

Pulmonary γ interferon production in patients with fibrosing alveolitis

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Abstract

Patients with fibrosing alveolitis have active inflammation within their lung interstitium. Previous studies have focused on the humoral (immune complex) driven processes. In this study increased pulmonary gamma interferon production has been evaluated. Bronchoalveolar lavage cells were obtained from 40 patients with fibrosing alveolitis, 22 with cryptogenic fibrosing alveolitis, and 18 with connective tissue disease associated (CTD) fibrosing alveolitis. Increased γ interferon production was seen in 12 (30%) patients and was similar in the two study groups. Up to 512 units/10⁶ cells were released over 24 hours, showing that the amounts of γ interferon released could be as large as those seen in other pulmonary diseases associated with active cellular immune processes, such as sarcoidosis. Spontaneous γ interferon production was related to increased serum concentrations of IgG and IgM but not to serum IgA, antinuclear antibody, or rheumatoid factor titres. There was no relation between γ interferon production and pulmonary uptake of gallium-67 citrate. The ratio of helper-inducer (Leu-3) to suppressor-cytotoxic (Leu-2) cells in bronchoalveolar lavage fluid was similar in the two study groups and was similar in patients whose cells produced γ interferon and those whose cells did not. These data suggest that γ interferon is released in the lungs of a proportion of individuals with cryptogenic fibrosing alveolitis and CTD-fibrosing alveolitis, suggesting a role for this cytokine in mediating these diseases.

Some patients with fibrosing alveolitis have an increased number of lymphocytes in the lung interstitium¹ and in fluid recovered by bronchoalveolar lavage,² suggesting that active cellular immune type processes may be present as in other pulmonary conditions, such as sarcoidosis, berylliosis, and extrinsic allergic alveolitis.² There is some evidence that these cells contribute to the disease process in that patients with an increased proportion of lymphocytes in lavage fluid have a better prognosis with treatment but a worse prognosis without treatment.³ Gamma interferon is a cytokine that has a central role in cellular immune responses.⁴ It is spontaneously released by T cells and macrophages in lavage fluid in sarcoidosis, a disorder with many

features that suggest a continuing cellular immune type process.⁵ Accordingly, to discover whether cellular immune processes are active in the lungs of patients with fibrosing alveolitis, we studied the spontaneous release of γ interferon by cells from lavage fluid from 40 patients with fibrosing alveolitis.

Methods

SUBJECTS

All patients were under the care of the Department of Respiratory Medicine. All had diffuse interstitial lung disease on their chest radiographs and no known exogenous cause for their pulmonary disease (dust, drugs, animal exposure). Forty consecutive patients with a diagnosis of fibrosing alveolitis were studied, 22 with cryptogenic fibrosing alveolitis and 18 with fibrosing alveolitis associated with connective tissue disease (CTD). Cryptogenic fibrosing alveolitis was defined as the presence of chronic diffuse interstitial lung disease on chest radiograph in the absence of hilar and pleural disease and with no clinical or laboratory features to suggest either a known cause or another type of interstitial lung disease.¹ Of the 22 patients in this category, 15 underwent transbronchial lung biopsy (none had granuloma) and five underwent open lung biopsy (all had features consistent with cryptogenic fibrosing alveolitis). None was receiving treatment at the time of the study. CTD-fibrosing alveolitis was defined as the presence of chronic diffuse interstitial lung disease in association with a connective tissue disease defined according to published criteria.⁶ In this group 10 underwent transbronchial and four open lung biopsy; none had granulomas. Treatment at the time of the study was as follows: nil 6, gold 1, penicillamine 3, azathioprine 1, methotrexate 1, non-steroidal anti-inflammatory drugs 6. None of the patients had evidence of airway disease (bacterial or viral infection, asthma, aspergillosis, chronic bronchitis) at presentation. The study was approved by the human rights committee of the University of Western Australia. Informed consent was obtained from all participants before the study. Concurrent control and normal values for lavage fluid and cytokine measurements in our laboratory are published elsewhere.^{5 7 8 10}

BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage was performed as described previously.⁷ Briefly, six 50 ml

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Table 1 Clinical and physiological features of the 40 patients with cryptogenic fibrosing alveolitis (CFA) and fibrosing alveolitis associated with connective tissue disease (CTD-FA) (where parentheses appear values are means with standard errors)

	No	M:F	No of smokers	Age (y)	No with		% of predicted values			
					clubbing	crackles	TLC	VC	TlCO	TLVA
CFA	22	15:7	10	60 (3)	5	21	86 (4)	90 (5)	53 (4)	67 (5)
CTD-FA	18	11:7	4	58 (3)	1	17	84 (4)	84 (6)	57 (5)	77 (6)

TLC—total lung capacity; VC—vital capacity; TlCO—transfer factor for carbon monoxide (single breath); TLVA—TlCO corrected for effective alveolar volume.

aliquots of warm (37°C) normal saline were instilled into either the middle lobe or a lingular subsegment via a fiberoptic bronchoscope and aspirated immediately. The fluid sample returned from the first 50 ml aliquot was processed separately from the sample returned from the remaining five 50 ml aliquots. Differential cell counts were performed on modified Wright-Giemsa (DiffQuick) stained cytospin preparations. All the data presented here were obtained from the second sample.

γ INTERFERON ASSAY

For release of γ interferon,⁵ lavage cells were suspended at 5×10^6 viable cells/ml in RPMI 1640 medium containing glutamine, antibiotics, and 10% fetal calf serum (all from Commonwealth Serum Laboratories, Melbourne). Cells were cultured in wells of 24 well tissue culture plates (Nunc, Roskilde, Denmark) for 24 hours at 37°C. The supernatants were then harvested, centrifuged, and frozen at -85°C until assayed. Gamma interferon titres were determined by a bioassay evaluating the dilution of a sample that produced a 50% reduction in encephalomyocarditis virus induced lysis of human amniotic WISH cells. Interferon was characterised as gamma or non-gamma interferon by evaluating the capacity of a monoclonal anti-γ interferon antibody (Meloy Laboratories, Springfield, Virginia) to inhibit interferon activity (we have previously established that this antibody is specific for γ interferon.⁵

IMMUNOFLUORESCENCE STUDIES

Lymphocyte subsets in lavage cell suspensions were evaluated by adding 50 μl aliquots of cells (10^7 /ml) to each well of a 96 well, round bottom plate (Nunc), adding a 10 μl aliquot of fluoresceinated anti-leu-3 or anti-leu-2 monoclonal antibody (Becton Dickinson, Sunnyvale, California) to each well (4°C, 30 minutes), and then washing the cells three times with cold (4°C) phosphate buffered saline and examining the cells using an ultraviolet microscope.⁸ At least 200 cells were routinely counted.

GALLIUM LUNG SCANNING

The patients being studied were injected with gallium-67 citrate (111 MBq) 48 hours before being evaluated by means of multiple views in a gamma counter (Searle LFOV). A scan was recorded as positive if counts in the lung were greater than those of a reference normal area (proximal upper limb).⁹

SYSTEMIC IMMUNOLOGICAL MEASUREMENTS

Serum immunoglobulins G, M, and A and rheumatoid factor were all measured by nephelometry. Serum antinuclear factor was assayed by immunofluorescence.

ANALYSIS

Results are presented as means with standard errors in parentheses unless otherwise stated. All statistical comparisons were performed with the two tailed Student's *t* test; values are recorded as not significantly different unless *p* was less than 0.05.

Results

The two groups of patients had similar clinical and physiological features (table 1).

LAVAGE FLUID CELLS

Differential counts on lavage cells in both the cryptogenic fibrosing alveolitis and CTD-fibrosing alveolitis groups showed changes similar to those described previously—that is, an increase in the proportion of neutrophils and a lesser increase in the proportion of eosinophils (table 2) by comparison with current controls.^{5,7,8,10} Some patients in each group (cryptogenic fibrosing alveolitis 3/32, CTD-fibrosing alveolitis 4/18) also had a greater proportion of lymphocytes than the controls.

SPONTANEOUS INTERFERON PRODUCTION

Increased interferon release was seen in 12 of the 40 patients (30%) (Figure 1). The remaining patients released little or no interferon, like the control subjects.⁵ Up to 512 units/ 10^6 cells of interferon was released over 24 hours. The

Table 2 Bronchialveolar lavage and systemic immunological data (mean (SEM) values) from patients with cryptogenic fibrosing alveolitis (CFA) and fibrosing alveolitis associated with connective tissue disease (CTD-FA)

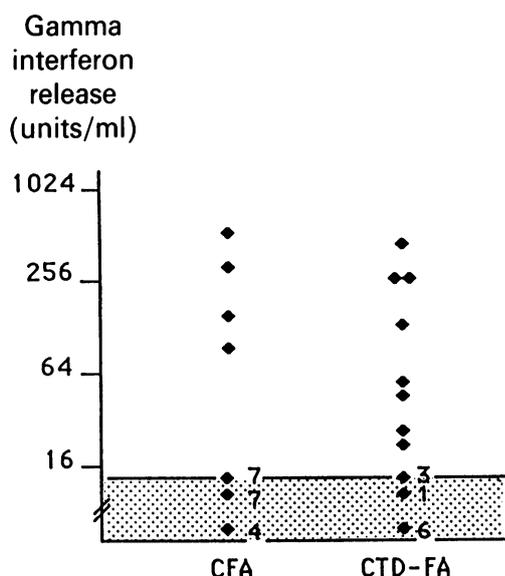
	Lavage fluid				Systemic				Number abnormal/No tested			
	Vol recorded (%)	Total cell No ($\times 10^6$)	Differential count (%)		Leu3/Leu2	IgG (g/l)	IgM (g/l)	IgA (g/l)	ANF	RF		
Normal range*	51 (5)	45 (10)	M	L							N	E
CFA	46 (4)	43 (10)	75 (3)	8 (2)	9 (2)†	7 (2)†	1.9 (0.6)	12.0 (0.7)	1.7 (0.3)	3.1 (0.4)	5/20	3/18
CTD-FA	48 (4)	23 (6)	69 (5)	11 (3)	17 (4)†	3 (1)†	1.0 (0.3)	13.0 (0.9)	1.7 (0.4)	2.7 (0.3)	7/16	6/16

*Established laboratory reference values^{6,10,14}

†*p* < 0.05 in the comparison with controls.

M—macrophages; L—lymphocytes; N—neutrophils; E—eosinophils; ANF—antinuclear factor; RF—rheumatoid factor.

Spontaneous release of γ interferon by bronchoalveolar lavage cells, measured in patients with cryptogenic fibrosing alveolitis (CFA) and connective tissue disease associated fibrosing alveolitis (CTD-FA). The shaded area represents the range of values seen in control individuals. For clarity, values falling within this normal range are shown as single points with the number of individuals beside each point.



amount of interferon released and the proportion of patients releasing interferon were similar in both groups. The interferon released was γ interferon in type as it was inhibited by the anti- γ interferon antibody of the CTD-fibrosing alveolitis group. Six of the eight γ interferon-producers were receiving either no therapy or non-steroidal anti-inflammatory drugs only.

RELATION OF γ INTERFERON TO SYSTEMIC IMMUNOLOGICAL MEASUREMENTS

Mean serum immunoglobulin concentrations were at the upper limits of the normal ranges (table 2). Patients with a clear increase in γ interferon production had significantly higher ($p < 0.05$) mean serum concentrations of IgG and IgM than non-producers (table 3). Serum IgA concentrations, antinuclear-factor, and rheumatoid factor titres did not differ significantly between patients whose cells did and did not produce increased amounts of γ interferon.

RELATION OF γ INTERFERON TO GALLIUM LUNG SCANNING

There was no relation between γ interferon production and uptake of gallium in subjects having gallium lung scans (table 3).

RELEASE OF γ INTERFERON, LYMPHOCYTE PROPORTIONS, AND LAVAGE LYMPHOCYTE SUBSETS

There was no significant correlation between γ interferon release and lavage lymphocyte sub-

populations. The ratio of Leu-3 to Leu-2 positive cells was similar in the cryptogenic fibrosing alveolitis and CTD-fibrosing alveolitis groups (table 2), and in the concurrent controls.⁸ It was also similar in producers and non-producers of γ interferon (table 3).

FOLLOW UP STUDIES

In the 13 patients who underwent follow up pulmonary function testing there was no significant difference between producers and non-producers of γ interferon (table 4). Numbers were too small to evaluate the predictive value of γ interferon measurements.

Discussion

Studies of the pathogenic processes underlying cryptogenic fibrosing alveolitis have highlighted the potential role for immune complexes in activating alveolar macrophages to produce mediators of inflammation and fibrosis.¹ Release of γ interferon may also contribute to the disease process. γ Interferon is released mainly by activated T cells⁴ but also in some circumstances by macrophages,^{5,11} and it induces changes in target cells that are consistent with known features of cryptogenic fibrosing alveolitis, particularly macrophage activation.⁴ γ Interferon may also contribute to enhanced fibrosis⁴ and induce expression of class II major histocompatibility complex (MHC) molecules,⁴ as noted in one study of lungs from patients with cryptogenic fibrosing alveolitis.¹² The enhanced expression of class II molecules may also contribute to rheumatoid lung, a class II MHC associated disease.¹³

In animal studies of lung fibrosis T cell deficiency partially protects mice from fibrotic challenge,¹⁴ suggesting a role for T cell products. There is evidence that γ interferon may represent one of these products because a recent study of drug induced pulmonary fibrosis found increased disease severity in animals treated with γ interferon in addition to the drug.¹⁵ A potential role for lymphocyte products is also implied in human disease, for in cryptogenic fibrosing alveolitis lymphocyte numbers parallel disease progression and response to treatment.³ Although alveolar macrophages are known to release γ interferon in response to several stimuli¹¹ and human alveolar macrophages in a proportion of patients with another interstitial lung disease, sarcoidosis, release γ interferon spontaneously,⁵ we have not undertaken the separa-

Table 3 Relation between γ interferon production and other immune indices* (where parentheses appear values are means with standard errors)

γ Interferon group	Serum IgG	Serum IgM	Serum IgA	Number abnormal/No tested		Positive gallium scan**	Leu 3/2 ratio††
				Serum ANF	Serum RF		
Producers†	15.2 (1.1)	2.6 (1.1)§	3.6 (0.4)	2/10	3/10	5/9	2.1 (0.9)
Non-producers‡	11.8 (0.6)	1.4 (0.2)	2.7 (0.3)	10/26	6/23	18/21	1.3 (0.5)

*Indices and normal ranges given in table 1.
 †Spontaneous production of ≥ 20 units of γ interferon/ 10^6 cells in 24 hours.
 ‡Spontaneous production of ≤ 10 units of γ interferon/ 10^6 cells in 24 hours.
 § $p < 0.05$ in the comparison with controls.
 **Increased pulmonary gallium uptake in a 48 hour scan.
 ††Lavage fluid Leu 3/Leu 2 ratio determined by fluorescence microscopy.
 ANF—antinuclear factor; RF—rheumatoid factor.

Table 4 Relation between γ interferon production and deterioration in pulmonary function

γ Interferon group	Mean (SEM) interval† (months)	No treated‡	Mean (SEM) % of original value	
			TLC	TLC _{CO}
Producers*	11 (4)	4/6	104 (2)	104 (3)
Non-producers*	14 (4)	1/7	100 (13)	98 (12)

*Defined as in table 3.

†Interval between initial evaluation and follow up pulmonary function tests.

‡Proportion of patients receiving immunosuppressive treatment during the observation period.

TLC—total lung capacity; TLC_{CO}—transfer factor for carbon monoxide.

tion steps required to purify T cells and alveolar macrophages from the lavage fluid from these patients; so we are unable to say which cells are responsible for release of γ interferon in our study.

The relation between immune complexes and production of γ interferon by human lung macrophages and T cells has not been studied, so we cannot say whether release of γ interferon in fibrosing alveolitis is related to immune complex mediated stimulation or whether it represents an entirely different immunopathogenic process.

Both the patients with cryptogenic fibrosing alveolitis and those with CTD-fibrosing alveolitis had increased proportions of neutrophils and eosinophils in lavage fluid samples with a relatively normal proportion of lymphocytes. This is consistent with findings in several previous studies.¹⁻³ Of the seven patients with an increased proportion of lymphocytes (mean 27% (SEM 4%)), four had increased production of γ interferon (mean γ interferon release 190 (66) units/10⁶ cells per 24 hours).

Release of γ interferon in our patients with fibrosing alveolitis was highly variable. In most of them large amounts of γ interferon were not released spontaneously; in some cases where they were, however, relatively large amounts were released—similar to the amounts released in patients with active pulmonary sarcoidosis.⁵

The association between spontaneous release of γ interferon and increased serum concentrations of IgG and IgM suggests that local release of γ interferon and production of immunoglobulins in the lung may be related. Our data suggest that spontaneous production of γ interferon in the lung may augment production of immunoglobulins by B cells, thus contributing to the production of local immune complexes in cryptogenic fibrosing alveolitis and CTD-fibrosing alveolitis.^{8,16,17} There was a trend towards an increase in helper: suppressor cell ratios in patients producing γ interferon, but this was not significant (table 3).

Increased pulmonary uptake of gallium was

not restricted to patients who were producing γ interferon. Active pulmonary uptake of gallium is thought to reflect macrophage activation within the lungs.⁹ This has been related to immune complex activation of macrophage Fc receptors in fibrosing alveolitis^{1,2} in contrast to pulmonary sarcoidosis, which is thought to be largely due to activation of macrophages by γ interferon derived from T cells.⁵ Both of these macrophage activation processes may be occurring in fibrosing alveolitis, but gallium data do not elucidate this point.

Longer follow up of more patients is required before the value of spontaneous production of γ interferon as a predictor of deterioration in pulmonary function or of response to treatment can be ascertained.

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