

Cryptogenic fibrosing alveolitis: prediction of fibrogenic activity from immunohistochemical studies of collagen types in lung biopsy specimens

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ABSTRACT Collagen immunofluorescence studies were performed on biopsy specimens from 25 patients with cryptogenic fibrosing alveolitis. We studied the relationship of these results to the clinical, radiological, and physiological assessments of disease activity during six-month periods before and after the lung biopsy; to the appearances on routine histological examination; to the inflammatory cell proportions in bronchoalveolar lavage fluid; and to the response to treatment. Positive associations were observed between the presence of type III collagen and disease activity before ($p < 0.015$) and after ($p < 0.02$) lung biopsy. These were independent of clinical and routine histological features. The type-III-positive group also contained nine of the 11 responding to treatment. None of the type-III-positive patients had a quiescent or stable course. In contrast, most patients with no type-III-collagen fluorescence had a stable course over the time of this study and did not improve on treatment. It is suggested that collagen typing may be an additional useful method of assessing potentially reversible disease in cryptogenic fibrosing alveolitis.

Patients fulfilling the clinical criteria for cryptogenic fibrosing alveolitis¹ form a heterogeneous group in respect of histological features, response to treatment, and rate of progression of disease. The variable survival rate was well illustrated in a recent large, retrospective study.² Half of the patients had died within 3.2 years of first hospital attendance, but a quarter were still alive after 10 years. Furthermore, the mean survival of untreated patients was longer than that of patients who were treated but failed to respond.³

In view of this variability, the assessment of activity and potential reversibility of disease becomes important if patients are to be treated at an appropriate time and before irreversible fibrosis has occurred. Clinical, physiological, and radiological data;^{2,4-8} grading of histological features;^{2,5,6} inflammatory cell proportions in bronchoalveolar lavage fluid;⁷⁻¹¹ and gallium-67-citrate lung scans⁸ have all been used for this purpose. Combinations of these are useful for determining disease severity,

and to a lesser extent for confirming disease activity. Their value in predicting response to treatment has, however, been poor.

Several, but not all, studies have emphasised that corticosteroid responsiveness tends to relate to more cellular and less fibrotic change in lung biopsies,^{3-6,12} but there are many exceptions and in particular not all patients with cellular disease respond. For example, 10 (38.5%) of 26 cases classified as desquamative interstitial pneumonitis failed to improve on corticosteroids.⁴ The need for more reliable methods remains.

Several idiotypes of the collagen molecule are found in the lung, each with its distinctive fibril form, functional characteristics, and biological properties. Type I collagen is the most plentiful collagen in normal organs and in scar tissue. During the early phases of fibrosis and scar formation in the lung, however, as in other tissues, the ratio of type III to type I collagen increases temporarily.¹³⁻¹⁵ On the basis of collagen immunofluorescence studies we have suggested that areas of mature collagen in sites of established (and probably irreversible) scar tissue in lung can be distinguished from areas of early active fibrosis, and that this information might be

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useful in the management of certain lung disorders.¹³ We report here a study of lung biopsy specimens from patients with cryptogenic fibrosing alveolitis and describe the relationship between results of collagen immunofluorescence studies and appearances on routine histological examination; clinical, radiological, and physiological data; inflammatory cell proportions from bronchoalveolar lavage fluid; and response to treatment. The value of these results in determining disease activity and hence identifying the patients who require treatment has been established.

Methods

Lung biopsy specimens with histological features in keeping with cryptogenic fibrosing alveolitis were obtained from 25 patients with clinical, physiological, and radiological evidence of the condition.¹ Widespread and persistent crepitations were present in 22 patients, breathlessness in 23, and finger clubbing in 18.

The group (table 1) included 18 men and 7 women and their mean age was 54.2 (± 8 SD). In 14 the lung alone was affected, the remainder having

associated disorders of other systems. Three had rheumatoid arthritis; one had arthralgia and was positive for rheumatoid factor and antinuclear antibodies; and three had systemic lupus erythematosus, two systemic sclerosis, one polymyositis, and one thyrotoxicosis and arthralgia without detectable autoantibodies.

LUNG SPECIMENS

Lung specimens were obtained by open biopsy in 18 cases, by drill biopsy in one, and from necropsies performed within one day of death in six cases. Specimens from different portions from the same lung or lobe were examined in 6 cases, and in one patient a lung biopsy and a necropsy specimen taken 17 months apart were available for comparison. Most biopsy specimens were obtained from comparable sites in the lateral aspect of the right lung in the region of the oblique fissure. Only one of four biopsy specimens from the left lung was from the lingula, as the changes in this area are frequently unrepresentatively severe.^{7,16} Specimens were snap-frozen fresh on carbon dioxide ice and stored at -70°C until examination.

Table 1 *Clinical features of the 25 patients with cryptogenic fibrosing alveolitis*

Patient No	Sex	Age at biopsy (y)	Smoking	Duration of symptoms (m)	FVC (% predicted)	KCO (% predicted)	Lavage (days before (+) or after (-) biopsy)	Lavage fluid lymphocytes (%)
<i>Group 1: positive for type III collagen</i>								
1	M	49	No	96	37	100	6+	1
2	M	48	No	9	52	37	-	
3	M	54	Ex	36	57	36	-	
4	F	59	Ex	12	52	53	50+	2.6
5	F	49	No	4	36	99	5+	18.3
6	M	61	Ex	72	72	48	-	
7	M	63	Ex	24	73	68	14+	15.7
8	M	26	Ex	24	55	79	5+	0.8
9	F	46	Ex	4	59	59	6+	21.5
10	M	57	Yes	240	78	33	240-	1.3
11	F	61	No	108	65	65	15+	15.7
12	M	57	Ex	36	63	105	-	
13	F	50	No	12	86	51	6+	6.2
14	M	55	Ex	16	72	78	-	
<i>Group 2: doubtfully positive for type III collagen</i>								
15	M	60	Yes	18	82	47	6+	0
16	M	49	Yes	24	82	74	334-	0
<i>Group 3: doubtfully negative for type III collagen</i>								
17	M	61	Yes	24	88	57	-	
18	M	54	Yes	168	100	57	36+	0.4
<i>Group 4: negative for type III collagen</i>								
19	F	67	No	36	65	58	-	
20	M	54	Ex	36	77	91	15+	0
21	F	55	No	12	70	85	14+	1
22	M	50	Yes	60	76	101	13+	2.9
23	M	58	Yes	120	73	78	35+	6.7
24	M	60	Yes	12	100	56	9+	7.1
25	M	51	Ex	48	88	86	183-	7.6

- Not done.

TYPE-SPECIFIC COLLAGEN IMMUNOFLUORESCENCE

Antibodies to native triple helical bovine collagen types I, II, and III were raised in guinea-pigs and isolated, and their specificity was verified.^{17,18} Highly purified antibodies to bovine type IV collagen raised in rabbits were obtained from Professor AJ Bailey (Agricultural Research Council, Langford, Bristol). These four type-specific collagen antibodies were used in an indirect-immunofluorescence technique.¹³ Optimal dilutions of reactants determined on different human adult and fetal tissues were used on all lung specimens. Eight serial 4- μ m sections from each specimen were cut on a Bright's cryostat. Four were fixed in formaldehyde and stained with haematoxylin and eosin (H and E), modified Glee's silver impregnation, Miller's elastin van Gieson, and Masson's trichrome stains respectively.¹³ The remaining four sections were treated with one of the four collagen antibodies in the indirect immunofluorescence technique.

HISTOLOGICAL ASSESSMENT

Microscopy of the immunofluorescence sections was performed under transmitted ultraviolet light.¹³ This combination provided contrast between the specific apple-green fluorescence of collagen binding the fluorescent conjugate and the non-specific blue "autofluorescence" caused by the refractile property of the tissues. The latter permitted histological orientation even in fibrotic lung with confusing distortion of architecture.

The following features were assessed: (1) *Presence of collagen types in corresponding sites*. (2) *Presence of type-III-collagen fluorescence in alveolar walls* (normally barely detectable by this method, with the batch of reactants used¹³): *negative* — none detected; *doubtful negative* — faint apple-green fluorescence of fibres, but of doubtful significance; *doubtful positive* — < 25% of tissue section abnormal, but with increased type III collagen; *positive* — increased type III collagen in greater part of section.

The H and E section and connective-tissue-stained specimens were examined by light microscopy and a four-point score was used to assess alveolar wall fibrosis, alveolar wall thickening, architectural destruction, intra-alveolar cellularity, and cellular infiltrate. In addition, each specimen was graded as "cellular" — abnormal increase in intra-alveolar and interstitial mononuclear cells, with little fibroblast or connective tissue deposition; "fibrotic" — connective tissue accumulation with variable numbers of interstitial fibroblasts but few inflammatory cells; or "mixed" — roughly equal proportions of the above features.

Owing to the subjective nature of these examina-

tions the following procedures were adopted: (1) All immunofluorescence and light microscopy was done by one observer without knowledge of the patients' clinical details. (2) Indirect immunofluorescence studies were performed on biopsy specimens in six batches, with previously determined optimal dilutions of the single set of reactants being used on each. The appropriate controls¹⁹ for indirect immunofluorescence and a "control tissue" (human fetal lung at 20 weeks' gestation) were included with each batch of lung samples. Six pathological specimens were processed on two different occasions. The same interpretation of fluorescence was made on each, confirming minimal inter-batch variation. (3) The indirect immunofluorescence and light microscopy studies were performed on separate occasions and repeated several days later.

CLINICAL COURSE AND RESPONSE TO TREATMENT

The patients' clinical course was considered in two parts: a period of six months before lung biopsies studied retrospectively, and (in all except those from whom necropsy specimens were obtained) a period of at least six months assessed prospectively after biopsy. All assessments were based on a four-point clinical grading of dyspnoea; measurement of forced vital capacity (FVC); measurement of transfer factor for carbon monoxide (TLCO) by the single-breath method and estimation of carbon monoxide transfer corrected for volume of ventilated lung (KCO) on the basis of lung volume determined by a helium dilution method; and chest radiographs read by two observers independently using the ILO/UICC scoring system.²⁰ Plates were read individually in random order and then in pairs to assess change. FVC and KCO were expressed as percentages of values predicted for sex, age, and height²¹ and only changes of greater than 10% were considered to indicate improvement or deterioration. The clinical assessment was repeated on two or more occasions during the six-month follow-up period. "Clinical change" was defined as change in two of these four parameters, sustained for six months. Initial and follow-up assessments and treatment were supervised by clinicians who did not know the results of the indirect immunofluorescence studies.

Corticosteroid treatment comprised a daily dose of 40-50 mg prednisone (adjusted for age, weight, and sex) given for 4 to 6 weeks. Where improvement was observed a gradual reduction over several months was attempted. When unacceptably high doses were required to maintain improvement, or when no response was observed, other treatments were attempted (table 2). During the course of this

Table 2 Response to treatment

	Course before biopsy*	Course after biopsy*	Treatment							
			Corticosteroid response				Response to other drugs†			
			Clinical	% FVC	% KCO	Radio-graph	Clinical	% FVC	% KCO	Radio-graph
Group 1: Positive for type III collagen										
1	↓		0	0	0	0	P	0	0	0
2	↓	PM	0	0	0	0	-	-	-	-
3	↓	PM	0	0	0	0	P	0	0	0
4	↓		+	+	0	0	A, C	+	0	0
5	↓		+	+	0	+	-	-	-	-
6	↓	PM	0	0	0	0	-	-	-	-
7	↓		+	0	0	+	C, Co, Cy	+	0	0
8	↓		+	0	0	+	C	+	0	0
9	↓		+	+	+	+	-	-	-	-
10	↓		0	0	0	0	P	0	0	0
11	↓		+	0	0	+	C, Co, Cy	+	0	0
12	↓	PM	+	+	0	0	-	-	-	-
13	↓		-	-	-	-	C, Co, Cy	+	0	+
14	↓	PM	+	0	0	+	-	-	-	-
Group 2: doubtfully positive for type III collagen										
15	→	→	0	0	0	0	P	0	0	0
16	→	→	0	0	0	0	P	0	0	0
Group 3: doubtfully negative for type III collagen										
17	→	↓	0	0	0	0	C, Co, P	0	0	0
18	→	→	-	-	-	-	-	-	-	-
Group 4: negative for type III collagen										
19	→	PM	-	-	-	-	P, A, C	0	0	0
20	↓		+	0	0	+	-	-	-	-
21	↓		+	+	0	+	-	-	-	-
22	→	→	0	0	0	0	P	0	0	0
23	→	→	-	-	-	-	C, Co, Cy	0	0	0
24	→	→	-	-	-	-	-	-	-	-
25	→	→	-	-	-	-	-	-	-	-

- Not done.

PM — necropsy specimen.

*↓ worse ↑ improved → stable. †P — penicillamine; C — cyclophosphamide; Co — colchicine; A — azathioprine; Cy — cyproheptadine.

investigation several regimens were being studied; they included cyclophosphamide (100-120 mg daily) in combination with colchicine (0.5 g three times daily) and cyproheptadine (4 mg three times daily), azathioprine (150 mg daily), and penicillamine (initially 125 mg daily increasing to 1 g daily). "Treatment response" was defined as an improvement on treatment in two of the four parameters mentioned above, sustained for six months. Treatment was instituted within one month of the

date of biopsy in 13 cases, and within two months in a further three; three were not treated. The six patients from whom necropsy specimens were obtained were treated from their date of presentation until death.

BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage was performed on 19 patients, mostly (12 cases) within the 15 days before the biopsy. Four lavages were undertaken from four

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Photomicrographs of type-specific collagen fluorescence in serial sections of lung from patients with cryptogenic fibrosing alveolitis, processed by an indirect immunofluorescence technique, with transmitting ultraviolet light achieving contrast between specific collagen fluorescence (apple-green), blue autofluorescence of unstained tissues, and light blue or white of elastic fibres.

(a) and (b) Patient 24: (a) Type-I-collagen fluorescence in thickened alveolar walls. (b) Absence of type-III-collagen fluorescence in the same area. Elastic fibres are abnormally prominent and represent accumulation or condensation (or both) in an area of fibrosis. In (a) pieces of alveolar wall in right upper and left lower corners of the field have been lost in the preparation of the section. This biopsy specimen was scored as negative for type III collagen. (About ×260.)

(c), (d), and (e) Patient 13: Fluorescence of type I, III, and IV collagens respectively. The intensity and profusion of type-III-collagen fluorescence is almost that of type I, and type III is present in all layers of the alveolar walls. The alveoli are reduced in size and contain cellular exudate. This biopsy specimen was scored as positive for type III collagen. (e) Type-IV-collagen fluorescence delineating alveolar and capillary (shown with arrow) basement membranes. (About ×190.)

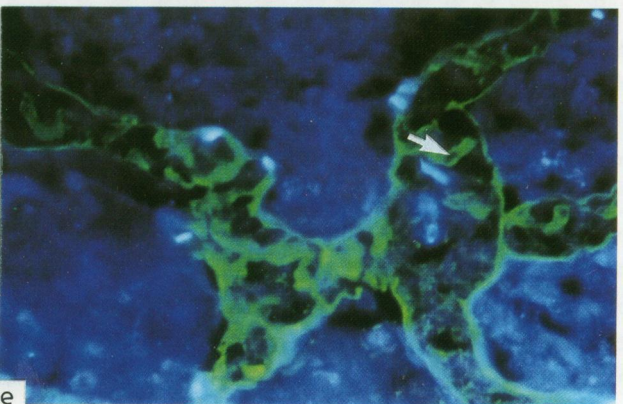
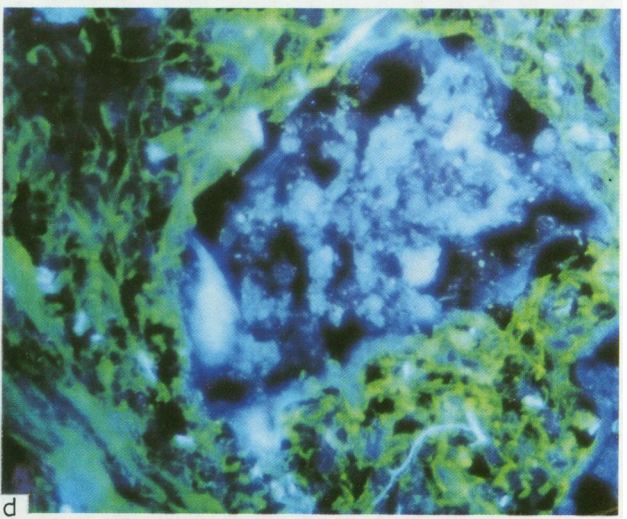
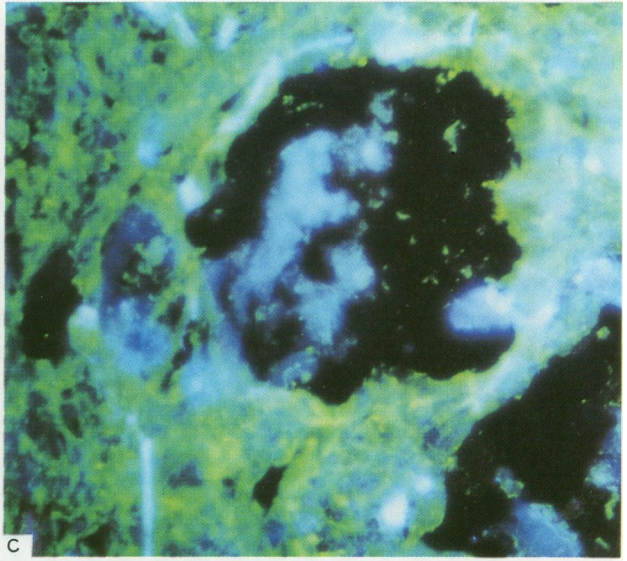
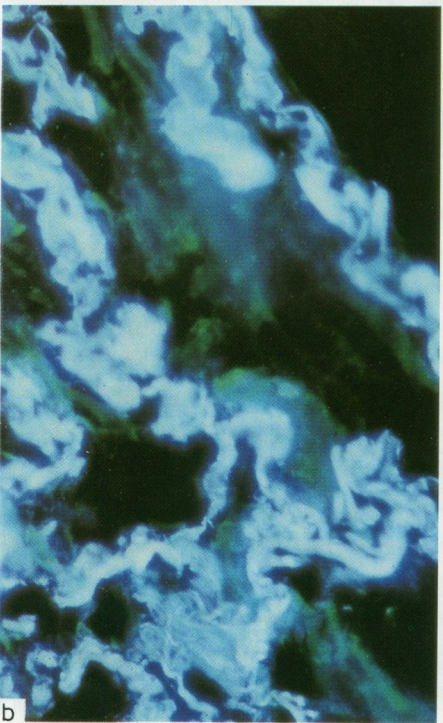


Table 3 Comparison between patients positive and negative for type III collagen

Features	Type III positive (No of cases or mean \pm SD)	%	Type III negative (No of cases or mean \pm SD)	%	p
Course before biopsy:					
Deterioration—symptomatic	14/14	100	2/11	18.2	< 0.0001
Deterioration \geq 2 criteria	6/6	100	1/5	20	< 0.015
Course after biopsy:					
Improved — \geq 2 criteria	7/9	77.8	2/10	20	< 0.02
Treatment response: \geq 2 criteria	9/14	64.3	2/8	25	NS
Duration of symptoms (m)	49.5 \pm 64.3		50.7 \pm 49.6		NS
Smokers	1/14	7.1	7/11	63.6	< 0.005
Associated autoimmune disease	4/14	28.6	6/11	54.5	NS
Rheumatoid factor (log RAHA)*	2.59 \pm 0.91		1.85 \pm 0.58		< 0.05
FVC % predicted	61.2 \pm 14.4		83.4 \pm 14.5		< 0.002
Bronchoalveolar lavage fluid lymphocytes \geq 11%	4/9	44.4	0/10		= 0.05
Histological score (H and E)†					
Wall fibrosis	3.07 \pm 0.62		3.0 \pm 0.77		NS
Wall thickness	3.21 \pm 0.7		2.91 \pm 0.83		NS
Intra-alveolar cellularity	2.93 \pm 0.92		2.46 \pm 1.04		NS
Interstitial cellularity	3.14 \pm 0.77		2.82 \pm 0.61		NS
Overall	13 mixed, 1 cellular		9 mixed, 1 cellular 1 fibrotic		

*RAHA — logarithm of reciprocal RAHA test titre (Fujizoki Pharmaceuticals).

†Four-point score: 1 — normal; 2 — mild; 3 — moderate; 4 — severe: see under "Methods."

to nine weeks and one six months before biopsy. In two patients lavage was performed outside the six-month period (8 and 11 months before), but the data have been included as their clinical course did not change during the observation period or up to the time of lavage.

STATISTICAL ANALYSIS

The Mann-Whitney U test or 2×2 tables with Fisher's exact test were used for group comparisons.

Results

TYPE-SPECIFIC COLLAGEN

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Type I collagen predominated in all sites of lung fibrosis. In areas where fibre bundles were closely packed the type-I-collagen fluorescence was weaker, type III collagen could not be seen, and a degree of blue, non-specific autofluorescence was observed in the ultraviolet light.¹³ In specimens from 14 patients, however, type III collagen could be easily identified, and was usually found in subepithelial sites or in loosely arranged fibrils between densely packed type I fibres in alveolar walls (fig). In several biopsy specimens it had a fluorescent intensity and widespread distribution like that of type I. Type III collagen was also found in its normal location in bronchial submucosa and subintima of vessels.^{13 14} Comparisons with H and E sections confirmed that the abnormal collections of type III collagen were in areas of cellularity (chronic inflammatory cells and fibroblasts), whereas relatively acellular areas of dense fibrous tissue contained only type I.

The grouping of patients on the basis of demon-

strable type III collagen is shown in tables 1 and 2. In 14 cases the features of activity were patchy, even within a single lung specimen. The unavoidable problem of lung sampling was illustrated in three of the six cases in which two biopsy specimens taken from different parts of the lung were examined. In two cases (5 and 11) one specimen was scored as "doubtful positive," whereas the second was definitely positive. In case 23 one sample was "negative" and the other "doubtful negative." In the remaining three cases, however, both specimens received the same score. The patients in the "doubtful positive", "doubtful negative" and "negative" categories were grouped together in the analysis. When the "doubtful" groups were omitted the associations with clinical features were similar.

HISTOLOGICAL ASSESSMENT OF FIBROSIS AND CELLULARITY

The biopsy specimens from all patients had features fulfilling the criteria for "usual interstitial pneumonia"¹⁴ but in different stages of fibrosis: two cases (4 and 21) were predominantly cellular, one was fibrotic (case 19) and the remainder were mixed. Comparison of collagen immunofluorescence (type I or III or both) and connective tissue stains confirmed that in areas of fibrosis with minimal type III collagen all stains compared favourably with fluorescence. In locations where type III collagen was more plentiful, however, the silver impregnation method correlated most accurately with type III collagen immunofluorescence.

CLINICAL COURSE AND RESPONSE TO TREATMENT

Steady deterioration in the six months before biopsy

was observed in 16 patients (table 2).

Nine of the 18 patients treated with corticosteroids (and one treated with a combination of corticosteroids and cyclophosphamide) during the six months showed a treatment response. Four of these, who were put on alternative treatment because of corticosteroid side effects, continued to improve.

Seven of the nine non-responders to corticosteroids were treated with alternative regimens but none improved. Three patients were not treated with corticosteroids but received either a combination of cyclophosphamide, colchicine, and cyproheptadine (one of two improved) or pencillamine and immunosuppressants (no response).

The terminal events in the six cases in which necropsy specimens were obtained were: ischaemic heart disease (case 19), pulmonary thromboembolism (case 2), *Haemophilus septicaemia* (case 14), fulminant influenza due to influenza B virus (case 3), herpes simplex (case 12), and measles pneumonia (case 6).

COMPARISON OF PATIENTS POSITIVE AND NEGATIVE FOR TYPE III COLLAGEN

All the patients who were positive for type III collagen (table 2) were deteriorating before biopsy, and continued to show signs of disease activity after biopsy or the start of treatment; there was continued deterioration in five and response to treatment in nine. In contrast, most patients who were negative for type III had a stable course before biopsy, only two showing features of deterioration. Most but not all the treatment responders were positive for type III, the trend not reaching statistical significance (table 3). Thus a strong positive association was observed between disease activity and the presence of type III collagen in lung biopsy specimens, but not between the latter and treatment response. This association was not accounted for by differences in histological score and there were no significant differences between the groups when analysed for age, sex, presence of crepitations, or the number of

postmortem specimens in each. The type-III-positive group contained fewer smokers ($p < 0.005$), but many were ex-smokers (eight out of 14). Cessation of smoking was possibly prompted by their deteriorating clinical course and poorer ventilatory function. The forced vital capacities were significantly lower than in the type-III-negative group.

All patients with an increased proportion of lymphocytes in bronchoalveolar lavage fluid (11% or higher) were type III positive. No difference in total cell yield, cell yield per millilitre of lavage fluid, or proportion of neutrophils or eosinophils was found.

COMPARISON OF TREATMENT RESPONDERS AND NON-RESPONDERS

Treatment responders (table 4) had had symptoms for a shorter time before treatment ($p < 0.05$). No significant group differences existed in clinical signs, lung function tests, symptoms of associated autoimmune disease, or proportions of cells in bronchoalveolar lavage fluid. All four patients with an increased percentage of lymphocytes in the lavage fluid were, however, in the responder group ($p = 0.07$), a trend that might have been significant had larger numbers of patients been studied. The light microscopy histological scores given for responders and non-responders were not significantly different.

Discussion

For the type-specific collagen immunofluorescence method highly purified type-specific antibodies are required. Cross-reactions between type I and type III antibodies are difficult if not impossible to eliminate, but a small degree of cross-reaction is acceptable since the object is to confirm only semi-quantitatively the presence or absence of type III collagen. Variations in specificity and avidity of different batches of antibody are usual, and each batch requires standardisation by comparison with previous batches on control substrates.^{22,23} As with histochemical stains, quantification is based on the area occupied by the positively stained tissue and its colour intensity, which cannot be measured accurately. An added difficulty with collagen immunofluorescence is its dependence on the coating of collagen fibres with fluorescent antibody complexes, a process favouring loose reticular fibres, which are accessible to the reactants, rather than tightly packed fibre bundles. Since type I has the latter form it is underestimated by comparison with type III.

Other potential weaknesses of this method are the subjective nature of the observations; the patchy distribution of the pathological process in cryptogenic fibrosing alveolitis, resulting in poor correla-

Table 4 Comparison of treatment responders and non-responders

Features	Responders (No of cases or mean \pm SD)	Non-responders (No of cases or mean \pm SD)	p
Sex male	5/11	10/11	< 0.05
Mean (\pm SD) duration of 1st symptom (m)	26.2 \pm 29.3	66.8 \pm 67.1	< 0.05
< 12 m	5/11	1/11	NS
> 36 m	1/11	5/11	NS
FVC (% predicted)	64.4 \pm 13.6	69.0 \pm 15.2	NS
Bronchoalveolar lavage fluid: lymphocytes \geq 11%	4/9	0/7	NS

tion between biopsy specimens from different lung segments; the infrequency with which biopsy specimens of adequate size are obtained; and the possibility that infections such as pneumonia, which occurred terminally in a few cases in this study, might alter the collagen ratios. Useful precautions to ensure accuracy and reproducibility have been mentioned in the paper and elsewhere.¹³

There are few published studies of the changes in collagen types in cryptogenic fibrosing alveolitis.^{24, 25} Seyer *et al*²⁴ performed biochemical assays of collagen types in postmortem lung specimens from five patients with idiopathic pulmonary fibrosis and showed that only 12-24% of collagen in diseased lungs was of type III, compared with 31% in normal lungs. Although apparently at variance with the results of the current study, these data might provide complementary information. In contrast to the immunofluorescence technique, biochemical extraction provides a quantitative measure of collagen content and of the ratio of collagen types in the total collagen solubilised.²⁶ The different solubility of the collagen types, however, introduces a potential source of error, types III and IV being more resistant to extraction than type I.²⁷ Because the immunofluorescence technique is highly sensitive and shows deposits of collagen which are outside the range of biochemical detection, a close correlation between biochemical and immunofluorescence findings need not be expected.

Other differences between the current study and that of Seyer *et al*²⁴ is that the latter was performed on lungs from patients who had died with cryptogenic fibrosing alveolitis, and no attempt was made to correlate biochemical findings with histopathological or clinical features.²⁴ Necropsy specimens of lung from patients with cryptogenic fibrosing alveolitis are known to contain more fibrosis and less inflammatory cellularity than biopsy specimens,² a trend also observed in the current study, and it is possible that Seyer *et al* studied lungs containing mature inactive fibrosis with predominantly type I collagen, which by the indirect immunofluorescence method would have been classed as "type III negative." A temporal transition of collagen fluorescence was observed in patient 7. The initial biopsy specimen was type III positive; he was treated and showed improvement, but died 17 months later from adenocarcinoma of the lung. The necropsy specimen was type III negative. Specimens showing "type-III-positive" immunofluorescence may not have a greater-than-normal proportion of type III collagen since, provided that the total collagen content (both type I and type III) rises, type III will become histologically evident without an alteration in proportions.

Although clinically active, the process was not uniformly early in the patients classed as type III positive. Physiological measurements of lung function gave worse results than in those negative for type III collagen and the grading of fibrosis on light microscopy was severe. The association of type III collagen with clinical activity was therefore not accounted for by an association with any of the usual details obtained from the history or even routine histological examination or by aspects of pulmonary physiology.

Furthermore, most patients who responded to treatment came from the type-III-positive group. The difference between responders and non-responders requires separate consideration. The former had had a shorter duration of symptoms³ and the group included all those with an increased percentage of lymphocytes in bronchoalveolar lavage fluid.⁷ These observations are consistent with those of Rudd *et al*,²⁸ who showed that responders had had a shorter duration of disease and worse FVC% at initial assessment than non-responders.

As in other series^{4-6, 12} the responder group also showed a trend towards more cellularity and less alveolar fibrosis and architectural destruction. Each of these associations has a varying but small value in predicting response to treatment. Together and in individual cases their predictive value increases. The presence or absence of type III collagen according to the immunofluorescence method is another useful parameter to add to this combined assessment.

Other clinically relevant observations were made in this study. No patient in the "type-III-positive" group had a quiescent or stable course, and none improved spontaneously. Those not responding to steroid treatment had a progressively downhill course. It is therefore recommended that "type-III-positive" patients who fail to respond to treatment should receive alternative forms of treatment in an attempt to arrest the process. On the other hand, most patients with no type-III-collagen fluorescence had a stable course during the observation period and were not affected by treatment, suggesting that they did not require treatment. Absence of type III collagen in biopsy specimens might therefore strengthen the decision to withhold potentially harmful treatment, at least until there is clinical evidence of disease activity.

Finally, because type III collagen is associated with active fibrogenesis, patients in this category might be more responsive to the effects of therapeutic agents such as penicillamine,^{29, 30} proline analogues, and colchicine, which act only during collagen synthesis or fibril formation (or both), and are ineffective in the presence of mature, dense scar tissue. Possibly by careful selection of patients for

treatment with these agents the evaluation of their therapeutic efficacy will improve and indications for their use emerge.

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