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Different cytokine profiles in cryptogenic fibrosing alveolitis and fibrosing alveolitis associated with systemic sclerosis

A quantitative study of open lung biopsies

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Different cytokine profiles in cryptogenic fibrosing alveolitis and fibrosing alveolitis associated with systemic sclerosis: a quantitative study of open lung biopsies. S. Majumdar, D. Li, T. Ansari, P. Pantelidis, C.M. Black, M. Gizycki, R.M. du Bois, P.K. Jeffery. ERS Journals Ltd 1999.

ABSTRACT: Differences in the inflammatory response and prognosis of cryptogenic fibrosing alveolitis (CFA) and that associated with systemic sclerosis (FASSc) are beginning to emerge. It is hypothesized that these differences may be reflected in a distinct pattern of T-helper (Th)-1 and Th-2-type cytokines.

Open lung biopsies were obtained from clinically well-documented cases of CFA and FASSc and, as a control, compared with grossly and histologically normal parenchyma obtained from smokers whose lungs were resected for cancer (n=5 in each group). *In situ* hybridization (ISH) was applied to the samples using anti-sense and sense ^{35}S-labelled riboprobes to detect messenger ribonucleic acid (mRNA) for interleukins (IL)-2, IL-4, IL-5 and interferon (IFN)-γ.

Between 52–91% of cells expressing the cytokines studied were present in the alveolar interstitium rather than in lumenal cells or the alveolar epithelial lining. The highest values for all four cytokines were present in the patients with FASSc, *i.e.*, 22–39 ISH positive cells·mm^{-2} alveolar tissue compared with 1–19 cells·mm^{-2} and 4–5 cells·mm^{-2} in CFA and control subjects, respectively. Whereas the proportions of the four cytokines in FASSc were similar to the control subjects, IL-4 and IL-5 predominated significantly in CFA ($p<0.001$). For example, the ratio of IL-5 to IFN-γ was 22:1 in CFA, significantly higher than in the cases with FASSc (2:1) or the control subjects (4:1) ($p<0.05$).

In conclusion, cryptogenic fibrosing alveolitis is an inflammatory condition which is characterized, like asthma, by a predominance of gene expression for T-helper-2-type regulatory cytokines, whereas cryptogenic fibrosing alveolitis associated with systemic sclerosis appears to have a distinct mixed T-helper-1/T-helper-2 functional phenotype and a greater number of cells expressing each of these pro-inflammatory cytokines.

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Cryptogenic fibrosing alveolitis (CFA) may occur alone or in association with systemic sclerosis (FASSc); the latter has a prognostic advantage as compared with CFA and there may be an underlying pathophysiological reason for this [1]. The alveolar interstitial inflammatory infiltrate of fibrosing alveolitis is composed of CD3-positive (T)-lymphocytes which are CD45Ro-positive (*i.e.* antigen-primed) and associated with the recruitment of polymorphonuclear leukocytes and macrophages [2, 3]. Cytokines which recruit eosinophils and neutrophils include interleukin (IL)-5 and I-L8, respectively. For both of these cytokines there are differences in gene expression between CFA and FASSc. For example, the concentrations of IL-8 in bronchoalveolar lavage (BAL) fluid and of IL-8 gene expression in lung tissues are significantly higher in CFA than in FASSc, given similar degrees of lung function impairment [4], and comparison of BAL in patients matched for severity of disease, has shown that more eosinophils are recruited to the lower respiratory tract in CFA than FASSc, again indicative of a distinctive pathogenesis [5].

The predominance of the CD4-positive (T-helper (Th)) lymphocyte has been shown in asthma and sarcoidosis. In asthma it is a Th-2-driven antibody-mediated condition associated with the production of IL-4, IL-5, IL-6 and IL-10; in contrast, Th-1 cells produce IL-2 and interferon (IFN)-γ, a profile associated with delayed type hypersensitivity (DTH) granulomatous reactions [6–9]. A predominance of the Th-2-type cytokine profile has been reported in CFA, following a qualitative examination of open lung biopsies with immunolocalization and *in situ* positivity for IL-4 and IL-5 [10] and this has been followed by an *in vitro* study demonstrating that lymphocytes cultured with autologous alveolar macrophages from patients with CFA have unexpectedly high levels of IL-4 and IL-5 [11].

Thus, the aims of the present study were: 1) to determine and quantify gene expression for the Th-1 and

Th-2-associated cytokines present in open lung biopsies from patients with fibrosing alveolitis; 2) to see if the balance of Th-1 and Th-2-type cytokine profiles was different in CFA and FASSc, considering their different activities and granulocyte patterns; and 3) identify the cell source of one of these cytokines, IL-4, for which there is evidence of a profibrotic role.

Materials and methods

Collection and preparation of tissue for in situ *hybridization*

Cytokine gene expression in open lung biopsies obtained from five patients with CFA (median age 56.8 yrs, range 43–67 yrs) and five patients with FASSc (median age 45.6 yrs, range 37–56 yrs) was determined. All included patients had evidence of fibrosing alveolitis and underwent clinical evaluation, including chest radiography and measurements of lung function. The results of high resolution computed tomography (CT) were available for two of the cases with CFA. Patients with systemic sclerosis fulfilled the American Rheumatism Association preliminary criteria for this diagnosis [12], and the presence of fibrosing alveolitis in this group was determined by CT and confirmed by surgical biopsy. As controls, pieces of macroscopically and histologically normal peripheral lung tissue were obtained from five smokers (median age 62.5 yrs, range 44–78 yrs) whose lungs were resected for the presence of localized carcinoma. Fresh tissues (~$1 \times 1 \times 0.5$ cm) were processed using a shortened processing schedule to maximize the retention of messenger ribonucleic acid (mRNA). Sections were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), followed by 2–3 changes of 15% PBS/sucrose rinse, then processed and embedded in paraffin wax. Sections 8 μm thick, were cut and mounted on cleaned, baked, sterile, ribonuclease (RNase)-free poly-L-lysine coated slides.

Isotopic in situ *hybridization*

In situ hybridization of tissue sections was carried out using isotopically labelled probes to maximize sensitivity and to enable double labelling experiments.

Probe preparation. To prepare the probes for *in situ* hybridization, complementary deoxyribonucleic acid (cDNA) for IL-4, IL-5, IL-2 and IFN-γ, were obtained in different vectors from Glaxo Wellcome Biomedical Research (Geneva, Switzerland) and first digested with appropriate restriction endonucleases. After gel electrophoresis, the fragments were recovered, subcloned into pGEM-4Z or pGEM-7Z vectors and propagated in JM 109 cells to give probe sizes as follows: IL-4 (500 bp), IL-5 (450 bp), IL-2(500 bp) and IFN-γ (490 bp). Single stranded sense (mRNA) or antisense (complementary (c)RNA) RNA probes were generated by *in vitro* transcription with linearized plasmids in the presence of adenosine, guanosine, cytidine and ^{35}S uridine triphosphate (ATP, GTP, CTP and ^{35}SUTP, respectively), and SP6 or T7 polymerase, respectively.

Hybridization. Sections were deparaffinized, rehydrated and taken through prehybridization steps for permeabilization (0.85% NaCl), digestion (Proteinase K 1 $\mu g \cdot mL^{-1}$ in 100 mM Tris-HCl pH 8.0, 50 mM ethylenediamine tetraacetic acid (EDTA) for 15 min at 37°C), post-fixation in 4% paraformaldehyde/PBS and acetylation (0.25% acetic anhydride, 0.1 M triethanolamine) to reduce non-specific binding. Hybridization mixture, 20 μL containing nine volumes of hybridization buffer and one volume of radiolabelled sense or antisense probe to give 0.5–1×10^6 counts per minute per section was applied [13]. Sections were covered with sialinized coverslips and hybridized overnight at 42°C in a moist environment. Posthybridization washes were carried out with two changes of $2 \times$ SSC and 10 mM dithiothreitol at 37°C. Unhybridized single stranded RNA was selectively removed with 20 $\mu g \cdot mL^{-1}$ RNase at 37°C. Sections were then dehydrated, dried and dipped in K5 emulsion (Ilford Scientific Products, Cheshire, UK), stored at 4°C in light tight boxes, and exposed to the radioisotope for 14 days. The auto-radiograms were developed in Kodak D-19 developer (Jemmerton Ltd, London, UK) and fixed in Ilford Hypam rapid fixer (Ilford Scientific Products) and counterstained with haematoxylin. In separate sets of slides, positive sections were treated with RNase (20 $\mu g \cdot mL^{-1}$ at 37°C) prior to hybridization to remove tissue RNA, as a control procedure. In addition, sense probes (having the same nucleotide sequence as the target tissue RNA) were used as a negative control procedure.

Double labelling: in situ *hybridization and immunohistochemistry*

To colocalize cytokine mRNA by isotopic *in situ* hybridization and immunolabel to establish the cell phenotype by immunohistochemistry, slides were selected during the final posthybridization washing stage and an alkaline phosphatse/anti-alkaline phosphatase (APAAP) immuno method [14] applied immediately. Sections were immersed in Tris-buffered saline (TBS; 150 mM NaCl, 50 mM Tris-HCl, pH 7.4) followed by incubation with mouse monoclonal anti-bodies (mAbs) to human mast cell tryptase AA1 (Dako M7052; Dako, High Wycombe, UK), or anti-CD3 (Novocastra NCL CD3PS1; Novocastra, Newcastle-upon-Tyne, UK), anti-CD45RO (Dako M742) MTI (Europath, Highland Comfort, Cornwall, UK) or anti-CD20 (Dako M755), respectively. Sections were washed with TBS, and rabbit anti-mouse unconjugated antibody (Z259 Dako) applied. The third layer APAAP (D651 Dako) diluted in normal human serum was applied to enhance the signal. Development of alkaline phosphatase to give an insoluble red end-product was carried out [15] using 3-hydroxy-2-napthoic acid 4-chloro-2-methylanilide (naphthol AS-TR) phosphate and hexazotized new fuchsin solution in presence of 1 mM levamisole to block endogenous activity. Sections were then air-dried and dipped in photographic emulsion for exposure in the dark to the isotope, followed by photographic development and counterstaining with haematoxylin. Negative controls for each immunohistochemistry run were also included.

Quantification. In situ signals, seen as tight clusters of autoradiographic grains, were identified and counted using a Carl Zeiss photomicroscope (Carl Zeiss, Jena, Germany).

An eyepiece graticule consisting of an etched square comprized of 0.1 mm sides with 10×10 points at equal intervals was used to mark areas of section for the purpose of quantification at a total magnification of $\times 375$. Slides were coded to avoid observer bias. Following examination of haematoxylin/eosin stained tissues, the slides which had been hybridized with the sense (negative) probe were examined first in order to detect any aberrant (background) autoradiographic signals. On a separate adjacent section, the numbers of positive antisense signals were then counted in consecutive grid areas by systematically moving the microscope stage until the entire area of the section had been scanned. Depending on their localization, antisense signals were classified as present in association with: alveolar interstitium, alveolar space, conducting airway (bronchiolar) lumen, vessels (artery, veins or lymphatic), pleura and/or connective tissue septa. When a signal was located at the interface of alveolar interstitium and alveolar space, half their total number was allocated to each of these two categories. To test the consistency of quantification and the inherent variation of repeated counts, one section was selected and counted five times over the period of the entire study. Signals with a well defined edge were counted and no attempt at grading was made in respect of the relative intensity or "size" of the signal. The total area of each tissue section and the area constituting the alveolar interstitium was then determined by interactive image analysis (Image 1.38; Apple Macintosh, Cupertino, CA, USA).

Statistical analysis

The coefficient of variation (CV=SD/mean $\times$ 100) for repeat counts of the same tissue section was 0.4%. The results of positive cell counts in the total area of section were expressed per square millilitre of alveolar interstitium in order to normalize for the expected variation in thickness of the alveolar wall. For each cytokine, the number of positive cells occurring in each tissue compartment was also expressed as a per cent of the total number of positive cells counted. The distribution of the data was non-normal and accordingly the Kruskall-Wallace analysis of variance and Mann-Whitney U-tests were applied to test for differences between the three groups. Changes in the proportions of cell numbers were analysed by the Chi-squared test. Assuming the null hypothesis, a p-value <0.05 was accepted as indicating a difference unlikely to have occurred by chance.

Results

The histological appearances of the lungs in control, CFA and FASSc are shown in figures 1a–c. Their appearances are in agreement with previous published descriptions [15–17] and the inflammatory cell profile was as previously described [18]. Cytokine *in situ* hybridization positivity was clearly identified by the tight cluster of autoradiographic grains over each positive cell (fig. 2a–c). The sense probes were negative (fig. 2d). Between 52 and 91% of the hybridization signals were present within the alveolar interstitium with the remainder associated with alveolar space, bronchiolar lumen, vessels, connective tissue septa or pleura.

For the alveolar interstitium, the mean number of each hybridization-positive cell per unit area of tissue varied within and between each group (table 1). On average, mRNA-positive cells for each of the four cytokines were much more frequent in the cases of FASSc than CFA or the controls, but due to the large between patient variability these differences did not reach statistical significance.

The means±SEM for all hybridization-positive cells in the total area of each section analysed for the three patient groups are shown in graphical form in figure 3. Cells expressing IFN-γ and IL-2 were statistically more frequent in the FASSc than the CFA group ($p<0.05$). The remaining two cytokines in the FASSc patients also showed a trend to high levels of expression.

When the relative proportions of the Th-1 to Th-2 cytokines were evaluated in the alveolar interstitium, striking differences emerged. Chi-squared analysis demonstrated that the proportions of the four cytokines in the FASSc group resembled those in the nonfibrotic control group, whereas the proportions in CFA showed a significantly different profile to either the FASSc or control groups ($p<0.001$). Table 2 shows the average ratios of Th-2 to Th-1-like cytokines in the three groups of patients. There

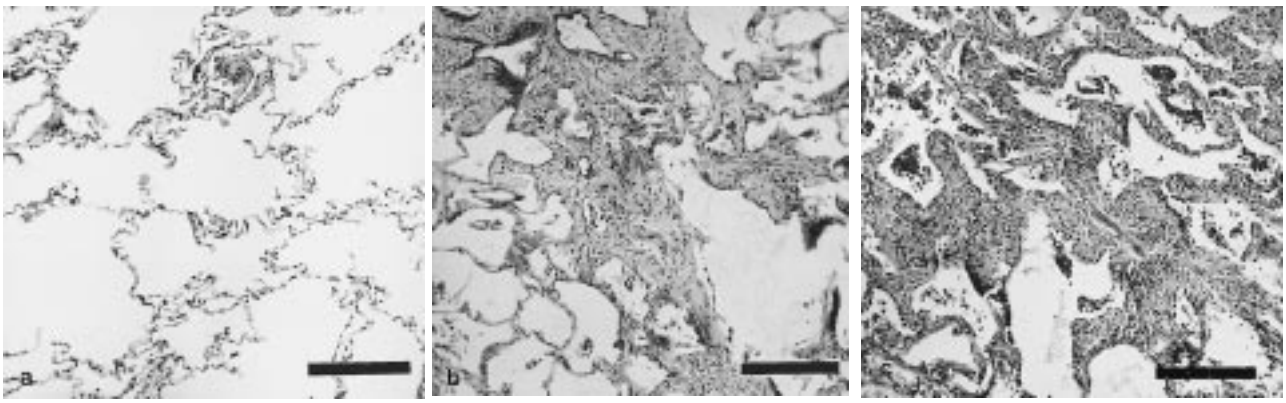

Fig. 1. – Haematoxylin/eosin stained histological sections of human lung. a) A control lung taken from a grossly normal area of a smoker′s lung resected for carcinoma showing alveoli of normal appearance; b) an open lung biopsy from a patient with cryptogenic fibrosing alveolitis (CFA) showing thickening of the alveolar walls due to excess connective tissue matrix and infiltration by inflammatory cells; c) open lung biopsy from a patient with fibrosing alveolitis associated with systemic sclerosis (FASSc) showing similarly thickened walls and inflammation as in (b). (Internal scale bars=400 μm.)

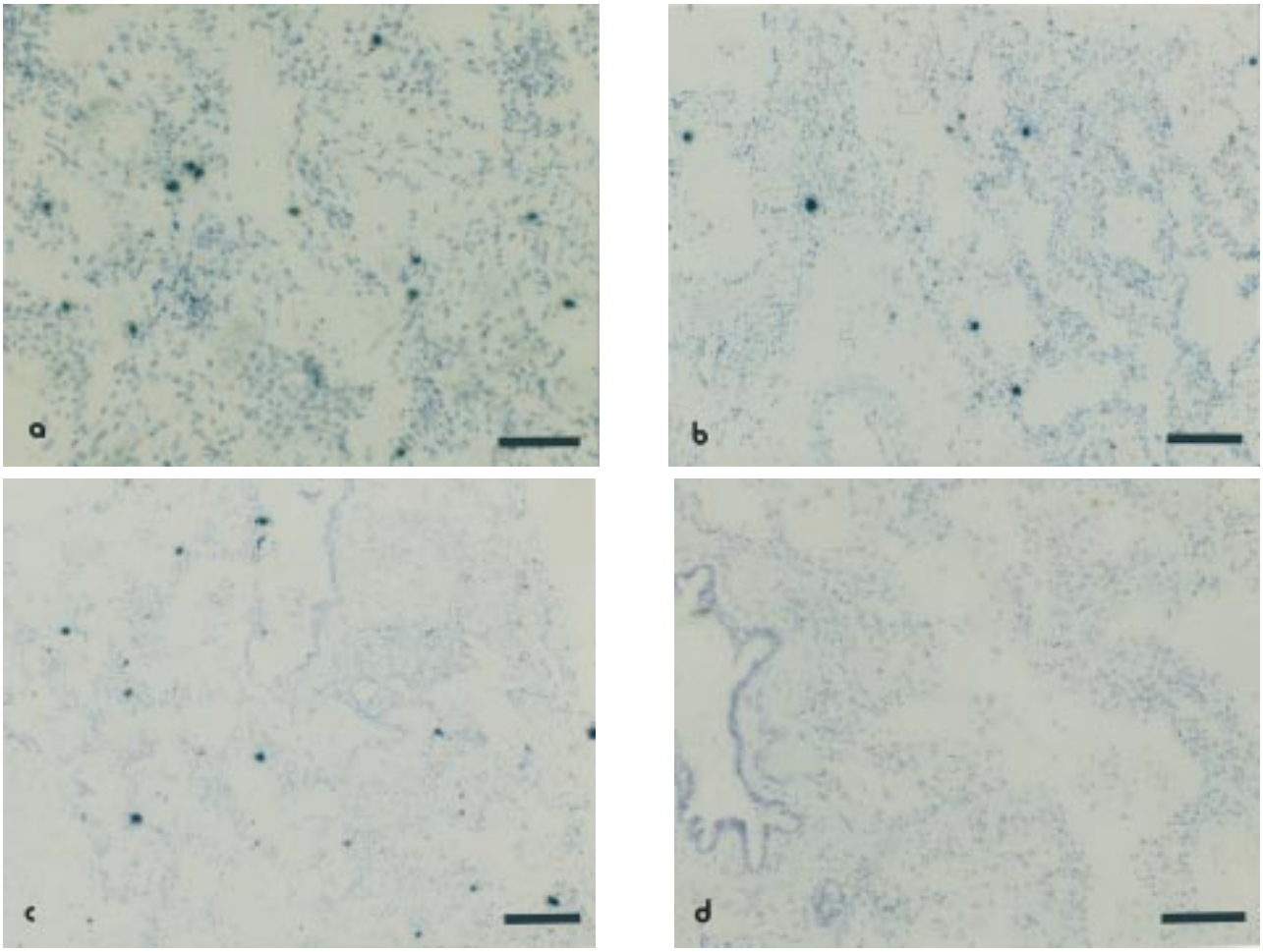

Fig. 2. – Autoradiograms showing the clusters of silver grains localized over cells retaining isotopically (^{35}S) labelled antisense riboprobes which have hybridized avidly with the intracytoplasmic messenger ribonucleic acid (mRNA) of three pro-inflammatory cytokines. a) Interleukin (IL)-4; b) IL-5 and c) interferon gamma (IFN-γ). Gene expression is mainly from cells located in the alveolar wall; d) a control procedure shows the absence of IL-5 signal in an adjacent section of the same case shown in b) but hybridized with an IL-5 sense probe (*i.e.* of the identical sequence as the mRNA of interest). Treatment of sections with ribonuclease (RNase) prior to hybridization with anti-sense probes also ablated the signal confirming signal specificity. (Internal scale bars=150 μm.)

were significantly increased ratios in favour of a Th-2 predominance in CFA compared with FASSc. Thus, the proportional differences in CFA were due to values for IL-4 and IL-5 being higher than the control, and lower values for IFN-γ and IL-2 than the control or FASSc groups.

Having established that CFA was distinct from the other groups in the disproportionate expression of Th-2-type cytokines, the identification of the cell source of the pro-fibrotic cytokine IL-4 was attempted. Double labelling experiments were carried out in an attempt to identify the cell type expressing mRNA for IL-4 by combining *in situ* hybridization and immunohistochemistry, the latter to identify mast cells (tryptase-positive), mononuclear phagocytes (CD-68 positive) and lymphocytes (MT-1 and CD45Ro-positive cells). Figure 4 shows the results of combining isotopic *in situ* hybridization for the Th-2-type cytokine IL-4 and in the same section, immunolabelling for tryptase as a marker of mast cells in a case of FASSc and the colocalization of IL-4 mRNA and mast cell tryptase in the same (mast) cell.

Table 3 shows the results of counts of cells in tissue sections stained for mast cells, monocytes, primed T-cells or T-cell/monocytes and doubled-labelled for IL-4 gene expression by *in situ* hybridization. For example, of 4,203 mast cells counted, only 40 (*i.e.* D/A × 100 or 1%) were also expressing the gene for IL-4. Yet in this same tissue section there was a total of 445 cells showing IL-4 gene expression, *i.e.* 9% of the IL-4 hybridization-positive cells were mast cells. Thus, 91% of the cells expressing the gene at this time were a population of cells other than the mast cell. In the section stained for monocytes/macrophages, 1% of the CD68-positive cells were IL-4-positive by hybridization, but of the total number of IL-4-positive cells, 38% were of the monocyte/macrophage lineage. In respect of CD45R0 (primed lymphocytes), <1% showed IL-4 gene expression, whereas 14% of IL-4-positive cells, were CD45R0-positive. Whilst only 2.5% of MT-1-positive cells were expressing IL-4, 66% of the cells expressing the IL-4 gene were MT-1-positive. As the MT-1 antibody detects both T-cells and monocytes, and 38% of the IL-4 gene-expressing cells were of the monocyte/macrophage lineage, this leaves ~28% of the IL-4 population which were T-cells. These results indicate, therefore, that ~9% of cells expressing the gene for IL-4 were mast cells, 28% were T-cells and 38% were of the monocyte lineage, leaving 25% of undetermined phenotype.

Table 1. – Number of *in situ* hybridization-positive cells per square millimetre of alveolar tissue

	Cytokine			
Group	IFN-γ	IL-2	IL-4	IL-5
CFA	0.9±0.2	4.9±1.6	14.0±7.8	18.5±7.2
FASSc	22.2±14.3	29.9±10.9	38.8±28.3	23.4±12.6
Control	3.7±1.1	5.4±1.4	3.9±1.1	3.9±1.1

Data are mean±SEM. IFN-γ: interferon gamma; IL: interleukin; CFA: cryptogenic fibrosing alveolitis; FASSc: fibrosing alveolitis associated with systemic sclerosis.

Table 2. – Ratios of T-helper (Th)-2/Th^{-1}-associated cytokines

	Cytokine			
Group	IFN-5/IFN-γ	IL-5/IL-2	IL-4/IFN-γ	IL-4/IL-2
CFA	21.7±9.5	8.3±4.6	20.7±8.7	3.2±1.2
FASSc	1.9±1.1 (p=0.01)$^{+}$	0.8±0.2 (p=0.07)$^{+}$	1.4±0.2 (p=0.09)$^{+}$	0.9±0.3 (NS)$^{+}$
Control	3.6±2.5 (p=0.05)$^{+}$	1.2±0.6 (NS)+	2.2±0.9 (NS)+	1.0±0.3 (NS)$^{+}$

Data are mean±SEM. IL: interleukin; IFN-γ: interferon gamma; CFA: cryptogenic fibrosing alveolitis; FASSc: fibrosing alveolitis associated with systemic sclerosis. $^{+}$: compared to CFA group. FASSc compared to the control group were all nonsignificant.

Discussion

Both CFA and FASSc are immunologically mediated conditions with chronic inflammation probably persisting due to an alveolar (auto) antigen expressed in response to viral infection, with Epstein-Barr virus the current suspect [15, 20, 21]. However the evidence supporting a viral aetiology in fibrosing alveolitis remains equivocal [22, 23]. Accordingly, the pathogenesis of both CFA and FASSc involve the infiltration of the alveolar interstitium by T-lymphocytes, a high proportion of which are activated [2, 22]. The present study provides novel data which demonstrate the upregulation of gene expression for four pro-inflammatory cytokines and shows that the relative proportions of gene expression for IFN-γ, IL-2, IL-4 and IL-5 are significantly different in CFA and FASSc. The quantitative molecular findings confirm the immunohistochemical study of WALLACE *et al.* [10] in which a Th-2 functional phenotype is indicated in CFA. The present results are also in accord with the *in vitro* findings of FURUIE *et al.* [11] who examined the accessory cell function of alveolar macrophages from patients with CFA. They demonstrated that when anti-CD3 mAb-stimulated T-cells were co-cultured with autologous alveolar macrophages from patients with CFA, they produced higher concentrations of IL-4 and IL-5 than those of normal nonsmoker or smoker volunteers. Interestingly, IL-10 failed to suppress IL-4 and IL-5 production in the cases of CFA but did so in the normal control subjects. By including nonfibrotic control lung tissue and patients with scleroderma in the present study, a distinctiveness between the profile of pro-inflammatory cytokines in CFA and FASSc has been demonstrated. Such comparisons are of relevance, as whilst CFA and FASSc have similar clinical, aetiological, physiological and histopathological features, they have marked survival differences [1].

The chronic inflammation of fibrosing alveolitis involves the recruitment of granulocytes and the proliferation and activation of alveolar wall fibroblasts associated with increased collagen production which occurs in excess of collagen degradation [25, 26]. Growth factors such as transforming growth factor-β, platelet-derived growth factor (PDGF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are clearly important in stimulating fibroblast proliferation and collagen synthesis. However, two key cytokines, IL-4 and IFN-γ, regulate the balance between Th-1 cell-mediated and Th-2 allergic inflammation and these also appear to be involved in fibrosing lung disease. The present results in humans

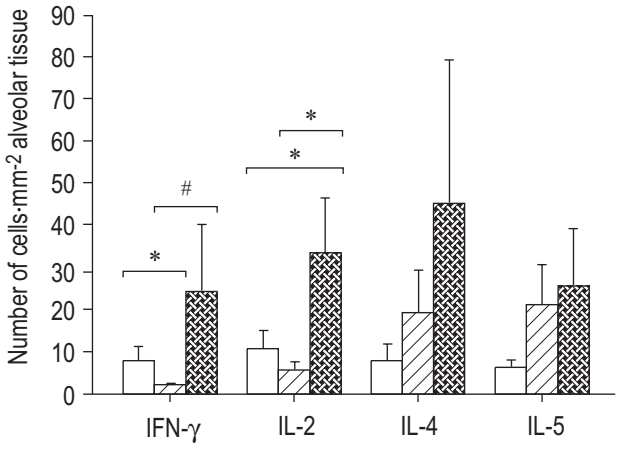

Fig. 3. – Number of *in situ* hybridization-positive cells for interferon (IFN)-γ, interleukin (IL)-2, IL-4 and IL-5 in control tissue samples (□), and tissue from cryptogenic fibrosing alveolitis (▨) and fibrosing alveolitis associated with systemic sclerosis (▩) patients. Data are mean±SEM. *: $p<0.05$; #: $p<0.07$.

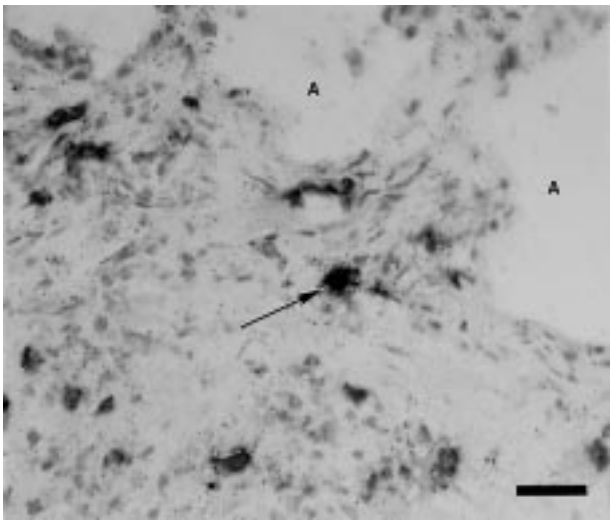

Fig. 4. – Double labelling experiment: the result of immunohistochemistry for mast cell tryptase by the APAAP technique using new fuschin (red) combined with *in situ* hybridization for interleukin (IL)-4 gene expression shown as dense clusters of silver grains in a case of fibrosing alveolitis associated with systemic sclerosis (FASSc). Many mast cells in the thickened alveolar interstitium stain for tryptase alone. A double labelled cell (arrow) is shown where clusters of silver grains are seen superimposed upon a red tryptase positive mast cell in the alveolar wall. Alveolar spaces (A). (Internal scale bar=50 μm.)

Table 3. – Numbers of immuno and interleukin (IL)-4 gene expression-positive cells per section

Cell	Marker	Total immunopositive	Total IL-4 positive	Only IL-4 positive	Double labelled (% cell type)	Immunopositive IL-4 cells %
Mast cell	Tryptase	4203	445	405	40 (<1)	9
Monocyte/macrophage	CD68	4216	135	84	51 (1)	38
Primed T-cell	CD45RO	5330	315	270	45 (<1)	14
T-cell/monocyte	MT-1	25068	941	324	617 (2.5)	66

confirm the studies in mice which demonstrate that the macrophage is one source of the pro-fibrotic cytokine IL-4 [27] by showing that the monocyte/macrophage lineage accounts for ~38% of cells expressing the gene for IL-4 in human fibrosing lung disease and that T-lymphocytes and mast cells account for ~28% and 9%, respectively. There remains, therefore, a further 25% of IL-4 gene expression whose source is at present unknown. Clearly, the upregulation of IL-4 occurs in multiple cell types and these could include structural cells such as fibroblasts or even epithelial cells but further work is required to ascertain this.

IL-4 can stimulate proliferation of extracellular matrix and IL-6 production by human dermal and synovial fibroblasts [28, 29]. These results have been extended to include the murine lung in which (Thy 1+) fibroblasts [30] have been shown to express mRNA for both the membrane bound and soluble form of the IL-4 receptor. The results of SEMPOWSKI *et al.* [30] indicate that elevated levels of IL-4 at a site of injury could result in the development of fibrosis by encouraging a fibroblast subset to proliferate and produce increased extracellular collagen. Further *in vivo* evidence for the proliferative role of IL-4 comes from the transgenic (knock-in) mouse in which increased expression of IL-4 by T-lineage thymic and peripheral lymphoid cells is associated with increased susceptibility to bleomycin-induced inflammation and pulmonary fibrosis which involves lymphocytes and, to a lesser extent, eosinophils. Recent experiments in the bleomycin-induced mouse model of pulmonary fibrosis demonstrate that there is also upregulation of IL-5 mRNA and protein by T-lymphocytes and eosinophils at sites of active fibrosis [31]. These results of IL-4 and IL-5 upregulation support the current findings and provides a role for them in the pathogenesis of human CFA.

In contrast, interferons are known to inhibit the growth of fibroblasts and their collagen synthesis *in vitro* [32–37]. PRIOR and HASLAM [38] have demonstrated that patients with fibrosing lung disorders with the highest levels of circulating IFN more frequently resolve in response to treatment with corticosteroids than those with low circulating levels; in CFA a high proportion of those whose blood lymphocytes showed impaired IFN production deteriorate spontaneously. These findings of blood-derived lymphocytes suggested that impaired production of IFN-γ might be a potentiating factor in the pathogenesis of pulmonary fibrosis. In comparison with the nonfibrotic controls group, the present results in FASSc show the trend to increased levels of not only Th-2 but also Th-1 associated cytokines, the latter which includes IFN-γ. The putative profibrotic effect of IL-4 would be expected, therefore, to be countered by the production of IFN-γ, which together with IL-2, showed an increase in FASSc when compared with either CFA or the nonfibrotic control groups. In respect of the mix of cytokines expressed, it might reasonably be concluded that whilst the inflammatory activity in FASSc would be greater, the fibrotic process might run a slower course with a more favourable outcome and response to corticosteroid therapy than that of CFA. In support of this hypothesis, the results of HEIN *et al.* [39] demonstrate beneficial effects of IFN-γ in the treatment of both the cutaneous and lung lesions of systemic sclerosis.

Finally, it is acknowledged that the differences between CFA and FASSc which are reported here may also be explained by sampling at different stages of the same disease process, as the patients with FASSc are generally seen and studied earlier in the course of their condition. In this case, the distinctions described may not be fundamental but rather reflect differences in the total numbers of activated cells at the differing times of sampling during the course of one disease process. Future studies will need to control for this by examining larger numbers of patients and careful selection and matching of the groups by both CT and measures of lung function.

In conclusion, cryptogenic fibrosing alveolitis appears to represent another inflammatory condition of the lung which is characterized by an infiltration of the alveolar interstitium by T-lymphocytes, predominantly of the T-helper-2 functional phenotype. These data highlight possibilities for a novel therapeutic modulation of the progression of lung fibrosis.

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