Effect of lung T lymphocytes on fibroblasts in idiopathic pulmonary fibrosis and extrinsic allergic alveolitis

Moises Selman, Georgina Gonzalez, Martin Bravo, Jose Sullivan-Lopez, Carlos Ramos, Martha Montano, Nora Barquin, Felipe Vadillo

Abstract

Increased fibroblast replication and interstitial collagen accumulation occur commonly in the interstitial lung disease that progress to fibrosis. The processes controlling lung fibrogenesis are not completely understood, however. This study was designed to analyse the influence of T lymphocytes from lung tissue obtained at open lung biopsy from four patients with idiopathic pulmonary fibrosis and four patients with extrinsic allergic alveolitis on fibroblast proliferation and collagen synthesis in vitro. Lung T cell supernatants from patients with both diseases induced a moderate but significant inhibition of human lung fibroblast cell line growth. In contrast, there was a clear difference in the effect of T cells from the two groups of patients in relation to collagen production. Lung T lymphocytes from all four patients with idiopathic pulmonary fibrosis produced a substantial increase in collagen synthesis (from 371% to 514% of control values), whereas T cells from three of the four patients with extrinsic allergic alveolitis induced a significant decrease in collagen production (to 35%, 36%, and 43% of control values); in the fourth case there was an increase in collagen synthesis but this was lower than that seen with T cells from any of the patients with idiopathic pulmonary fibrosis. Peripheral T cells from six patients and control subjects caused a small increase in fibroblast proliferation and no change in collagen synthesis. The findings suggest that at least two types of interaction occur between lung T cells and fibroblasts in these disorders. A variable degree of inhibition of cell proliferation is observed in response to lung T cell supernatants from patients with both idiopathic pulmonary fibrosis and extrinsic allergic alveolitis; a substantial increase in collagen synthesis is triggered by lymphokines from patients with idiopathic pulmonary fibrosis.

The development of interstitial lung fibrosis depends on a complex series of poorly understood cellular and molecular mechanisms. A critical step in this pathological response is the proliferation of fibroblasts followed by alterations in collagen metabolism that lead to

the excessive accumulation of interstitial collagens.¹⁻³ Much attention has been directed to the production of mediators that may be responsible for the unregulated replication of fibroblasts and the exaggerated synthesis of interstitial matrix proteins.

In chronic interstitial lung disease pulmonary macrophages are known to release at least three growth factors for fibroblasts spontaneously: fibronectin, alveolar macrophage derived growth factor, and platelet derived growth factor.⁴⁻⁷ All three growth factors have a potent effect on the duplication of fibroblasts, acting as competence or progression growth factors; only platelet derived growth factor, however, appears to modify collagen synthesis.⁸ These three mediators have been identified in macrophages, but the role of other inflammatory or immune cells in the development of lung fibrosis has not been evaluated.

Lung T lymphocytes are increased, in terms of absolute numbers, in almost all interstitial lung diseases, though their presence is more prominent in some disorders, such as sarcoidosis and extrinsic allergic alveolitis.⁹⁻¹¹ Analysis of these cells has almost always been restricted to changes in the regulatory subpopulations and their participation in the inflammatory response. Their possible role in the control of lung fibroblast proliferation and collagen synthesis has received little attention.

Recently, Cathcart *et al*¹² reported that circulating T cells from patients with idiopathic pulmonary fibrosis enhanced fibroblast replication more than T cells from normal control subjects, but they did not study collagen synthesis. Several in vitro studies have shown that lymphocytes from non-lung sources have the ability to stimulate or inhibit fibroblast migration, proliferation, and collagen synthesis, depending on the experimental conditions.¹³⁻¹⁶ These observations suggest that, in addition to the known immune and inflammatory functions, T lymphocytes may have a role in fibrogenesis regulation. Many of these studies were performed with peripheral blood mononuclear leucocytes, however, and because the source of lymphocytes may not be representative of the lung inflammatory response the meaning of these findings is not clear.

The aim of the present study was to examine the influence of T lymphocytes, obtained from the pulmonary parenchyma obtained at open lung biopsy from patients with idiopathic pulmonary fibrosis and extrin-

Instituto Nacional de
Enfermedades
Respiratorias

M Selman
G Gonzalez
M Bravo
J Sullivan-Lopez
C Ramos
M Montano
N Barquin

Instituto Nacional de
Perinatología, Mexico
DF, Mexico
P Vadillo

Address for reprint requests:
Dr Moises Selman, Instituto
Nacional de Enfermedades
Respiratorias, Tlalpan 4502,
CP 14080, Mexico DF,
Mexico.

Accepted 22 February 1990

sic allergic alveolitis, on fibroblast proliferation and collagen synthesis *in vitro*.

Methods

STUDY POPULATION

We studied four patients with idiopathic pulmonary fibrosis (mean (SD) age 55 (10) years), three of them female, and four female patients with chronic extrinsic allergic alveolitis (mean age 34 (6) years). None of the subjects smoked. The duration of the disease ranged from seven to 12 months for patients with extrinsic allergic alveolitis, and from 10 to 18 months in idiopathic pulmonary fibrosis.

The diagnoses of both diseases was based on history, clinical and radiological features, and the functional patterns of interstitial lung disease according to previously described criteria,^{2,9,17} and confirmed by the histological analysis of a lung biopsy specimen obtained through an open thoracotomy. No patient had systemic manifestations or was receiving treatment before biopsy. We did not include subjects with normal lungs because few lymphocytes are obtained in a tissue biopsy sample.⁹ We used peripheral blood T cells obtained from four healthy volunteers (mean age 43 (9) years; two female) as controls.

PURIFICATION OF LUNG T CELLS

Tissue samples were treated with trypsin (Gibco, Grand Island, New York) 0.025% for 20 minutes with continuous agitation. The supernatants were discarded and the tissues were treated again in the same manner. Afterwards 1% fetal calf serum was added to inhibit enzymatic activity. The cells were washed with RPMI 1640 and mononuclear cells were separated by gradient density centrifugation (700 *g*, 30 min, room temperature) of the cell suspension diluted 1:1 with ficoll-hypaque (Pharmacia, Piscataway, New Jersey).

To obtain T lymphocytes, the lung mononuclear cells were washed twice in RPMI 1640, suspended at 1×10^4 cells/ml, and mixed with 0.1 ml sheep red blood cells treated with 2-aminoethylisothiuronium bromide (Sigma, St Louis, Maryland). The mixture was first incubated for 15 minutes at 37°C and then for four hours at 4°C, resuspended and subjected to the same gradient density centrifugation. The pelleted sheep red blood cell rosetting cells were treated with an ammonium chloride solution (0.14 M NH_4Cl in phosphate buffered saline) to lyse the red cells, and the T lymphocytes were recovered after being washed three times in RPMI 1640. After this procedure the T lymphocyte purity was greater than 98% according to indirect immunofluorescence staining with the monoclonal antibody OKT3 (Ortho Pharmaceutical Corporation). In addition, the T cell population showed more than 95% viability by the trypan blue exclusion-phase contrast criterion.

Isolated T cells were suspended to a final concentration of 1×10^5 cells/ml in RPMI

1640 with 1% fetal calf serum, pH 7.4, supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. They were then incubated in 24 well cluster dishes at 37°C for 24 hours in 5% carbon dioxide, 95% air atmosphere. The supernatants were collected and stored at -70°C until they were tested for their effects on fibroblast proliferation and collagen synthesis.

In addition, in three patients with extrinsic allergic alveolitis, three with idiopathic pulmonary fibrosis, and four normal, healthy volunteers, blood samples were obtained and T cells were isolated and assayed as described above.

ASSAY FOR FIBROBLAST MITOGENIC ACTIVITY

Normal human lung fibroblasts (MRC-5) obtained from the American Type Culture Collection were used as target cells to assess the degree of fibroblast proliferation induced by lung T cell supernatants. The cells were maintained in Earle modified Eagle's medium (Flow, McLean, Virginia) containing 10% fetal calf serum and supplemented with non-essential aminoacids, 50 U/ml penicillin, and 50 µg/ml streptomycin and subcultured by trypsinisation. Fibroblasts were used in passage 20. After 2.5×10^3 cells/well had been seeded in a 96 flat bottom microtitre well plate (Flow), the cells were incubated for three days in the same conditions. At this time 5×10^3 cells/well were present in subconfluency. The medium was then substituted by serum free medium (100 µl/well) and 100 µl/well of T cell supernatants were added (the final concentration of fetal calf serum was 0.5%). Control samples consisting of medium with 0.5% fetal calf serum and medium with 7.5% fetal calf serum were incubated simultaneously. As a positive control we also used 2.5 ng/ml of purified platelet derived growth factor.

Each experimental condition was examined in triplicate. Fibroblasts were then cultured for 24 hours. Eight hours before the end of the incubation period DNA synthesis was assessed by labelling the cells with $1 \mu\text{Ci}$ ^3H thymidine (New England Nuclear, Boston, Massachusetts). At the end of the incubation period the medium was discarded and the bottom layer cells washed once with cold 0.15 M sodium chloride and then four times with cold distilled water, to eliminate all free ^3H thymidine. Fibroblasts were treated with 150 µl of 10% trichloroacetic acid solution for 15 minutes, and with 150 µl of warm 0.1% sodium dodecyl sulphate solution for another 15 minutes for lysis. The cells of each well were harvested on to glass filter strips with an automatic cell harvester. The filter discs were immersed in scintillation fluid and counted in a beta scintillation counter (Beckman LS 100 C). The mean uptake of ^3H thymidine in the presence of medium with 0.5% fetal calf serum was 1237 (SD 106) cpm and in the presence of 7.5% fetal calf serum 3938 (408) cpm. The data are expressed in terms of cpm/well, and of a growth index obtained as follows:

$$\frac{(\text{cpm in presence of T cell supernatants}) - (\text{cpm in media plus 0.5\% FCS})}{(\text{cpm in media plus 7.5\% FCS}) - (\text{cpm in media plus 0.5\% FCS})} \times 100,$$

where FCS = fetal calf serum.

COLLAGEN SYNTHESIS ASSAY

The medium of confluent fibroblasts cultured in 24 well plates was replaced with 0.4 ml of fresh medium containing 0.5% fetal calf serum. One hundred microlitres of lung T lymphocyte conditioned medium, as described above, were added to the wells. Basal controls contained medium plus 0.5% fetal calf serum and the positive induction controls 2.5 ng/ml of platelet derived growth factor. All experiments were performed in triplicate. After 20 hours' incubation at 37°C in 95% air and 5% carbon dioxide atmosphere, a mixture of 10 µg/ml β aminopropionitrile, 50 µg/ml L-ascorbic acid, and 20 µCi ³H proline was added to each well. The incubation was continued for four hours, 20 mM N-ethylmaleimide (NEM), 1 mM phenylmethylsulphonyl fluoride (PMSF), and 100 mM ethylenediamine tetracetic acid (EDTA) (all final concentrations) were added to each well and all media were recovered in separate phials. Two hundred microlitres of 0.1% Triton X-100 were added to each well and after one hour at 4°C the lysed cells and the supernatants were collected and pooled. The samples were dialysed exhaustively against water containing 2.5 mM NEM, 10 mM EDTA, 0.1 mM PMSF, and 0.02% sodium azide at 4°C until all free radioactive material was eliminated. After a final dialysis against 0.05 M Tris-HCl, pH 7.4, the samples were separated into two equal parts and assayed to quantify collagen production with highly purified bacterial collagenase (type VII, Sigma Company, St Louis, Missouri), with a slight modification of the method suggested by Peterkofsky and Diegelman¹⁸; 40 µg/ml of collagenase and 5 mM CaCl₂ were added to one tube (collagenase tube) and 0.05 M Tris-HCl, pH 7.4, and 100 mM EDTA to the control tube. NEM was added to both tubes at a final concentration of 2.5 mM. Samples were incubated for 20 hours at 37°C and 100 mM EDTA plus 1 mg/ml of collagen type I purified from rat tendon were subsequently added to the collagenase tube. Finally, 50% dioxane was added to both tubes. The samples were placed at 4°C for one hour and centrifuged at 10 000 g for 10 minutes. The supernatants were counted

in a Beckman LS 100 C scintillation counter. The amount of incorporated ³H OH-proline, as an index of newly synthesised collagen, was obtained by subtracting the cpm of the control tube from the cpm of the collagenase tube. Results were expressed as mean (SD) cpm/1 × 10⁵ fibroblasts and as percentages of control fibroblasts.

STATISTICAL ANALYSIS

The significance of the difference between values obtained from experimental and control fibroblasts was tested by Student's *t* test. Probabilities of 0.05 or less were considered significant.

Results

EFFECT OF T CELL SUPERNATANT ON FIBROBLAST GROWTH INDEX

The effects of exposure to lung T lymphocyte supernatants on fibroblast replication are shown in table 1. Under subconfluent culture conditions there was a moderate but significant inhibition of cell growth in the eight cases studied by comparison with control levels. After 24 hours of culture the growth index (%) ranged from -6.8 to -19.5 (mean (SD) -15.2 (5.8)) for T lymphocyte supernatants from patients with idiopathic pulmonary fibrosis, and from -12 to -27.7 (mean (SD) -21.1 (6.9)) for those from patients with extrinsic allergic alveolitis. The inhibition of fibroblast duplication was significant in all patients (*p* < 0.05- < 0.01).

To determine whether toxicity derived from T cell protein products might have influenced these results, fibroblast viability was assayed at the end of the incubation period by the trypan blue exclusion technique. Cell viability was greater than 95%, and was similar in the experimental and control wells.

In contrast to the findings with lung T cell supernatants, there was increased fibroblast proliferation in cultures containing peripheral T cell lymphokines from the six patients studied, the mean (SD) percentage increase being 23.5 (8.3) for patients with idiopathic pulmonary fibrosis and 17.6 (5.5) for those with extrinsic allergic alveolitis. T cell supernatants from control subjects caused a mean 13.8% (6.3%) increase in fibroblast duplication.

Finally, under the same culture conditions, 2.5 ng/ml of purified platelet derived growth factor, used as a positive control, enhanced the rate of DNA synthesis and induced fibroblast replication of 74% (table 1).

EFFECT OF T CELL SUPERNATANTS ON FIBROBLAST COLLAGEN SYNTHESIS

This analysis was performed under confluent culture conditions in stationary growth fibroblasts with low serum concentration. Under these conditions fibroblasts are sensitive to factors that influence collagen synthesis but are unresponsive to growth modulating signals.

In contrast to the findings with fibroblast replication, there was a clear difference between the response of these cells to unstimulated T lymphocyte samples obtained

Table 1 Effect of lung T cell supernatant on fibroblast replication in patients with idiopathic pulmonary fibrosis (IPF) and extrinsic allergic alveolitis (EAA)

Diagnosis	Cpm/well (mean (SD))	Growth index (%)	<i>p</i> value*
IPF	708 (57)	-19.5	<0.01
IPF	967 (66)	-6.8	<0.05
IPF	806 (101)	-15.9	<0.01
IPF	737 (92)	-18.5	<0.01
EAA	912 (73)	-12.0	<0.05
EAA	555 (105)	-25.2	<0.01
EAA	487 (94)	-27.7	<0.01
EAA	706 (63)	-19.6	<0.01
Platelet derived growth factor (2.5 ng/ml)	3236 (321)	74.0	
Control	1237 (106)		

*Obtained from the differences in cpm/well between experimental and control wells.

Table 2 Effect of lung T cell supernatants on collagen synthesis in patients with idiopathic pulmonary fibrosis (IPF) and extrinsic allergic alveolitis (EAA)

Diagnosis	Cpm $\times 10^5$ fibroblasts (mean (SD))	% of control	p value*
IPF	4253 (159.5)	371	<0.01
IPF	3874 (1110)	338	<0.05
IPF	5890 (1610)	514	<0.01
IPF	5558 (1572)	485	<0.01
EAA	2147 (297)	187	<0.05
EAA	405 (106)	35	<0.05
EAA	421 (119)	36	<0.05
EAA	492 (101)	43	<0.05
Platelet derived growth factor (2.5 ng/ml)	5521 (1637)	481	
Control	1145 (325)		

*Obtained from the differences in cpm/well between experimental and control wells.

from patients with idiopathic pulmonary fibrosis and from patients with extrinsic allergic alveolitis (table 2). At a concentration of 100 μ l/ml there was a substantial increase in collagen synthesis in the cultures exposed to T lymphocyte supernatants from the four patients with idiopathic pulmonary fibrosis. The percentage increase in collagen synthesis varied from 371 to 514 over control values (mean (SD) 427 (85)). When lung T cells from patients with extrinsic allergic alveolitis were used there was a significant decrease in collagen production in three patients (to 35%, 36%, and 43% of control values), whereas the fourth patient showed an increase in collagen production (187% over the control value), though it was still lower than that seen in any of the experiments using T lymphocyte supernatants from patients with idiopathic pulmonary fibrosis.

Supernatants from peripheral blood T cells from the six patients studied and the normal subjects caused no significant changes in collagen synthesis, mean (SD) values being 1225 (228) cpm for idiopathic pulmonary fibrosis, 1036 (207) cpm for extrinsic allergic alveolitis, and 1241 (302) cpm for healthy individuals, compared with a control value of 1145 (325) cpm.

The results of fibroblast viability at the end of the assay indicated that the inhibition of 3 H hydroxyproline-protein synthesis after exposure to T cell supernatants from the three patients with extrinsic allergic alveolitis was not due to a toxic effect on the fibroblasts.

Discussion

Increased fibroblast proliferation and interstitial collagen accumulation occur commonly in the interstitial lung diseases that progress to fibrosis.

As much evidence suggests that soluble mediators released by inflammatory cells, mainly macrophages,⁴⁻⁷ affect fibroblast behaviour during lung fibrogenesis, most studies in this field have focused on macrophage-fibroblast interactions. The role of lung T lymphocytes in the development of fibrosis in interstitial lung diseases has not been evaluated. The present investigation was designed to analyse possible interactions between lung T lymphocytes obtained from patients

with idiopathic pulmonary fibrosis and extrinsic allergic alveolitis and normal lung fibroblasts.

In our study supernatants from lung T cells obtained from patients with idiopathic pulmonary fibrosis increased collagen synthesis several fold. This effect was observed when they had been exposed to the supernatants of unstimulated cells after incubation for only 24 hours. In contrast, the T cell supernatants from three of the four patients with extrinsic allergic alveolitis decreased collagen synthesis. Collagen production was stimulated in the fourth patient, though the rate was still lower than that seen after exposure to T cell supernatants from all the patients with idiopathic pulmonary fibrosis. T cells obtained from peripheral blood of patients with idiopathic pulmonary fibrosis and extrinsic allergic alveolitis and from normal subjects did not modify collagen synthesis, suggesting that the changes observed are due to activation of lung T cells.

Although more patients are needed before firm conclusions can be drawn, a dissimilar stimulus received by T cells from patients with allergic alveolitis and idiopathic pulmonary fibrosis or different immune cell subpopulations (or both) seem to produce a different effect on collagen production. The modifications that occur in the lung parenchyma microenvironment in patients with idiopathic pulmonary fibrosis induce T lymphocytes to produce a factor or factors that increase fibroblast collagen synthesis, whereas in extrinsic allergic alveolitis T lymphocytes are modified to inhibit collagen production. These results support our previous findings, which suggested that alterations in collagen degradation, but not in collagen synthesis, have a critical role in the progression to interstitial fibrosis in some patients with chronic extrinsic allergic alveolitis.¹⁷ The cell surface phenotype of the E rosetted cells could not be analysed in this investigation owing to the small number of lung lymphocytes; in a previous study in our laboratory the ratio of helper (CD4⁺) to suppressor (CD8⁺) lymphocytes in lung tissue differed in the two diseases, being 0.61 in patients with allergic alveolitis and 1.1 in those with idiopathic pulmonary fibrosis.⁹ We are unable to say whether such differences in T cell populations are responsible for the different behaviour of T cells with respect to collagen synthesis in the present investigation.

The products of lung T lymphocytes from patients with idiopathic pulmonary fibrosis and extrinsic allergic alveolitis did not activate fibroblast proliferation. Our results suggest that signals are released that decrease fibroblast replication slightly. In contrast, the supernatants from peripheral T cells stimulated fibroblast replication, as described previously.¹² The use of lung T cells is likely to represent conditions in the lung in patients with interstitial lung disease more precisely.

It has been proposed that the antireplicative effect of mononuclear cell derived inhibitors on fibroblast proliferation is due to activation of prostaglandin E₂ synthesis by fibroblasts.¹⁹

Inhibition of lung fibroblast replication and collagen synthesis by alveolar macrophages appears to be mediated by prostaglandin E_2 ,¹⁹⁻²¹ which is apparently produced by the fibroblast itself. The effect we observed with lung T lymphocyte supernatants from patients with idiopathic pulmonary fibrosis do not appear to have been induced by prostaglandin E_2 because decreased fibroblast replication was accompanied by a substantial increase in collagen synthesis.

Our preliminary results in this small group of patients suggest at least two types of interaction between T cells and fibroblasts. Firstly, a variable degree of inhibition of cell proliferation is observed with lung T cell supernatants from patients with idiopathic pulmonary fibrosis and extrinsic allergic alveolitis and, secondly, a substantial increase in collagen synthesis occurs in response to lung T lymphocytes in patients with idiopathic pulmonary fibrosis.

Fibroblasts have a major role in lung fibrogenesis because they replicate at the site of tissue injury and secrete collagenous proteins. Although there is some evidence that "fibrotic" fibroblasts may function autonomously,²² their behaviour in vivo must be the net effect of both stimulatory and inhibitory mediators in the damaged tissue. The secretory products of lymphocytes take part in many immunological and inflammatory processes. Our findings provide some evidence that they have a role in the development of fibrosis, at least in the evolution of idiopathic pulmonary fibrosis.

- 1 Goldstein RH, Fine A. Fibrotic reactions in the lung: the activation of the lung fibroblast. *Exp Lung Res* 1986;11:245-61.
- 2 Selman M, Montañó M, Ramos C, Chapela R. Concentration, biosynthesis and degradation of collagen in idiopathic pulmonary fibrosis. *Thorax* 1986;41:355-9.
- 3 Laurent GJ. Lung collagen: more than scaffolding. *Thorax* 1986;41:418-28.
- 4 Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG. Production of fibronectin by the human alveolar macrophage: mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung diseases. *Proc Natl Acad Sci USA* 1981;78:7147-51.
- 5 Bitterman PB, Adelberg S, Crystal RG. Mechanisms of pulmonary fibrosis. Spontaneous release of the alveolar macrophage-derived growth factor in the interstitial lung disorders. *J Clin Invest* 1983;72:1801-13.
- 6 Martinet Y, Rom WN, Grotendorst GR, Crystal RG. Exaggerated and spontaneous release of platelet-derived growth factor by alveolar macrophages from patients with idiopathic pulmonary fibrosis. *N Engl J Med* 1987;317:202-9.
- 7 Avila R, Selman M, Bravo M, Antoniadis H. Detection of platelet-derived growth factor in situ in human pulmonary fibrosis by indirect immunofluorescence [abstract]. *J Cell Biol* 1987;105:275A.
- 8 Ross R, Raines EW, Bowen-Pope DF. The biology of platelet-derived growth factor. *Cell* 1986;46:155-69.
- 9 Barrios R, Selman M, Franco R, Chapela R, Sullivan-López J, Fortoul T. Subpopulations of T cells in lung biopsies from patients with pigeon breeder's disease. *Lung* 1987;165:181-7.
- 10 Crystal RG, Bitterman PB, Rennard SI, Hance AJ, Keogh BA. Interstitial lung diseases of unknown cause. Disorders characterized by chronic inflammation of the lower respiratory tract. *N Engl J Med* 1984;310:235-44.
- 11 Crystal RG, Gadek JE, Ferrans VJ, Fulmer JD, Line BR, Hunninghake GW. Interstitial lung disease: current concepts of pathogenesis, staging and therapy. *Am J Med* 1981;70:542-68.
- 12 Cathcart MK, Edmur LI, Ahtiala-Stewart K, Ahmad M. Excessive helper T-cell function in patients with idiopathic pulmonary fibrosis: correlation with disease activity. *Clin Immunol Immunopathol* 1987;43:382-94.
- 13 Jimenez SA, Rosenbloom J. Production of collagen synthesis inhibitory lymphokine by human leukemic T-lymphocyte cell lines. *Immunol Lett* 1985;11:69-74.
- 14 Parrott DP, Goldberg RL, Kaplan SR, Fuller GC. The effect of lymphokines on proliferation and collagen synthesis of cultured human synovial cells. *Eur J Clin Invest* 1982;12:407-15.
- 15 Postlethwaite AE, Smith GN, Mainardi CL, Seyer JM, Kang AH. Lymphocyte modulation of fibroblast function in vitro: stimulation and inhibition of collagen production by different effector molecules. *J Immunol* 1984;132:2470-7.
- 16 Seyer JM. Mediators of increased collagen synthesis in fibrosing organs. *Fund Appl Toxicol* 1985;5:228-39.
- 17 Selman M, Montañó M, Ramos C, Chapela R, González G, Vadillo F. Lung collagen metabolism and the clinical course of hypersensitivity pneumonitis. *Chest* 1988;94:347-53.
- 18 Peterkofsky B, Diegelman R. Use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. *Biochemistry* 1971;10:988-94.
- 19 Elias JA, Rossman MD, Zurier RB, Daniele RP. Human alveolar macrophage inhibition of lung fibroblasts growth. A prostaglandin dependent process. *Am Rev Respir Dis* 1985;131:94-9.
- 20 Bitterman PB, Wewers MD, Rennard SI, Adelberg S, Crystal RG. Modulation of alveolar macrophage-derived fibroblast proliferation by alternative macrophage mediators. *J Clin Invest* 1986;77:700-8.
- 21 Goldstein RH, Polgar P. The effect and interaction of bradikinin and prostaglandin on protein and collagen production by lung fibroblasts. *J Biol Chem* 1982;257:8630-3.
- 22 Jordana M, Schulman J, McSharry C, et al. Heterogeneous proliferative characteristics of human adult lung fibroblast lines and clonally derived fibroblasts from control and fibrotic tissue. *Am Rev Respir Dis* 1988;137:579-84.