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Corticosteroids restore the balance between locally produced Th1 and Th2 cytokines and immunoglobulin isotypes to normal in sarcoid lung

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SUMMARY

In this study, we have investigated the balance between Th1- and Th2-like activity in the lungs in sarcoidosis and have determined the effect of corticosteroid treatment on this. Twenty-one patients with acute untreated sarcoidosis were investigated by bronchoalveolar lavage (BAL) and compared with 11 normal volunteers. Sixteen of the sarcoid patients required corticosteroid therapy and seven of these were reinvestigated after 2–3 months' treatment. In order to assess Th1- and Th2-like activity in the lungs, IgG subclasses and IgE were measured in BAL fluid and serum, and IL-2, IL-4 and interferon-gamma (IFN-γ) in BAL. In patients with untreated sarcoidosis, albumin-corrected BAL/serum ratios for IgG4 and IgE were significantly reduced (IgG4, $1{\cdot}04 \pm 0{\cdot}18$ (mean ± s.e.m.); IgE $9{\cdot}58 \pm 3{\cdot}11$) compared with those in normal controls (IgG4 $5{\cdot}3 \pm 0{\cdot}72$, $P < 0{\cdot}001$; IgE $67{\cdot}7 \pm 28{\cdot}9$, $P < 0{\cdot}01$). Estimates of actual levels of immunoglobulins produced in the lungs were also made and showed extremely high levels of total IgG in sarcoid patients ($39{\cdot}56 \pm 8{\cdot}2$ mg/l) compared with controls ($1{\cdot}17 \pm 0{\cdot}5$ mg/l, $P < 0{\cdot}001$). Although there was no difference between the groups in amount of IgG4 locally produced, the proportion of total IgG which was IgG4 was greatly reduced in those with sarcoidosis ($1{\cdot}6 \pm 0{\cdot}4$% compared with $38{\cdot}5 \pm 3{\cdot}2$%; $P < 0{\cdot}001$). Lavage levels of IL-4 were also reduced in sarcoid patients (IL-4 $2{\cdot}103 \pm 0{\cdot}21$ pg/ml) compared with those in normals (IL-4 $6{\cdot}8 \pm 1{\cdot}05$; $P < 0{\cdot}001$). Levels of IL-2 were lower ($7{\cdot}63 \pm 0{\cdot}51$ pg/ml compared with $9{\cdot}4 \pm 0{\cdot}95$ pg/ml), but this difference was not significant. IFN-γ, however, could not be detected above 0·4 pg/ml in any of the normal lavage fluid, but was detectable in 12/21 patients with sarcoidosis ($\chi^2 = 7{\cdot}74$; $P < 0{\cdot}001$). These changes reverted towards normal on treatment with oral corticosteroids. The mean albumin-corrected BAL/serum ratio for IgG4 before treatment was $0{\cdot}88 \pm 0{\cdot}33$ compared with $5{\cdot}5 \pm 2{\cdot}1$ ($P < 0{\cdot}05$) on treatment, and for IgE before treatment $9{\cdot}52 \pm 2{\cdot}15$ compared with $50{\cdot}8 \pm 17{\cdot}9$ ($P < 0{\cdot}05$) on treatment. Total IgG produced in the lung fell from $26{\cdot}16 \pm 7{\cdot}9$ to $6{\cdot}12 \pm 2{\cdot}4$ mg/l ($P < 0{\cdot}001$) on treatment, and the proportion of IgG4 locally produced rose from $2{\cdot}3 \pm 0{\cdot}8$% to $23{\cdot}9 \pm 6{\cdot}1$% ($P < 0{\cdot}01$). The mean level of IL-4 in lavage before treatment was $2{\cdot}53 \pm 0{\cdot}34$ pg/ml compared with $4{\cdot}7 \pm 0{\cdot}34$ ($P < 0{\cdot}001$) on treatment. Levels of IL-2 also rose significantly on treatment from $8{\cdot}74 \pm 0{\cdot}95$ pg/ml before to $14{\cdot}44 \pm 1{\cdot}38$ pg/ml ($P < 0{\cdot}001$) on treatment. Levels of IFN-γ fell from $1{\cdot}65 \pm 0{\cdot}43$ pg/ml before treatment to undetectable levels in all patients ($P < 0{\cdot}001$) on treatment. These results demonstrate an imbalance between Th1- and Th2-like activity in the lungs in sarcoidosis, with suppression of Th2 and increase in Th1. Corticosteroid therapy restores the normal balance between Th1 and Th2 cytokines and immunoglobulins in the lungs, suggesting an effect on local immune regulation.

Keywords sarcoidosis Th1/Th2 bronchoalveolar lavage corticosteroids

INTRODUCTION

Sarcoidosis is a systemic granulomatous disorder involving the lungs in over 90% of patients. Although the etiology is uncertain, possible causes include a transmissible agent [1,2], genetic susceptibility [3,4] and an abnormal host response to inhaled antigen. As intrathoracic involvement is common, it seems highly likely that the lungs are the port of entry for any transmissible agent which could result in stimulation of local cell-mediated immunity in susceptible patients.

Immunization with a soluble protein antigen results in production of different types of effector $CD4^+$ T cells. These have been termed T helper 1 (Th1) and T helper 2 (Th2) on the basis of the cytokines they secrete [5]. Th1 cells in the mouse make interferon-gamma (IFN-γ), IL-2 and tumour necrosis factor-alpha (TNF-α). These cells effect cell-mediated immunity and support IgG2a

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antibody production. Th2 cells make IL-4, IL-5, IL-6 and IL-10 and support IgE production. Although much less information is available on cytokine profiles of different human T cells, there is increasing evidence that Th1 and Th2 responses occur [6,7]. For example, most but not all T cell clones prepared from peripheral blood of atopic donors are Th2-like [8,9] while those from non-atopic individuals are more commonly Th1-like [8–10]. In addition to IgE, the Th2 cytokine IL-4 has been shown to switch human peripheral blood B cells to IgG4 [11]. Cytokine gene expression for IL-3, IL-4, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF), but not IL-2 or IFN-γ, have been detected *in vivo* in allergen-induced late-phase reactions of both nose and skin [12,13]. Furthermore, Robinson *et al*. [14] demonstrated a Th2-like pattern of cytokine mRNA expression in bronchoalveolar lavage (BAL) T cells from atopic asthmatics.

In granulomatous diseases, however, there is evidence for a predominant Th1-type response. In leprosy, *Mycobacterium leprae*-specific T cell clones have been demonstrated to be of the Th1 phenotype [15], and skin biopsies of tuberculin reactions exhibit a predominantly Th1 pattern of cytokine mRNA [16]. Furthermore, in pulmonary tuberculosis there is evidence for a Th1-like bronchoalveolar T cell subset [17]. In sarcoidosis, IFN-γ was released from both alveolar macrophages and T lymphocytes recovered by BAL [18], and IL-6 production by monocytes and alveolar macrophages is also elevated [19].

Local production of both IgE and IgG4 by B cells is indicative of a Th2-type response and requires IL-4 but is inhibited by IFN-γ [20]. IgG4 is a subclass of IgG involved in the normal response to chronic antigen stimulation, and is thought to be a blocking rather than a sensitizing antibody [21]. Normal individuals produce high levels of IgG4 in their lungs [22–24], but Rankin *et al*. [25] found considerably reduced levels of IgG4 in BAL fluid (BALF) from patients with sarcoidosis, despite elevation of total IgG. This suggests dysfunction of immune regulation and possible depression of Th2 responses.

In the present study we have examined local production of Th1- and Th2-associated immunoglobulins and cytokines to test the hypothesis that there is an imbalance between Th1- and Th2-like responses in the lungs in sarcoidosis, and whether corticosteroid therapy will restore the balance to normal.

PATIENTS AND METHODS

Patients

Twenty-one patients with acute untreated sarcoidosis and 11 normal, non-smoking volunteers were studied. None of the normal controls had experienced an upper respiratory tract infection in the 6 weeks before investigation. One had a history of eczema and one of asthma; neither were on any medication including any form of steroid therapy. Twenty patients with sarcoidosis had radiological evidence of intrathoracic disease (Table 1), and all had evidence of non-caseating granulomata on transbronchial biopsy. One had a previous history of asthma and one of hayfever. Neither were on any medication for these conditions. In one patient, mycobacteria were seen microscopically in the lavage fluid but failed to grow in culture. She had no risk factors for tuberculosis and failed to respond clinically to anti-tuberculous therapy. When steroids were added to the regimen, however, there was a dramatic improvement both clinically and radiologically. Eleven patients were male and 10 female, and their ages ranged from 18 to 72 years. Fifteen patients showed abnormalities of lung function at presentation. Sixteen patients were later treated with corticosteroids on the basis of clinical deterioration, including declining lung function or extrapulmonary manifestations of disease. Seven of these were bronchoscoped a second time 2–3 months after starting treatment. At the time of the second bronchoscopy, all demonstrated clinical evidence of improvement.

Table 1. Staging of chest radiographs in patients with sarcoidosis at presentation

Stage	Chest x-ray	Number of patients
0	Normal chest x-ray	1
I	Lymphadenopathy only	3
II	Lymphadenopathy plus interstitial shadowing	12
III	Interstitial shadowing no lymphadenopathy	5
IV	Fibrosis	0

All except one patient in the study had radiological evidence of pulmonary disease and none had end stage fibrotic disease.

Specimen collection

Fibreoptic bronchoscopy with BAL was performed as previously described [26]. Nine 20-ml aliquots of warmed normal saline were instilled into the right middle lobe or lingular and aspirated into silicone-coated glass bottles maintained at 4°C. The same volume was used for all patients and controls and all lavages were performed by one operator (H.J.M.). There was no significant difference in fluid recovered for both groups (Table 2). The whole lavage fluid was centrifuged at 300 ***g*** for 10 min at 4°C and the supernatant stored in aliquots at −20°C for future analysis. Serum collected at the time of bronchoscopy was similarly stored.

Measurement of IgG subclasses

The four subclasses of IgG were measured by ELISA using Bindazyme human immunoglobulin G subclass enzyme immunoassay kits (The Binding Site, Birmingham, UK). Briefly, samples

Table 2. Bronchoalveolar lavage (BAL) volumes instilled and recovered

	Normals ($n = 11$)	Sarcoids at presentation ($n = 21$)	Sarcoids before treatment ($n = 7$)	Sarcoids on treatment ($n = 7$)
Volume instilled (ml)	180	180	180	180
Volume recovered (ml)	130 (100–150)	120 (100–150)	110 (100–130)	130 (100–150)

Figures given are medians, with range in parentheses. There were no significant differences between any of the groups.

were applied in duplicate to wells coated with mouse MoAb to human IgG1, IgG2 and IgG3, and sheep polyclonal antibodies for IgG4 estimation. Serum was used in the assay at 1 : 25 000 dilution and BALF used neat and at 1 : 2 and 1 : 10 dilutions. Incubations were for 2 h at 37°C. Plates were washed three times between stages. The conjugate used was a sheep anti-human IgG conjugated to horseradish peroxidase and the colour developed with 3,3′,5,5′-tetramethyl benzidine (TMB) substrate. The reaction was stopped with phosphoric acid after 30 min at room temperature, which converted the blue reaction product to yellow. Optical density (OD) was read at 450 nm on a Multiskan ELISA plate reader. Calibration curves were constructed for each plate using a stabilized human serum calibrator calibrated against the SPS01 and WHO 67/97 reference calibration for IgG subclasses. The assay ranges were as follows: IgG1 2·6–168 mg/*l*; IgG2 17–1080 mg/*l*; IgG3 3·1–196 mg/*l*; IgG4 2·1–132 mg/*l*.

Measurement of albumin

Albumin was measured in serum and lavage fluid using human Albumin Radial Immunodiffusion kits (The Binding Site). RID plates were optimized for normal concentrations for serum samples and for low concentrations for BAL samples. Specimens were applied in duplicate to the plates in dilutions according to the manufacturer's instructions. None of the paired samples differed by more than 5%. Standard curves were constructed using human high and low albumin standards (The Binding Site). Plates were incubated at room temperature until the diameter of the undiluted standard reached the appropriate size indicating complete diffusion. This took up to 1 week for lavage samples before diffusion rings became visible by side lighting. All the resulting ring diameters were measured by one operator using a calibrated eye piece and standard curves made by plotting the square of the diameters of the precipitates formed by the standards against their protein concentration in mg/*l*.

Measurement of IgE

IgE was measured using a previously described ELISA [27]. Briefly, plates were coated with purified anti-human IgE (clone 7.12, a kind gift from Professor A. Saxon, UCLA, Los Angeles, CA) at a concentration of 1 mg/ml. Serum samples were diluted 1 : 10 with PBS–Tween–5% horse serum and BAL specimens applied neat. The plates were incubated overnight at 4°C, washed three times, then incubated overnight with a rabbit anti-IgE conjugated to alkaline phosphatase at 1 : 300 dilution. Plates were washed three times and developed with *p*-nitrophenyl phosphate 1 mg/ml in diethanolamine buffer pH 9·8. The OD of the resulting product was read after 3 h at 405 nm. The minimum detection limit of the assay was 100 pg/ml.

Measurement of cytokines

IL-4, IL-2 and IFN-γ were measured in BALF only. Using neat BALF no IL-4 could be detected, so the samples were concentrated 10 times using Microcon 10 concentrator vials (Amicon Inc., Beverly, MA). The concentrated samples were assayed using a quantitative sandwich ELISA (Amersham, Aylesbury, UK) with an assay range of 15–2000 pg/ml. Colour was developed using tetramethylbenzidine and OD read at 450 nm. IL-2 was also undetectable in neat BALF. Samples were concentrated five times as above and measured using a similar ELISA method (R & D Systems, Inc., Minneapolis, MN). The assay range was 30–2000 pg/ml. IFN-γ was detectable in neat BALF from four patients with sarcoidosis. The remaining samples were concentrated five times as above and assayed using a similar ELISA method (AMS Biotechnology, Europe Ltd., Witney, UK). The assay range was 2–10 000 pg/ml. The size of filters in the concentrator vials was chosen to retain those cytokines we were interested in. All samples were treated identically, therefore any limitations of the method (e.g. molecules sticking to the filter) would have applied equally to controls and patients. It is widely accepted that all methods of concentrating BALF have limitations, and we have used the best technology available [28] and taken care to ensure that the same experimental conditions were applied to all samples.

Correction for transudation of serum proteins into BALF

All immunoglobulin classes were represented in the blood. A proportion of these proteins in lung secretions, therefore, will have been derived from the vascular compartment by diffusion across lung tissue, which acts as a semipermeable membrane with respect to proteins in solution. Some proteins such as albumin were derived almost exclusively from the blood. In order to assess whether an immunoglobulin found in lavage fluid is in excess of that which could be present by simple diffusion from serum, an albumin correction can be used. The formula:

$$\frac{(\text{immunoglobulin in BAL})}{(\text{albumin in BAL})} \div \frac{(\text{immunoglobulin in serum})}{(\text{albumin in serum})}$$

was applied. Taking into account the size of immunoglobulins, a ratio of approximately 0·6 would be expected from diffusion alone. Thus any result > 0·6 implies some local production of antibody in excess of that diffused from serum [29], or active transport from serum to secretions. It then becomes possible to calculate an estimated amount of each immunoglobulin which is locally produced and the proportion of IgE and each subclass of IgG of the total immunoglobulin measured.

In inflammatory lung disease there is increased leakage of serum proteins into the lungs. In order to compare local production of a protein in the lungs of normals with that in patients with sarcoidosis, it is necessary to relate levels of those proteins also found in the blood to a standard substance such as albumin. Albumin gives a measure of the degree of that leakage, thus allowing an estimation of the proportion of locally produced protein. Several other reference markers have been tried to overcome the problems of dilution of soluble components in BALF and leakage from serum. All have associated problems and most investigators use albumin [28].

Levels of cytokines are expressed as a ratio of picograms of cytokine per μg of albumin in lavage without reference to serum levels.

Statistical analysis

Results are expressed as the mean ± s.e.m. Comparisons of the levels and ratios of immunoglobulins and cytokines were made by both non-parametric statistics (Mann–Whitney *U*-test) and by paired or unpaired Student's *t*-tests, as data were sufficiently normally distributed, and using the unequal variance technique to allow for unequal variance between groups where appropriate. Both methods yielded the same results. Wilcoxon rank sum tests were used to analyse data relating to estimates of quantity and proportion of immunoglobulins locally produced. Where data were scored as positive and negative, they were analysed using the χ^2 test.

Table 3. Levels of immunoglobulins and albumin in serum

	Normals ($n = 11$)	Sarcoids at presentation ($n = 21$)	Sarcoids before Rx ($n = 7$)	Sarcoids on Rx ($n = 7$)
IgG1, mg/ml, normal range (4·2–12·9)	10·2 (1·3)	13·1 (1·2)	13·1 (2·3)	8·3 (1·7)
IgG2, mg/ml, normal range (1·2–7·5)	4·0 (0·3)	6·5 (0·8)	7·9 (1·6)	6·1 (0·8)
IgG3, mg/ml, normal range (0·4–1·3)	1·0 (0·1)	1·3 (0·3)	1·4 (0·5)	1·0 (0·5)*
IgG4, mg/ml, normal range (< 2·9)	0·4 (0·05)	0·6 (0·1)	0·6 (0·1)	0·4 (0·1)*
IgE, U/ml, normal range (1–100)	12·5 (1·8)	8·13 (1·96)	4·79 (1·62)	2·23 (0·58)*
Albumin, mg/ml, normal range (35·0–45·0)	36·9 (2·0)	37·1 (2·0)	30·0 (3·0)	31·8 (3·0)

Subclasses of IgG, IgE and albumin were within or very close to the normal range in serum for both patients with sarcoidosis and controls. There was, however, a tendency for higher levels of all the IgG subclasses in serum of patients with sarcoidosis. Values given are mean with s.e.m. in parentheses; Rx, Treatment. * $P < 0{\cdot}05$.

RESULTS

IgG subclass levels

Serum IgG subclass levels fell within the normal range in normal volunteers (Table 3). Patients with sarcoidosis, however, tended to have higher serum levels of all the four subclasses, consistent with the higher levels of immunoglobulins normally found in this disease (Table 3). Serum levels fell in patients reassessed after 2–3 months treatment with oral corticosteroids. Furthermore, patients not requiring steroid treatment (i.e. 'well' patients, $n = 5$) tended to have lower serum levels of IgG1 ($7{\cdot}14 \pm 1{\cdot}3$ g/l), IgG2 ($5{\cdot}56 \pm 0{\cdot}88$ g/l) and IgG3 ($0{\cdot}84 \pm 0{\cdot}5$ g/l) than those requiring treatment ('sick' patients, $n = 16$) (IgG1 $10{\cdot}24 \pm 1{\cdot}27$; IgG2 $6{\cdot}08 \pm 0{\cdot}8$; IgG3 $1{\cdot}2 \pm 0{\cdot}25$ g/l), although these differences were not statistically significant.

All subclasses of IgG could be detected in BALF from both normal volunteers and patients with sarcoidosis by the methods used. Absolute levels of immunoglobulins in BALF, however, were not comparable between normal and diseased groups, as these are partially dependent on lung capillary leakiness. This is shown by the large difference found between normals and patients with sarcoidosis for lavage albumin: $9{\cdot}8 \pm 2{\cdot}1$ mg/l in normals, $159{\cdot}5 \pm 42{\cdot}1$ mg/l in patients ($P < 0{\cdot}01$). Again, those patients reassessed while on treatment showed a fall in levels of BAL albumin from $147{\cdot}8 \pm 57{\cdot}9$ mg/l to $47{\cdot}2 \pm 45{\cdot}8$ mg/l ($P < 0{\cdot}05$).

Table 4. Immunoglobulin lavage : serum ratios corrected for albumin

	Normals ($n = 11$)	Sarcoids at presentation ($n = 21$)	Sarcoids before treatment ($n = 7$)	Sarcoids on treatment ($n = 7$)
IgG1	2·0 (0·84)	1·3 (0·27)	1·13 (0·16)	1·57 (0·33)
IgG2	0·29 (0·12)	0·42 (0·09)	0·43 (0·06)	0·46 (0·16)
IgG3	1·26 (0·57)	0·54 (0·17)	0·4 (0·18)	0·045 (0·019)
IgG4	5·3 (0·72)	1·04 (0·18)***	0·88 (0·33)	5·46 (2·07)*
IgE	67·67 (28·9)	9·58 (3·11)**	9·52 (2·15)	50·8 (17·9)*

There was a significant reduction in corrected lavage : serum ratios for both IgG4 and IgE in patients with sarcoidosis compared with normals. In the seven patients re-evaluated following 2–3 months treatment with corticosteroids, these levels rose significantly. Values given are mean (s.e.m.). * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

Local production of IgG subclasses in the lungs

The BAL/serum ratio corrected for albumin gives some measure of local immunoglobulin production or the concentration of a specific protein in the lungs. All normal volunteers showed evidence of local production of IgG1 and IgG4 in their lungs, with particularly high levels of IgG4 (Table 4). Three also had evidence of local production of IgG3, but the majority had low levels of IgG2 and IgG3 in their lungs relative to serum. None demonstrated local production of IgG2. In patients with sarcoidosis, the patterns of local production of IgG subclasses were similar to normals for IgG1, IgG2 and IgG3. Corrected BAL/serum ratios for IgG4, however, were greater than 1 in only nine of the 21 patients, and ratios for this subclass were significantly lower than those found in normal volunteers (Table 4; Fig. 1). Using the absolute concentrations of IgE and the different subclasses of IgG in BAL, it is possible to calculate an estimated amount of protein locally produced, and these figures are presented in Table 5. It is immediately clear that patients with sarcoidosis were making excessive quantities of total IgG in their lungs, most of which was in the form of IgG1. In normal lungs, there was a greater proportion of locally produced IgG4 than IgG1 ($P < 0{\cdot}05$). Whilst there was no difference in the estimated amount of locally produced IgG4 in mg/l between patients and controls, as a proportion of totally produced IgG, it was significantly lower in sarcoid patients ($P < 0{\cdot}001$).

IgE levels and local production

Serum levels of IgE were quite variable in both normal and sarcoid groups, with both two normals and two sarcoids having high serum levels: mean $12{\cdot}5 \pm 2{\cdot}8$ U/ml, range 0·81–54 U/ml, in normals; and $8{\cdot}13 \pm 2{\cdot}96$ U/ml, range 0·35–40 U/ml, in untreated patients with sarcoidosis. All individuals with high serum levels of IgE were those with evidence of atopic disease.

There was, however, a significant reduction in corrected BAL/serum ratios for IgE in patients with sarcoidosis: mean $9{\cdot}58 \pm 3{\cdot}11$ U/ml compared with $67{\cdot}67 \pm 28{\cdot}9$ U/ml in normals ($P = 0{\cdot}01$) (Fig. 1). Estimates of amount of IgE locally produced were no different in the two groups, but as a proportion of total protein measured there was a significant reduction in patients with sarcoidosis ($P < 0{\cdot}001$; Table 5).

Levels of cytokines in BAL

IL-4 levels in BALF were significantly reduced in patients with acute untreated sarcoidosis ($2{\cdot}53 \pm 0{\cdot}34$ pg/ml) compared with

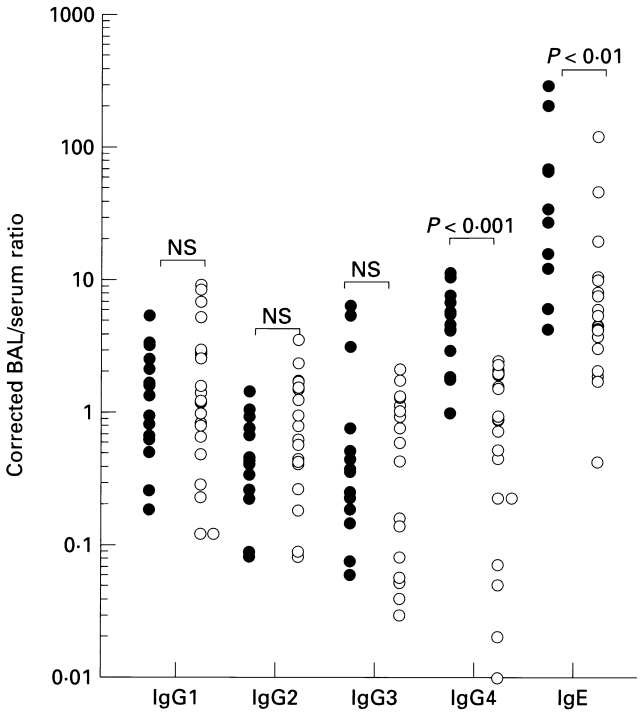

Fig. 1. The concentration of IgG1–4 and IgE in bronchoalveolar lavage (BAL)/serum corrected for albumin in patients with sarcoidosis (○) and normal controls (●). There was no difference between normals and patients with sarcoidosis for IgG1, IgG2 or IgG3, but levels of IgG4 and IgE were significantly reduced in patients with sarcoidosis.

normals (4·7 ± 0·34 pg/ml; $P < 0·001$) (Fig. 2). Levels of IL-2 in BALF of patients with sarcoidosis were reduced, but not significantly (7·63 ± 0·51 pg/ml compared with 9·4 ± 0·95 pg/ml). IFN-γ, however, could not be detected in concentrated BALF from controls, but was detectable at levels above 0·4 pg/ml (BALF × 5 concentrated) in 12/20 patients with acute sarcoidosis ($\chi^2 = 7·74$; $P < 0·01$) (Fig. 2). Levels of all cytokines measured in BALF were low, and in most cases the fluid had to be concentrated five or 10 times before they could be detected.

Differences between 'sick' and 'well' sarcoid patients

Sixteen patients were treated with corticosteroids on clinical evidence of severe or deteriorating disease, and are hitherto referred to as 'sick'. Five patients did not require treatment on clinical grounds and are hitherto referred to as 'well'. There were no significant differences between these two groups of patients for serum levels of albumin, IgG subclasses, IgE or IL-4, but BAL albumin levels in 'sick' patients were 179·5 ± 48·1 mg/*l* compared with 68·7 ± 11·7 mg/*l* in 'well' patients. Furthermore, despite the disparate numbers in each group, 'well' patients had significantly higher BAL/serum ratios of IgG4 (1·87 ± 0·24) and IgE (45·85 ± 27·12) compared with the 'sick' group (IgG4 0·82 ± 0·18, $P < 0·05$; IgE 6·12 ± 1·53, $P < 0·01$).

Effect of corticosteroids

Seven patients were reassessed after 2–3 months treatment with oral corticosteroids. All showed some improvement in chest x-ray and lung function at this time. In addition to a fall in serum immunoglobulins (Table 3), BALF albumin levels fell from 147·8 ± 57·9 mg/*l* to 47·2 ± 45·8 mg/*l* ($P < 0·05$). There was no

Table 5. Estimate of immunoglobulin produced in the lungs

		Normals ($n = 11$)	Sarcoids at presentation ($n = 21$)	Sarcoids before treatment ($n = 7$)	Sarcoids on treatment ($n = 7$)
Total IgG locally produced (mg/ml)		1·17 (0·5)	39·56*** (8·2)	26·16 (7·9)	6·12*** (2·4)
IgG1	a	41 (11)	53 (9)	42 (9·4)	37 (13)
	b	0·35 (0·12)	30·1 (8·6)***	25·4 (10)	3·9 (2·6)*
	c	30 (8)	76·1 (21)***	97·1 (15)	63·7 (14)*
IgG2	a	28 (9·9)	33 (9·1)	9 (8)	16·6 (10)
	b	0·29 (0·14)	7·73 (3·1)**	0·13 (0·09)	0·75 (0·5)
	c	25 (9)	19·5 (8)	0·5 (0·2)	12·3 (6)
IgG3	a	29 (14)	26 (9·6)	12 (8)	8 (5)
	b	0·08 (0·04)	1·11 (0·53)*	0·16 (0·02)	0·01 (0·01)*
	c	6·8 (2·6)	2·8 (1·5)	0·06 (0·01)	0·1 (0·03)
IgG4	a	82 (4·7)	30 (6·6)***	15 (10)	63 (13)**
	b	0·45 (0·09)	0·62 (0·32)	0·18 (0·1)	1·46 (0·39)**
	c	38·5 (3·2)	1·6 (0·4)***	2·3 (0·8)	23·9 (6·1)**
IgE	a	96 (1·2)	83 (5·7)	89 (3·7)	95 (2·9)
	b	0·08 (0·007)	0·2 (0·03)	0·036 (0·02)	0·026 (0·01)
	c	6·7 (1·8)	0·5 (0·2)**	0·14 (0·02)	0·42 (0·02)*

Estimate of amount of immunoglobulins produced in the lungs using levels measured in bronchoalveolar lavage (BAL). (a) Percent of immunoglobulin measured in BAL which is locally produced. (b) Estimate of amount locally produced (mg/ml). (c) Proportion of total IgG which is produced in the lungs. Figures given are mean with s.e.m. in parentheses.

* $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

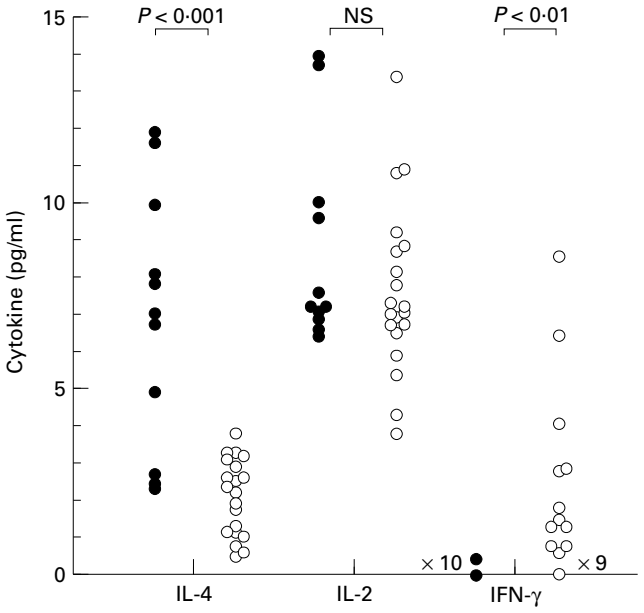

Fig. 2. The concentration of cytokines IL-4, IL-2 and IFN-γ in bronchoalveolar lavage fluid (BALF) from patients with sarcoidosis (○) compared with normal controls (●). Patients with acute sarcoidosis had significantly depressed levels of IL-4, but elevated levels of IFN-γ could be measured in 12/21 patients, while IFN-γ could not be detected in BALF from normal controls.

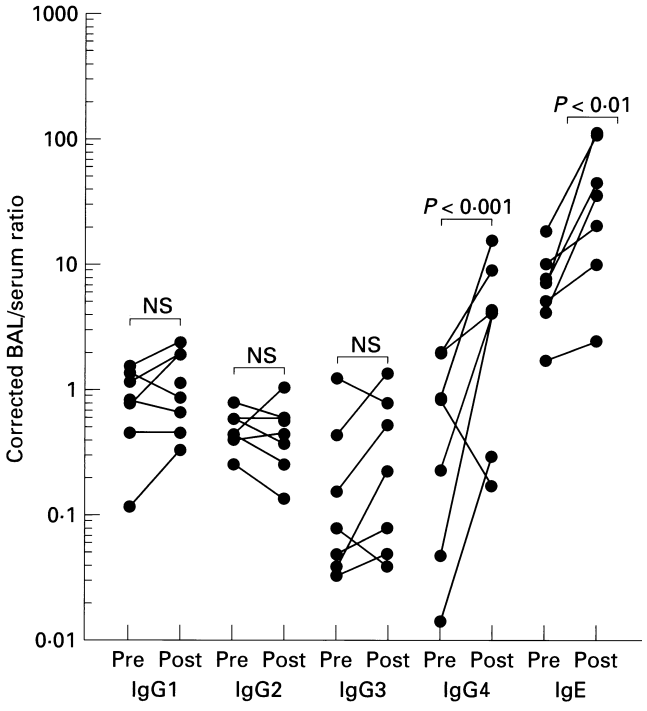

Fig. 3. The effect of corticosteroid treatment on the bronchoalveolar lavage (BAL)/serum ratio of IgG1–4 and IgE in patients with sarcoidosis. There was no change in levels of IgG1, IgG2 or IgG3 on corticosteroid treatment, but levels of both IgG4 and IgE rose significantly in 6/7 patients (IgG4) and 7/7 patients (IgE) on treatment.

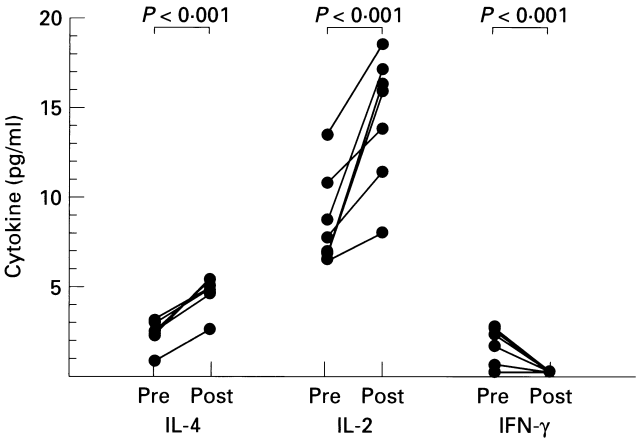

Fig. 4. The effect of corticosteroid treatment on levels of cytokines in lavage fluid in patients with sarcoidosis compared with normals. There was a significant increase in levels of IL-4 in all seven patients on treatment with corticosteroids ($P < 0{\cdot}001$). IL-2 levels also rose significantly ($P < 0{\cdot}001$). IFN-γ could be detected in bronchoalveolar lavage fluid (BALF) from 6/7 patients with acute untreated sarcoidosis, but these levels became undetectable following 2–3 months treatment ($P < 0{\cdot}001$).

detectable change in corrected BAL/serum ratios for IgG1, IgG2 or IgG3 on treatment, but significant increases in ratios for IgG4 ($0{\cdot}88 \pm 0{\cdot}33$ to $5{\cdot}46 \pm 2{\cdot}07$, $P = 0{\cdot}05$) and IgE ($9{\cdot}52 \pm 2{\cdot}15$ to $50{\cdot}75 \pm 17{\cdot}88$, $P < 0{\cdot}05$) (Fig. 3). Estimated concentrations of IgG4 and IgE as a proportion of total immunoglobulin measured confirmed results using BAL/serum ratios corrected for albumin (Table 5). These show significantly low proportions of both IgG4 and IgE before treatment, which rose to near normal levels on treatment. Furthermore, levels of IL-4 in BALF rose significantly on corticosteroid therapy from $2{\cdot}53 \pm 0{\cdot}34$ pg/ml to $4{\cdot}7 \pm 0{\cdot}34$ pg/ml ($P < 0{\cdot}001$), while levels of IFN-γ in BALF fell from $1{\cdot}65 \pm 0{\cdot}43$ pg/ml to unrecordable levels in all patients ($P < 0{\cdot}001$) (Fig. 4). Levels of IL-2 also rose significantly in all those reassessed on treatment from $8{\cdot}74 \pm 0{\cdot}95$ pg/ml to $14{\cdot}44 \pm 1{\cdot}38$ pg/ml ($P < 0{\cdot}001$) (Fig. 4).

DISCUSSION

This study shows depressed local production of IgG4 and IgE in the lungs of patients with pulmonary sarcoidosis. BAL levels of IL-4 were also reduced in patients compared with normals. IFN-γ could not be detected in concentrated BALF of normal controls, but was detectable in 12/20 patients with acute sarcoidosis. These abnormalities reverted towards normal on treatment with corticosteroids. Furthermore, patients with more severe disease showed a greater degree of abnormality than those with mild disease. These results could be explained by the depression of a local Th2-like response, the promotion of a Th1-like response, or a combination of both in this disease. Corticosteroids go some way to restoring the normal balance.

Sarcoidosis, although a systemic disease, affects the lungs in >90% of patients. In sarcoidosis, there is leakage of serum proteins into the lungs, as confirmed by levels of albumin found in BALF. The aim of this study was to examine the effect of corticosteroid treatment on local production of immunoglobulins and cytokines associated with Th1- and Th2-type immune responses, and to advance our understanding of the regulation of the local lung environment in this disease. We therefore, as others have done,

elected to equate our immunoglobulin data with albumin in both BAL and serum to reflect local production. This shows relative reduction in local production of IgG4 and IgE in active sarcoidosis but maintenance of normal production of IgG1, IgG2 and IgG3. Had we not done this, levels between patients and controls of particularly IgG4 and IgE would not have been comparable. Actual levels of IgE and IgG4 measured in serum *versus* lavage fluid represent a 100-fold differential, so that small changes in vascular leakage would swamp local production. Therefore, in order to detect local production, it is necessary to control for such leakage. In normals, there was a similar ratio of measurements of IgG subclasses and IgE in serum *versus* lavage, so the pressure to move from serum to lung is the same. This pattern, however, was different in sarcoidosis, thus supporting the hypothesis that the local lung environment is different in this disease.

The pattern of IgG subclasses found in BALF from the normal volunteers in this study was similar to that found in previous studies [22–24], with IgG4 being the main locally produced subclass in the lower respiratory tract. This was confirmed when estimates of the quantity of IgG4 and the proportion of total IgG produced in the lungs was made. In patients with sarcoidosis, IgG4 levels relative to albumin were considerably reduced, as has been noted in one previous study [25]. Those patients reassessed after a relatively short time on corticosteroid therapy showed a significant increase in BAL IgG4 levels and in local production of IgG4, although values were still lower than in normal volunteers. The second measurements were, however, made after a relatively short period of treatment. The function of IgG4 in the lungs is unclear, but serum deficiency of this antibody subclass is associated with increased susceptibility to infection [30,31] and has also been found in a number of conditions in which an immunoregulatory abnormality is present [32,33]. IgG4 is also involved in the normal response to chronic antigen stimulation [21]. The lungs are constantly exposed to antigens, which may explain why IgG4 is found in abundance in normal lungs. Although patients with sarcoidosis are able to produce IgG4 in their lungs, this was such a tiny proportion of the total IgG being produced that its efficacy may well be compromised. This may be the result of the disease process, or alternatively could be one factor in allowing a granulomatous response to antigen stimulation.

Both IgG4 and IgE synthesis by human B cells is induced by IL-4 and IL-13 [20,34]. In this study, not only was lung IgG1 production overwhelmingly increased in sarcoidosis with no corresponding increase in IgG4 production, but also there was no increase in IgE production in proportion to the total immunoglobulin being produced. Furthermore, levels of IL-4 were also significantly reduced in BALF from these patients, suggesting the dominance of cells producing a Th1 repertoire of cytokines over those producing Th2 cytokines. Th1-type cells are thought to be involved in cell-mediated immune reactions, while Th2-like cells are implicated in allergic inflammatory processes [12,35] and favour IgE and IgG4 immune responses. The formation of Th2 clones is enhanced *in vitro* by IL-4 [36], which also promotes the growth of committed Th2 cells [37]. Th2 cell development is inhibited by IFN-γ [38], which enhances differentiation of Th1 cells [39]. We found elevated levels of IFN-γ in BALF in sarcoidosis, which supports the hypothesis of a dominant Th1 response and a depressed Th2 response. It is now recognized that, in humans, Th1 and Th2 cytokines can be produced by cells other than T cells. The patients investigated in this study, however, all had elevated numbers of lymphocytes, particularly of the CD4 phenotype, and no increase in, for example, mast cells or basophils (data not shown).

It has previously been shown that T cells within inflamed tissue replicate faster than normal in sarcoidosis [40] and that T cells accumulating in the lung interstitium can release IFN-γ [18]. Sarcoid T cells also spontaneously release IL-2 *in vitro* [41,42] and this can be suppressed by corticosteroids [43]. The same workers were, however, unable to detect IL-2 in concentrated BALF from these patients with sarcoidosis [41]. We were able to measure IL-2 in concentrated BALF, but levels of all cytokines measured were extremely low. We did not, however, find raised levels of IL-2 in patients compared with normal subjects, but a slight reduction and a significant increase on treatment. Although initially this result may seem contradictory, as IL-2 is considered to be a Th1-like cytokine, it should be remembered that both IL-2 and IL-4 are also produced by Th0 or uncommitted T cells. Our findings are supported by the work of Devergne *et al*. [44], who found that cells expressing the IFN-γ gene were 32 times more frequent than those expressing the IL-2 gene in the lymph nodes of sarcoid patients. There was also a high proportion of cells containing IFN-γ mRNA inside sarcoid granulomata. It would of course be interesting to investigate lavage cells and lung granulomata from patients with sarcoidosis by *in situ* hybridization for detection of mRNA relevant to Th1- and Th2-type cytokines. This may represent the basis of a further study.

It is generally assumed that the obviously aberrant immune response in the lungs in sarcoidosis is triggered by antigenic insult. Various candidates have been proposed for this role, but particular attention has focused on *M. tuberculosis*. The disease caused by this organism is pathologically very similar to sarcoidosis, the two can coexist, and it is not uncommon for a patient treated for tuberculosis to then develop sarcoidosis. Robinson *et al*. [17] looked for evidence of a Th1-like bronchoalveolar T cell subset in pulmonary tuberculosis, and were unable to demonstrate any increased proportion of BAL cells expressing IL-2 mRNA despite a six-fold increase in IFN-γ mRNA-positive cells. Furthermore, human Th1-like T lymphocyte clones react to purified protein derivative of *M. tuberculosis* or *M. leprae* by producing predominantly IFN-γ and variable amounts of IL-2 [7,15]. Increased release of IFN-γ from Th1-like cells will promote macrophage activation and granuloma formation. If there is a concurrent failure to produce IL-2 and IL-4, then T cell survival may be compromised, as IL-2 has been shown to reduce expression of bcl-2 and enhance T cell survival [45]. Thus lack of IL-2 may in itself prevent both the growth of Th2-like T cells and lead to premature programmed cell death (apoptosis). If, as has been suggested, it is the cytokines of Th2-like cells (particularly IL-10) that regulate macrophage activation and differentiation [46], their absence may allow macrophage differentiation to proceed unchecked and result in an ever increasing granulomatous tissue reaction.

The increase in Th1 and depression of Th2 responses in the lungs of our patients were reversed by corticosteroid treatment. The role of corticosteroids in the clinical management of sarcoidosis is well established and their interference in lymphocyte and macrophage function and in macrophage/lymphocyte interactions has been demonstrated [43,47–51]. In the current study, corticosteroid therapy promoted Th2-like activity as demonstrated by a proportionate increase in local IgG4, IgE and an absolute increase in IL-4 production, while depressing Th1-like activity, as shown by reduced IFN-γ production. Steroids also decrease the levels of serum IFN-γ in some patients with sarcoidosis [52].

It is widely accepted that attempts to quantify soluble components in BALF are difficult, because no completely reliable denominator or reference substance is available. As certainly the immunoglobulins can be detected in serum and are also derived from local lung production, comparison with a standard substance is necessary, as set out in the European Respiratory Society's Technical Recommendations for BAL [28]. The data presented here show that, despite serum leakage, there was considerable proportionate decrease in production of both IgG4 and IgE in untreated patients. The levels of IL-4 were also reduced, while local production of IFN-γ was increased. These positions were reversed on treatment and both the differences between patients and normals and the changes found on corticosteroid treatment were highly significant. Differentiation of Th2-like cells and production of IL-4 are inhibited by IFN-γ [38]. If the activated T cells in sarcoidosis are Th1 and steroids regulate the activity of activated cells, then the inhibition of IL-4 production by IFN-γ will be removed. Thus it is quite logical in this disease for production of IL-4 in the lungs to be increased on steroid treatment. Furthermore, *in vitro* data by Wu *et al*. [53] demonstrate an increase in IgE production in response to steroids. This, thus further supports the argument that not only lung leakage but also the local immunological environment in sarcoidosis is altered by corticosteroids.

These drugs, however, are also effective in the treatment of atopic disease, which is associated with Th2-type responses, suggesting non-specific immunomodulatory mechanisms for corticosteroids. The apparent paradox of reduction of Th2 responsiveness by steroids in atopic disease and reduction of Th1 responsiveness in sarcoidosis can be explained, as activated cells are more susceptible to steroid inhibition.

The fact that serum IgG4 and IgE measurements were lower after treatment with steroids is not at odds with the finding of restored Th2-like activity in the lungs. In fact, it further strengthens the argument that the local immune environment can act independently of the systemic circulation. There is ample evidence from work on AIDS, cytomegalovirus pneumonitis and asthma, for example, that the lung is capable of behaving as an immunological organ.

The data presented here indicate that there is an imbalance in the normal levels of Th1 and Th2 cytokines and in the Th2-dependent immunoglobulins IgG4 and IgE in the lungs of patients with sarcoidosis. This local imbalance is corrected by corticosteroid treatment, which may therefore suppress granuloma formation.

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REFERENCES

1 Mitchell DW, Rees RJW. Sarcoidosis. Oxford: Pergamon Press, 1983: 132.

2 Hills SE, Parkes SA, Baker SB. Epidemiology of sarcoidosis in the Isle of Man—2: Evidence for space-time clustering. Thorax 1987; **42**:427–30.

3 Pasturenzi L, Martinetti M, Cuccia M, Cipriani A, Semenzato G, Luisetti M, the Pavia-Padova Sarcoidosis Study Group. HLA class I, II and III polymorphism in Italian patients with sarcoidosis. Chest 1993; **104**:1170–5.

4 Abe S, Yamaguchi E, Makimura S, Okazaki N, Kunikane H, Kawakami Y. Association of HLA-DR with sarcoidosis: correlation with clinical course. Chest 1987; **92**:488–90.

5 Mosmann TR, Cherwinski H, Bond MW, Gieldin MA, Coffman RL. Two types of murine helper T cell clones. J Immunol 1986; **136**:234–57.

6 Wierenga EA, Snoek M, De-Groot C, Chretien I, Bos JD, Jansen HM, Kapsenberg ML. Evidence for compartmentalisation of functional subsets of $CD4^+$ T lymphocytes in atopic patients. J Immunol 1990; **144**:4651–6.

7 Del Prete G-F, De Carli M, Mastomauro C, Macchia D, Biagotti R, Ricci M, Romagnani S. Purified protein derivative of *Mycobacterium tuberculosis* and excretory-secretory antigens of *Toxocara canis* expand *in vitro* human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. J Clin Invest 1991; **88**:346–50.

8 Parronchi P, Macchia D, Piccinni MP *et al*. Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. PNAS 1991; **88**:4538–42.

9 Romagnani S. Human Th1 and Th2 subsets: doubt no more. Immunol Today 1991; **12**:256–7.

10 Wierenga EA, Snoek M, Bos JD, Jansen HM, Kapsenberg ML. Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. Eur J Immunol 1990; **20**:1519–26.

11 Aversa G, Punnonen J, Cocks BG, de Waal Malefyt R, Vega F Jr, Zurawski SM, Zurawski S, de Vries JE. An interleukin 4 (IL-4) mutant protein inhibits both IL-4 or IL-13 induced human immunoglobulin G4 (IgG4) and IgE synthesis and B cell proliferation: support for a common component shared by IL-4 and IL-13 receptors. J Exp Med 1993; **178**:2213–8.

12 Kay AB, Sun Y, Varney V, Gaga M, Durham SR, Moqbel R, Wardlaw AJ, Hamid Q. Messenger RNA expression of the cytokine gene cluster interleukin-3 (IL-3), IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor in allergen-induced late-phase cutaneous reactions in atopic subjects. J Exp Med 1991; **173**:775–8.

13 Durham SR, Sun Y, Varney VA, Jacobson MR, Sudderick RM, Mackay IS, Kay AB, Hamid Q. Cytokine messenger RNA expression for IL-3, IL-4, IL-5 and granulocyte/macrophage colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to tissue eosinophilia. J Immunol 1992; **148**:2390–4.

14 Robinson DS, Hamid Q, Sun Y *et al*. Predominant Th2-like bronchoalveolar T lymphocyte population in atopic asthma. N Engl J Med 1992; **326**:298–304.

15 Haanen JBAG, de Waal Malefijt R, Res PCM, Kraakman EM, Ottenhoff THM, de Vries RRP, Spits H. Selection of human T helper 1-like T cell subset by mycobacteria. J Exp Med 1991; **174**:583–92.

16 Tsicopoulos A, Hamid Q, Varney V, Sun Y, Moqbel R, Durham SR, Kay AB. Preferential messenger RNA expression of Th1-type cells (IFN-γ^+, IL-2^+) in classical delayed-type (tuberculin) hypersensitivity reactions in human skin. J Immunol 1992; **148**:2058–61.

17 Robinson DS, Sun Y, Taylor IK, Wangoo A, Mitchell DM, Kay AB, Hamid Q, Show RJ. Evidence for a Th1-like bronchoalveolar T-cell subset and predominance of interferon-gamma gene activation in pulmonary tuberculosis. Am J Respir Crit Care Med 1994; **149**:989–93.

18 Robinson BWS, McLemore TL, Crystal RG. Gamma interferon is spontaneously released by alveolar macrophages and lung T lymphocytes in patients with pulmonary sarcoidosis. J Clin Invest 1985; **75**:1488–95.

19 Sahashi K, Ina Y, Takada K, Sato T, Yamamoto M, Morishita M. Significance of interleukin 6 in patients with sarcoidosis. Chest 1994; **106**:156–60.

20 Del Prete G, Maggi E, Parronchi P *et al*. IL-4 is an essential factor for the IgE synthesis induced *in vitro* by human T cell clones and their supernatants. J Immunol 1988; **140**:419–38.

21 Van der Zee JS, Van Swieten P, Aalberse RC. Inhibition of complement activation by IgG4 antibodies. Clin Exp Immunol 1986; **64**:415–22.

22 Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY. Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects. Am Rev Respir Dis 1985; **131**:584–7.

23 Out TA, Van der Graaf EA, Van den Berg NJ, Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. Scand J Immunol 1991; **33**:719–27.

24 Milburn HJ, Prentice HG, Grundy JE. IgG subclasses in the lungs of patients with interstitial pneumonitis following bone marrow transplantation. Eur Respir J 1993; **6**:944–50.

25 Rankin JA, Olchowski J, Naegel GP, Merrill WW, Reynolds HY. Immunoglobulin G subclasses in sarcoidosis. Ann NY Acad Sci 1986; **465**:122–9.

26 Milburn HJ, Prentice HG, du Bois RM. Role of bronchoalveolar lavage in the evaluation of interstitial pneumonitis in recipients of bone marrow transplants. Thorax 1987; **42**:766–72.

27 Kemeny DM, Richards D, Johannsson A, Durnin S. Ultrasensitive enzyme-linked immunosorbent assay (ELISA) for the detection of picogram quantities of IgE. J Immunol Methods 1989; **120**:251–5.

28 Klech L, Pohl W (eds) for the European Society of Pneumonology Task Group on BAL. Technical recommendations and guidelines for bronchoalveolar lavage (BAL). Eur Respir J 1989; **2**:561–5.

29 Wiggins J, Stockley RA. Variability in sputum sol phase proteins in chronic obstructive bronchitis. The value of using albumin for standardisation. Am Rev Respir Dis 1983; **128**:60–64.

30 Beck CS, Heiner DC. Selective immunoglobulin G4 deficiency in recurrent infections of the respiratory tract. Am Rev Respir Dis 1981; **124**:94–96.

31 Bentur L, Nisbet-Brown E, Levison H, Roifman CM. Lung disease associated with IgG subclass deficiency in chronic mucocutaneous candidiasis. J Paediatr 1991; **118**:82–86.

32 Oxelius VA. Immunoglobulin G (IgG) subclasses and human disease. Am J Med 1984; **76**:7–18.

33 Heiner DC, Lee SI, Short JA. IgG4 subclass deficiency syndromes. Monogr Allergy 1986; **20**:149–56.

34 Punnonen J, Aversa G, Cocks BG, McKenzie AN, Menon S, Zurawski G, de Waal Malefyt R, de Vries JE. Interleukin 13 induces interleukin 4-independent IgG4 and IgE synthesis and CD23 expression by human B cells. Proc Natl Acad Sci USA 1993; **90**:3730–4.

35 Street NE, Mosmann TR. Functional diversity of T lymphocytes due to secretion of different cytokine patterns. FASEB J 1991; **5**:171–7.

36 Swain SL, Weinberg AD, English M, Huston G. IL-4 directs the development of Th2-like helper effectors. J Immunol 1990; **145**: 3796–806.

37 Greenbaum LA, Horowitz JB, Woods A, Pasquali T, Reich E-P, Bottomley K. Autocrine growth of $CD4^+$ T cells. Differential effects of IL-1 on helper and inflammatory T cells. J Immunol 1988; **140**:1555–60.

38 Fernandez-Botran R, Sanders VM, Mosmann TR, Vitetta ES. Lymphocyte-mediated regulation of the proliferative response of clones of the T helper 1 and T helper 2 cells. J Exp Med 1988; **168**:543–58.

39 Gajewski TF, Schell SR, Nau G, Fitch FW. Regulation of T-cell activation: differences among T cell subsets. Immunol Rev 1989; **111**:79–110.

40 Bitterman PB, Saltzman LE, Adelberg S, Ferrano VJ, Crystal RG. One mechanism for the expansion of the mononuclear phagocyte population in the chronically inflamed lung. J Clin Invest 1984; **74**: 460–9.

41 Pinkston P, Bitterman PB, Crystal RG. Spontaneous release of interleukin 2 by lung T lymphocytes in active pulmonary sarcoidosis. N Engl J Med 1983; **308**:793–800.

42 Hunninghake GW, Bedell GH, Zavala DC, Monick M, Brady M. Role of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. Am Rev Respir Dis 1983; **128**:634–8.

43 Pinkston P, Saltini C, Muller-Quernheim J, Crystal RG. Corticosteroid therapy suppresses spontaneous interleukin-2 release and spontaneous proliferation of lung T lymphocytes of patients with active pulmonary sarcoidosis. J Immunol 1987; **139**:755–60.

44 Devergne O, Emilie D, Peuchmaur M, Crevon MC, D'Agay MF, Galanaud P. Production of cytokines in sarcoid lymph nodes: preferential expression of interleukin-1 beta and interferon-gamma genes. Hum Pathol 1992; **23**:317–23.

45 Akbar AN, Borthwick M, Salmon M *et al*. The significance of low bcl-2 expression by CD45RO T cells in normal individuals and patients with acute viral infections. The role of apoptosis in T cell memory. J Exp Med 1993; **178**:427–38.

46 de Waal-Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10. J Exp Med 1991; **174**:1209–20.

47 Ceuppens JC, Laquet LM, Marien J, Demeds M, van der Eeckhout A, Stevens E. Alveolar T-cell subsets in pulmonary sarcoidosis. Correlations with disease activity and effect of steroid treatment. Am Rev Respir Dis 1984; **129**:563–8.

48 Hance AJ, Douches S, Winchester RJ, Ferrans VJ, Crystal RG. Characterisation of mononuclear phagocyte subpopulations in the human lung by using monoclonal antibodies. Changes in alveolar macrophage phenotype associated with pulmonary sarcoidosis. J Immunol 1985; **134**:284–92.

49 Ton CM, van Maarseveen T, Mullink H, Stam J, de Groot J. Macrophage-T suppressor cell interference in the lungs of steroid-treated sarcoidosis patients. Am Rev Respir Dis 1991; **143**:839–43.

50 Baughman RP, Lower EE. The effect of corticosteroid or methotrexate therapy on lung lymphocytes and macrophages in sarcoidosis. Am Rev Respir Dis 1990; **142**:1268–71.

51 Spatafora M, Mirabella A, Rossi G. Bonanno A, Riccobono L, Merendino A, Bellia V, Bonsignore G. Effects of oral steroids on immunoglobulins in bronchoalveolar lavage fluid in active sarcoidosis. Eur Respir J 1988; **1**:505–9.

52 Prior C, Haslam PL. Increased levels of serum interferon-gamma in pulmonary sarcoidosis and relationship with response to corticosteroid therapy. Am Rev Respir Dis 1991; **143**:53–60.

53 Wu CY, Safarti M, Heusser C, Fournier S, Rubio-Trujillo M, Peleman R, Delespesse G. Glucocorticoids increase the synthesis of immunoglobulin E by interleukin-4-stimulated human lymphocytes. J Clin Invest 1991; **87**:870–7.