Bronchoalveolar lavage in patients with mild and severe rheumatoid lung disease

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Abstract

The reported prevalence of interstitial lung disease in patients with rheumatoid arthritis has varied from 10% to 50%, yet less than 5% of patients with arthritis develop severe fibrosing interstitial lung disease. This suggests that subclinical disease may not always presage progressive disease. Bronchoalveolar lavage fluid from patients with rheumatoid arthritis and either clinically evident interstitial lung disease or subclinical disease was examined for the presence of factors with a putative role in the development of interstitial fibrosis. Patients with subclinical disease were identified by prospective radiographic and lung function screening of 93 patients with rheumatoid arthritis. Fourteen patients were identified in this manner and an association between subclinical disease and smoking history was noted. Eleven patients with established interstitial lung disease had increased neutrophils ($p < 0.05$), collagenase, and type III procollagen N terminal peptide levels ($p < 0.01$) in the bronchoalveolar lavage fluid. Preliminary characterisation of the bronchoalveolar lavage collagenase suggested that it originated from neutrophils. Ten patients with subclinical interstitial lung disease underwent bronchoalveolar lavage. Of these, one had increased neutrophils and two had increased collagenase concentrations—abnormalities associated with advanced interstitial lung disease and a poor prognosis. These results suggest that in arthritis patients with evidence of subclinical pulmonary interstitial disease bronchoalveolar lavage might be useful in identifying those who may require careful monitoring in the hope that early treatment will prevent severe fibrosis.

Interstitial lung disease is the most common and most serious pleuropulmonary complication of rheumatoid arthritis.¹ Patients may develop a severe, progressive fibrosing alveolitis which is clinically and histologically similar to cryptogenic fibrosing alveolitis.² Screening of patients with rheumatoid arthritis for interstitial lung disease shows prevalences of 10–50%.^{3–6} This wide variation probably reflects differences in the methods used to assess lung disease and in the popula-

tions of patients examined. Whichever value is accepted, the proportion of patients with rheumatoid arthritis who develop severe fibrosing alveolitis is clearly lower. Patients identified by screening are usually symptomless and have mild radiographic or pulmonary function abnormalities.^{3,4,6} It would be of great clinical benefit to identify patients in whom mild pulmonary abnormality is likely to progress to more advanced disease.

Bronchoalveolar lavage allows detection of inflammation in the lower respiratory tract and may provide prognostic information in cryptogenic fibrosing alveolitis.^{7,8} Bronchoalveolar lavage studies in patients with rheumatoid arthritis and established interstitial lung disease have shown abnormalities similar to those of cryptogenic fibrosing alveolitis.^{9,10} Bronchoalveolar lavage in patients with rheumatoid arthritis but little or no lung disease showed lymphocytic alveolitis in some patients, which it was suggested might proceed to more severe lung disease.^{9,11} Other studies, however, suggest that increased lymphocytes in bronchoalveolar lavage fluid are associated with a good pulmonary prognosis in patients with collagen vascular disorders¹² and cryptogenic fibrosing alveolitis.⁷ Assessment of lavage fluid from patients with rheumatoid arthritis for markers implicated in the development of severe fibrosing disease may help to identify patients likely to develop serious pulmonary complications. In this study the levels of several such markers—neutrophils, collagenase, type III procollagen peptide, and fibronectin^{7,13–15}—were determined in lavage fluid from patients with rheumatoid arthritis with and without established interstitial lung disease.

Methods

PATIENTS

We prospectively screened 93 patients with rheumatoid arthritis as defined by the American Rheumatism Association¹⁶ for subclinical pulmonary abnormalities. We selected them from consecutive patients attending a rheumatology outpatient clinic or admitted to an inpatient rehabilitation unit during October 1987–May 1988. Patients were excluded if they were under 20 or over 65 years, if there was any doubt about the diagnosis of rheumatoid arthritis, or if they were severely debilitated—that is, grade 4 on the American Rheumatism Association classification.¹⁶ Posteroanterior and lateral chest radiographs were obtained. Interstitial changes were classified according to

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the International Labour Office (ILO) classification.¹⁷ Spirometry was performed with either a bellows vitalograph or a portable electronic vitalograph. Values were expressed as a percentage of the normal for sex, age, and height.¹⁸ This system of screening was chosen because it could be performed during a follow up assessment in a rheumatology clinic. A patient was considered to show evidence of interstitial lung disease if two observers agreed that there was an interstitial infiltrate on the chest radiograph or if the forced vital capacity (FVC) was under 80% while the FEV₁/FVC ratio was normal (that is, over 70%).

For each patient we obtained a detailed history of the diagnosis, course, and treatment of their rheumatoid arthritis and current respiratory symptoms and also a smoking history. A physical examination was performed to determine the extent of joint lesions and the presence or absence of subcutaneous nodules, finger clubbing, and chest signs.

Ten patients with evidence of subclinical lung disease underwent bronchoalveolar lavage. Eleven patients with known interstitial lung disease and rheumatoid arthritis were identified from the records of bronchoalveolar lavage performed by us from 1984 to 1988. These patients and their hospital case notes were reviewed (notes only if the patient had died). Identical information to that of the screened patients was obtained plus details of the course and therapy of their pulmonary disease. Four healthy volunteers and 11 hospital inpatients who had no lung disease were included as a control group. They had normal results in their pulmonary function tests, performed before lavage.

The study was approved by the ethics committee of St Vincent's Hospital. Patients gave oral consent to the initial tests and written consent to bronchoscopy and bronchoalveolar lavage.

BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage was performed during fiberoptic bronchoscopy under local anaesthesia. The lavage was performed in a segmental or subsegmental right middle lobe bronchus. Premedication with atropine (0.6 mg) and pethidine (50 mg) was routine and further sedation with 5–10 mg intravenous diazepam was given as required. Before lavage the bronchus was anaesthetised with 2 ml of 1% lignocaine hydrochloride. The bronchoscope was wedged in a subsegmental bronchus and 180 ml of 0.9% saline (at 37°C) was infused in three 60 ml aliquots with gentle suction after each infusion. The volume of the aspirated fluid was recorded and the fluid strained through sterile surgical gauze to remove mucus. The fluid was then centrifuged at 400 *g* for 10 minutes and the supernatant stored at –20°C for subsequent analysis.

CELLULAR ANALYSIS

The recovered cells were washed twice in Hanks' balanced salt solution and resuspended in RPMI 1640 to a concentration of 2×10^6 cells/ml. Total T lymphocytes and T lymphocyte subsets were analysed by fluorescent microscopy, murine monoclonal antibodies being used.¹⁴ Differential counts of lymphocytes, macrophages, and neutrophils were performed on Wright-Giemsa stained cell smears by a second observer. Where there was a difference between the methods of over 10% for the percentage of lymphocytes an esterase stained smear was used to distinguish small macrophages from large lymphocytes.

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ANALYSIS OF LAVAGE FLUID

Before analysis fluid was centrifuged at 1000 *g* for 15 minutes, phenylmethylsulphonylfluoride (PMSF, final concentration 0.1 mmol/l) was added to prevent protease digestion, and the solution was concentrated 20 fold by ultrafiltration in CF25 Centrifo membrane cones (Amicon Corporation, Danvers, USA). Average protein recovery after concentration was 73.2% (*n* = 20, coefficient of variation = 18%). Fluid was analysed for collagenase, procollagen peptide, fibronectin, protein, and urea.^{14,19} As no satisfactory reference component for normalising lavage fluid proteins is available,²⁰ concentrations of collagenase, procollagen peptide, and fibronectin were calculated per ml of lavage fluid, per mg of bronchoalveolar lavage protein, and per μ g of bronchoalveolar lavage urea.¹⁴ Only where differences between groups were statistically significant for all three calculations were they accepted as being truly significant.

SUBSTRATE SPECIFICITY OF BRONCHOALVEOLAR LAVAGE COLLAGENASE

For investigating the substrate specificity of lavage fluid collagenase, samples containing collagenase were pooled and assessed for their ability to digest radiolabelled type I (specific activity 3×10^5 cpm) and type III (specific activity 8.4×10^5 cpm) collagen. Assay of type III collagen digestion was the same as for type I collagen¹⁴ except that all incubations were carried out for 24 hours. In addition, digestion of both types of collagen was performed at 25°C and digestion products were analysed by SDS-polyacrylamide gel electrophoresis.

STATISTICAL ANALYSES

Statistical analyses were performed with Statgraphics (Statistical Graphics Corporation, Rockville, Maryland, USA), a statistical software package for use with IBM personal computers. Comparisons were made by non-parametric statistical analysis (Wilcoxon rank test). Yates's corrected χ^2 test was used for comparison of proportions.²¹ Data on lavage fluid are expressed as medians and absolute ranges.

Results

Of the 93 patients screened for lung disease, 14 had subclinical interstitial lung disease. Their clinical features are shown in table 1. Three of the 14 patients had abnormal chest radiographs and restrictive impairment, one had restrictive impairment alone, and 10 had an abnormal chest radiograph without evidence of pulmonary restriction. The radiograph infiltrate was

Table 1 Clinical features of patients with rheumatoid arthritis (RA) found to have subclinical pulmonary disease at screening

Patient No	Age (y), sex	Duration (y) of RA	RF	ARA class	Treatment (not NSA)	Smoking history	Dyspnoea (NYHA)	Signs	Chest radiograph score*	FVC (% pred)	Overall BAL result
1	38 F	9	—	3	Au	Cr	1	—	S, 2/1	107	Normal
2	55 F	32	—	2	PC, Au, ST	Ex	1	—	T, 1/2	36	Increased % T cells
3	52 M	18	+	2	Au	Ex	2	cr	T, 1/2	90	Normal
4	58 M	2	+	2	—	Ex	1	—	T, 1/2	83	Not available
5	53 M	11	+	1	Au, PC, ST	Cr	1	—	T, 1/1	98	Increased collagenase
6	54 M	27	+	3	Au, ST, MX	Cr	1	Pleural rub, cl	Y, 2/2	83	Not available
7	52 F	11	—	2	Au	Never	1	cl	T, 1/2	71	Increased collagenase
8	62 M	6	+	3	ST	Ex	3	cl, cr	U, 2/2	35	Not available
9	54 M	18	+	2	Au	Ex	2	—	T, 2/2	84	Increased % neutrophils
10	59 F	1	+	2	Au	Cr	1	—	Y, 2/1	103	Not available
11	27 F	4	+	2	Au, PC, ST	Cr	1	—	0/0	61	Normal
12	60 M	12	+	3	Au, PC, ST	Cr	1	—	T, 1/1	93	Normal
13	38 F	5	+	2	Au	Cr	1	—	Y, 2/1	100	Normal
14	57 F	3	+	2	Au, ST	Cr	1	—	Y, 2/1	86	Normal
Mean (SD)	51 (10)	11 (9)		2.2 (0.6)			1.3 (0.6)			81 (23)	

*ILO.¹⁷

RF—rheumatoid factor; ARA—American Rheumatology Association; NSA—non-steroidal anti-inflammatory drug; Au—gold; PC—penicillamine; ST—steroid; MX—methotrexate; Cr—current smoker; Ex—previous smoker (> 6 months before); NYHA—New York Heart Association; cl—clubbing; cr—crackles; BAL—bronchoalveolar lavage.

usually bilateral, situated in the middle and lower zones, but varied in type.

Comparison of the clinical histories of patients with (n = 14) and without (n = 79) subclinical pulmonary disease indicated that the only significant difference between the two groups was a much higher frequency of current or past cigarette smoking in patients with pulmonary disease (p < 0.05; table 2). The clinical features of the 11 patients with established interstitial lung disease are shown in table 3. These patients had more symptoms, higher profusion scores on the chest radiograph, and worse pulmonary function than patients identified from screening. The proportion of current or past smokers among patients with established interstitial lung disease was similar to that in the screened group. The prognosis in the group with established disease was poor: five patients had died since initial bronchoalveolar lavage and three of the deaths were

related to interstitial lung disease or its treatment. Corticosteroid alone (n = 4) or corticosteroid plus azathioprine (n = 3) had not produced a sustained improvement in any patient. The addition of cyclophosphamide in one patient and methotrexate in another was associated with some improvement in pulmonary symptoms (table 3).

Bronchoalveolar lavage results from both groups of patients were compared with those from the 15 control subjects. Cellular analyses (table 4) showed no differences between the control and the rheumatoid arthritis group in the proportion of T lymphocytes recovered or the T cell phenotype. The proportion of neutrophils recovered was significantly greater in the patients with rheumatoid arthritis and established interstitial lung disease than in the control subjects. Only two patients with subclinical disease had abnormal cell counts (table 1). One (patient 9) had a raised neutrophil count (43% of inflammatory cells); the other (patient 2) had an increased proportion of T lymphocytes (52% of inflammatory cells).

The volume of bronchoalveolar lavage fluid recovered (table 4) was smaller in both rheumatoid arthritis groups. Patients with rheumatoid arthritis also had greater protein concentrations than control subjects; urea concentrations were similar in the three groups. Patients with established interstitial lung disease had a greater procollagen peptide concentration (p < 0.02) and collagenase activity (p < 0.01) in the lavage fluid than either the control group or those with subclinical disease (figure). These differences remained when results were expressed per mg of protein or per µg of urea in the lavage fluid. In patients with established interstitial lung disease collagenase activity and procollagen peptide concentration were positively correlated (r = 0.64, p < 0.05, Spearman rank test). Only one patient with established interstitial lung disease (patient 9, table 3) had no detectable levels of either lavage fluid

Table 2 Comparison of the clinical characteristics of screened patients with rheumatoid arthritis with and without subclinical lung disease

	Subclinical lung disease (n = 14)	No lung disease (n = 79)
Mean (SD) age (y)	53.6 (10.7)	51.2 (10.1)
Sex	7M, 7F	26M, 53F
Mean (SD) disease duration (y)	13.4 (9.8)	11.1 (7.5)
No (%) with rheumatoid factor	12 (86)	62 (79)
No (%) with erosive disease	4 (28.5)	13 (17)
Treatment (No (%))*		
Gold	12 (86)	68 (86)
Penicillamine	5 (36)	38 (48)
Corticosteroid	8 (57)	51 (65)
Smoking history (No (%))		
Non-smoker	1 (7)	30 (38)
Current smoker	8 (57)	23 (29)
Ex-smoker	5 (36)	26 (33)

*Current or previous treatment.

Table 3 Clinical features of patients with rheumatoid arthritis (RA) and established interstitial lung disease (ILD)

Case No	Age* (y), sex	Duration (y) of RA*	RF	ARA class*	Treatment* (not NSA1)	Smoking history*	Dyspnoea† (NYHA)	Signs†	Chest radiograph score†
1	70 M	20	+	2	—	Ex	3	cl, cr	U, 3/2
2	62 M	1	+	2	Au	Ex	2	cr	T, 2/2
3	59 M	9	+	2	—	Cr	2	cl, cr	N/A
4	66 M	Coincident	+	2	—	Ex	3	cl, cr	N/A
5	61 M	2	—	3	Au	Ex	3	cr	T, 2/3
6	58 M	8	+	2	Au	Cr	2	cl, cr	S/T, 2/2
7	58 M	9	+	2	Au	Ex	3	cr	U, 3/2
8	51 M	6	+	2	DXT to feet	cr	3	cr	U, 2/2
9	56 F	14	+	3	Au, PC, ST	cr	2	Pleural rub	T, 1/2
10	51 F	10	+	3	Au, PC, ST, DXT	Never	3	cl, cr	T, 2/1
11	54 F	7/12	+	1	—	Ex	2	cr	T, 2/2
Mean (SD)	59 (9)	7 (6)		2.2 (0.6)			2.5 (0.5)		

*At the time of diagnosis of interstitial lung disease.

†At the time of bronchoalveolar lavage.

N/A—radiograph not available for review; DXT—radiotherapy; AZ—azathioprine; CP—cyclophosphamide. Other abbreviations as in table 1.

collagenase or procollagen peptide. This patient had the least lung infiltration in this group, and was the only patient to remain stable with corticosteroids alone. This patient's main pulmonary problem was pleurisy, which contributed to the low FVC% observed.

Lavage fluid concentrations of fibronectin were similar in control subjects and patients. Median lavage procollagen peptide concentrations were similar in the control subjects and the patients with subclinical interstitial lung disease. Collagenase was not detected in bronchoalveolar lavage fluid from control subjects, but was found in the lavage fluid from two patients with subclinical interstitial lung disease (Nos 5 and 7; table 1 and figure). The four patients with subclinical lung disease and abnormal lavage fluid profiles did not differ from other patients in this group with respect to the duration, severity, or treatment of their rheumatoid arthritis or their smoking history.

The rate of degradation of type I collagen by a pooled sample of lavage fluid collagenase was 250 µg collagen degraded per ml of the sample.

No degradation of type III collagen occurred after incubation for 24 hours. This specificity for type I collagen was confirmed by SDS-PAGE analysis of digestion products, which showed collagen fragments from type I but not from type III collagen. The failure of lavage fluid collagenase to cleave type III collagen strongly suggests that the enzyme is of neutrophil origin.²²

Discussion

Screening of 93 patients with established rheumatoid arthritis identified 14 patients with subclinical lung disease. An association between the presence of subclinical interstitial disease and a history of cigarette smoking was observed. In addition, 10 of the 11 patients with rheumatoid arthritis and established interstitial lung disease were current smokers or ex-smokers. Previous studies have also shown that current smokers with rheumatoid arthritis have a higher incidence of pulmonary

Table 4 Analysis of bronchoalveolar lavage samples from patients with rheumatoid arthritis (RA) and control subjects (medians (absolute ranges))

		RA patients	
	Controls	Subclinical ILD	Established ILD
CELLULAR ANALYSIS			
Total cells × 10 ⁶	8.8 (3.6– 30.3)	19.0 (0.8– 54.8)	16.1 (3.2 – 31.4)
° T lymphocytes	7.5 (0.7– 23.4)	5.6 (0.5– 52.4)	10.0 (1.3 – 30.2)
° Leu 2a + ve cells	4.3 (0.7– 21.8)	2.2 (0.4– 22.8)	5.9 (0.5 – 28.1)
° Leu 3a + ve cells	4.0 (0 – 11.7)	2.2 (0.3– 33.2)	3.3 (0.5 – 13.8)
° alveolar macrophages	91.2 (71.9– 97.9)	92.3 (18.0– 97.3)	75.7 (28.0 – 95.2)
° neutrophils	0 (0 – 2.0)	1.0 (0 – 43.0)	1.7 (0 – 61.0)*
PROTEIN ANALYSIS			
Volume (ml)	120.0 (61.0–130.0)	93.5 (50.0–120.0)	74.0 (38.0 –100.0)**
Protein (mg/ml)	53.0 (11.0–110.0)	104.0 (44.0–170.0)	87.0 (22.0 –190.0)*
Urea (μg/ml)	3.3 (1.3– 9.1)	5.3 (2.7– 7.4)	4.2 (2.1 – 16.0)
Fibronectin (μg/ml)	0.18 (0 – 0.98)	0.17 (0 – 0.28)	0.46 (0.06– 0.83)
Procollagen peptide (ng/ml)	0 (0 – 0.05)	0 (0 – 0.04)	0.39 (0 – 1.62)**
Collagenase (munit/ml)	0	0 (0 – 53.5)	54.8 (0 –220)**

*p < 0.05, **p < 0.01 compared with controls (Wilcoxon rank sum test).

abnormal bronchoalveolar lavage fluid profiles—one had an increased lymphocyte count, one an increased neutrophil count, and two increased collagenase in the lavage fluid. Garcia *et al*⁹ observed increases in lavage fluid lymphocytes in patients with rheumatoid arthritis who had no clinical evidence of pulmonary disease, and so did Tishler *et al*¹¹ in four patients with rheumatoid arthritis and radiographic signs of disease. The meaning of the increased lavage fluid lymphocytes in patients with rheumatoid arthritis is not, however, clear. Wallaert *et al*,¹² in a study of 61 patients with various collagen vascular disorders but with no pulmonary disease (including four patients with rheumatoid arthritis), found that patients with increased lavage fluid lymphocytes showed no functional deterioration over 12 months. These investigators concluded that “the presence of a lymphocyte alveolitis does not lead to a clinically significant pulmonary interstitial process per se” in these disorders. Increase in lavage fluid lymphocytes in cryptogenic fibrosing alveolitis patients is associated with a good prognosis and responsiveness to corticosteroids.^{7 28}

In contrast, an increase in lavage fluid neutrophils in patients with or without clinical signs of pulmonary disease is associated with a poor prognosis and lack of responsiveness to treatment.^{7 8 12 27 28} Although only one patient with subclinical interstitial lung disease had a raised neutrophil count in the lavage fluid, a further two patients had increased lavage fluid collagenase, which appears to be of neutrophil origin and may reflect the presence of activated neutrophils in the lower respiratory tract. Thus three of our patients with rheumatoid arthritis and subclinical lung disease had markers that are found in the lavage fluid of patients with rheumatoid arthritis and established pulmonary disease and are associated with a poor prognosis in cryptogenic fibrosing alveolitis. As only a small proportion of patients with rheumatoid arthritis and subclinical lung disease develop chronic fibrosing interstitial lung disease the results of this study suggest that bronchoalveolar lavage in these patients may help to identify those who require careful monitoring in the hope that early treatment may prevent the development of severe interstitial disease.

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