free 45 kD molecule).8,9 Finally, neopterin, a metabolite of guanosine triphosphate produced and released by monocyte-macrophages stimulated by γ interferon, has been proposed as a reliable marker of cell mediated immunity.14

To assess the clinical and prognostic significance of these serological markers of cell mediated immunity, we have assessed the levels of soluble IL-2 receptor and CD8 and of neopterin in a group of patients with fibrosing alveolitis. We also tested the serum for the presence of tumour necrosis factor, as this cytokine, which is released by macrophages and T cells, stimulates neutrophil activation and behaves as a growth factor for fibroblasts, in addition to stimulating collagenase and prostaglandin E2 production by these cells.15

Methods

PATIENTS
We obtained serum from 20 patients (nine female) with idiopathic pulmonary fibrosis, with a mean age of 58 (range 29–75) years, 11 of whom were having immunosuppressive treatment. Serum was also obtained from 12 patients with sarcoidosis (seven female) with a mean age of 44 (range 33–55) years, seven of whom were having immunosuppressive treatment. Control serum was obtained from 20 healthy age and sex matched subjects (blood donors and normal elderly subjects selected according to the Senieur protocol).10

The diagnosis of idiopathic pulmonary fibrosis was made from clinical, radiological, physiological, and histological findings. The
diagnosis of sarcoidosis was confirmed histo-
logically.17 All patients with idiopathic pul-
monary fibrosis or sarcoidosis had a trans-
bronchial or open lung biopsy. No associated
well defined connective tissue disease was
present in the patients with idiopathic pul-
monary fibrosis ("lone" idiopathic pulmonary
fibrosis). There was no history of occupational
exposure or of sensitivity changes to drugs or
organic dust. Clinical, radiological, scintigraphic,
physiological, and pathological data from the
patients with idiopathic pulmonary fibrosis
are shown in the table.

Gallium-67 scans were scored as described
by Nosal et al18 and the histological appear-
ances of lung tissue according to the system
of Watters et al.19 The single breath transfer
factor for carbon monoxide (TLCO) corrected
for alveolar ventilation (TLCO/VA) was measured
as described,20 and results are given as a
predicted. Clinical improvement was also cal-
culated serially from a clinical-radiographic-
physiological score (CRP), as proposed by
Watters et al.19 The score is derived from
seven variables, each weighted according to an
estimate of its relative importance derived
from published reports. The components and
the corresponding percentages of the score are:

1. historically derived activity required to
cause dyspnoea (20%) 
2. chest radiograph (10%) 
3. forced vital capacity (10%) 
4. forced expiratory volume in one second
(5%) 
5. thoracic gas volume or functional residual
capacity (10%) 
6. transfer factor for carbon monoxide (5%) 
7. the resting arteriovenous oxygen gradient
(A-aPo2) (10%) 
8. exercise induced reduction in oxygen
saturation indexed to the fraction of
predicted maximal oxygen consumption
achieved during exercise (30%).

The score rises as impairment increases; the
maximum score is 100.

After a mean 23 (SEM 3-9) months all
patients were reassessed clinically; 12 had
deteriorated (nine died), six were stable, and
two had improved. Clinical assessment of
improvement or deterioration was made
according to whether one or more of the
following changes had occurred: a change in
dyspnoea score of more than 6, a change in
FVC, predicted of more than 8, a change in
CRP score of more than 10.

Bronchoalveolar lavage specimens were
obtained from 14 patients with idiopathic pul-
monary fibrosis by lavaging the right middle
lobe through a flexible fibreoptic broncho-
scope with 60 ml aliquots of normal saline
buffered to pH 7-0 with 8-4 pH sodium bicar-
bonate.21 The mean (SD) volume of fluid
introduced was 360 (66) ml and of fluid
retrieved was 124 (36) ml. A centrifuged
specimen stained with Wright-Giemsa was
used to obtain a differential cell count (table).

### ASSAYS OF SOLUBLE CD8 AND IL-2R AND OF
tumoR necrosis factor

Serum concentrations of sCD8 and sIL-2R
and of tumour necrosis factor were assessed
with commercially available enzyme linked
immunoassays (T Cell Sciences, Cambridge,
Massachusetts), which use two monoclonal
antibodies directed against different epitopes
of the molecule.22-25 The tests were per-
formed according to the manufacturer's instruc-
tions. All serum samples were tested in
duplicate. Absorbance values from patients'
samples were plotted on a standard curve
obtained from reference samples. The stan-
dard curves contained 0, 100, 400, 1000, and
2000 units of soluble CD8; 0, 400, 800, and
1600 units/ml of sIL-2R; and 0, 40, 150, 500
and 1000 pg/ml of recombinant human

tumour necrosis factor. The detection limit of
the assays was 10 pg/ml. rTNF (1 mg) used as

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CRP—clinical, radiological, pathological score (see under "Methods"); a—cellular infiltration score (0-21); b—fibrosis score (0-24); c—total score (0-45); FVC—forced vital capacity; TLCO—carbon monoxide transfer factor; M—macrophages; L—lymphocytes; N—neutrophils; E—eosinophils; ND—not determined.

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Clinical, radiological, scintigraphic, physiological, and pathological data from patients with idiopathic pulmonary fibrosis
sCD8 (U/ml)

Figure 1 Soluble (s) CD8 serum levels in controls and patients.
○ Untreated; □ corticosteroid treated; ▪ not corticosteroid treated; NC—normal controls; IPF—patients with idiopathic pulmonary fibrosis; Sarc—patients with sarcoidosis.

sIL-2R (U/ml)

Figure 2 Interleukin (IL)—2R serum levels in controls and patients. Symbols and abbreviations as in figure 1.

Neopterin (nmol/l)

Figure 3 Neopterin serum concentrations in controls and patients. Symbols and abbreviations as in figure 1.

Results
The concentrations of sCD8, sIL-2R, and neopterin were significantly greater in patients with idiopathic pulmonary fibrosis than in the control subjects (figs 1–3). Patients with sarcoidosis had higher concentrations of sIL-2R and neopterin than did those with idiopathic pulmonary fibrosis, though the differences were not significant; sCD8 was similar in the two groups. The patients with idiopathic pulmonary fibrosis who were receiving corticosteroids had lower concentrations of sIL-2R (mean 574 (SD 253·2) U/ml) than the untreated patients (850 (401) U/ml; p < 0·05). sCD8 and neopterin concentrations did not differ in the patients who were and were not receiving corticosteroids. Detectable levels of tumour necrosis factor were present in seven of 20 patients with idiopathic pulmonary fibrosis and in four of 12 patients with sarcoidosis (fig 4). sCD8 and sIL-2R concentrations did not correlate with the clinical, radiological, and physiological data (shown in the table).

Neopterin concentrations correlated negatively with pathological scores and positively with FVC % predicted. Patients with detectable circulating tumour necrosis factor had a lower percentage of neutrophils in lavage fluid (those with tumour necrosis factor: mean 8·8% (SD 5·1%) neutrophils; those without: 15·1% (5·3%); p < 0·05). There was a linear correlation (r = 0·32, p < 0·05) between sIL-2R and neopterin concentrations in the patients with idiopathic pulmonary fibrosis. There was no correlation between sCD8, sIL-2R, neopterin, or tumour necrosis factor and the clinical outcome at the end of the follow up period.

Discussion
Activated T lymphocytes have recently been found in lung biopsy specimens from patients with idiopathic pulmonary fibrosis.8 T cells
with the suppressor-cytotoxic phenotype (CD8+) constituted the great majority of the cells in alveolar septa and in the mucosa of small inflamed airways.6

CD8+ T cell activation is associated with a parallel release of CD8 protein, which can be measured in the serum of patients.27 Our patients with idiopathic pulmonary fibrosis had increased concentrations of serum sCD8, which probably reflected the presence of activated suppressor-cytotoxic cells in the lung parenchyma.

Other markers of cell mediated immunity, sIL-2R and neopterin, are also increased in the sera of patients with idiopathic pulmonary fibrosis. The positive correlation between concentrations of sIL-2R and neopterin could reflect primary activation of monocytes/macrophages because expression of IL-2R has been found on normal and malignant monocyes,28 and neopterin is a guanosine triphosphate metabolite synthesised by γ interferon activated macrophages.29 Serum concentrations of sIL-2R and neopterin tended to be higher in patients with sarcoidosis than those in patients with idiopathic pulmonary fibrosis, as has been shown previously.11

The fact that sCD8 concentrations were similar in the two interstitial lung diseases is interesting; it suggests that there is a higher percentage of activated CD8+ cells in patients with idiopathic pulmonary fibrosis than in those with sarcoidosis.

The lack of correlation between sCD8 or sIL-2R concentrations and the clinical, pathological, and physiological measures or the clinical outcome in the patients with idiopathic pulmonary fibrosis may be because the circulating concentrations of these markers does not mirror local inflammatory reactions completely. In sarcoidosis, where a trend towards higher concentrations of IL-2R was found, Lawrence and coworkers11 found only a weak association between IL-2R levels and gallium-67 lung scans among the various measures of disease activity that they evalu-ated. Alternatively, the lack of any correlation may be explained by a closer association of clinical severity with the degree of fibrosis than with inflammation. It is noteworthy that many of our patients had disease of relatively long duration. The finding that neopterin concentrations correlated positively with FVC "o, predicted values and inversely with total pathological scores supports this hypothesis; immune mediated inflammatory processes may burn out in the late (fibrotic) stage of the disease.

Activated T cells and macrophages synthesise and release a number of cytokines whose main function is immunoregulation. The biological activities30 of tumour necrosis factor would be compatible with a role in idiopathic pulmonary fibrosis. The finding of detectable amounts of tumour necrosis factor in serum in only a third of the patients with idiopathic pulmonary fibrosis may support a pathogenetic role for this cytokine in a few patients, particularly those with non-neutrophilic alveolitis. The association of tumour necrosis factor in the serum with a low percentage of neutrophils in lavage fluid is consistent with the recent finding that tumour necrosis factor promotes adherence of neutrophils and inhibition of chemotactic migration.31 In the patients with high concentrations of tumour necrosis factor neutrophils may be more inclined to adhere to the interstitium of the alveolar wall rather than migrate into the alveolar spaces.

In conclusion, the results of our study support the hypothesis that cell mediated immune phenomena occur in idiopathic pulmonary fibrosis, but none of the markers we studied provided a useful measure of clinical activity.

We thank Mrs Anne Collins for revising the English manuscript and Mrs Patrizia Rappini for typing assistance.


23 Rubin LA, Kurman CC, Biddison WE, Goldman ND, Nelson DL. A monoclonal antibody, 7G7/B6, binds to an epitope on the human interleukin-2 receptor that is distinct from that recognized by IL-2 or anti-Tac. Hybridoma 1985;4:91–102.


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Thorax 1990 45: 536-540
doi: 10.1136/thx.45.7.536

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