Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial responsiveness

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Abstract

Background Bronchial mucosal inflammation and epithelial damage are characteristic features of asthma. Activation of T helper lymphocytes may contribute to this process by mechanisms including the release of cytokines promoting eosinophil infiltration and activation.

Methods Bronchial washings and bronchoalveolar lavage fluid were obtained from 29 atopic asthmatic patients (19 with current symptoms and 10 symptom free) and 13 normal volunteers. Flow cytometry was used to assess T cell phenotype and activation status in bronchoalveolar lavage fluid and peripheral blood, and differential cell counts were made on bronchial washings and bronchoalveolar lavage fluid. Findings were related to severity of disease as reflected by symptom scores, baseline lung function, and airway responsiveness.

Results CD4 T lymphocytes in bronchoalveolar lavage fluid and blood from asthmatic patients were activated by comparison with controls (CD4 CD25, median 16.8% v 8.7% for bronchoalveolar lavage fluid, and 15.3% v 8.7% in blood). Bronchoalveolar lavage fluid CD4 T cells from both asthmatic patients and controls were of memory phenotype (95.8% and 96.8% CD45RO and 1.7% and 0.4% CD45RA respectively), whereas both CD45RO and CD45RA T cells were present in blood. Patients with asthma and current symptoms showed increased bronchoalveolar T cell activation compared with patients without symptoms (CD4 CD25 18.7% v 12.3%). Within the asthmatic group there was a significant association between CD4 CD25 lymphocytes and asthma symptom scores ($r_s = 0.75$), airway methacholine responsiveness ($\log PC_{20}$, $r_s = -0.43$) and baseline FEV₁ ($r_s = -0.39$). A correlation was also found between CD4 CD25 lymphocytes and eosinophils in bronchoalveolar lavage fluid ($r_s = 0.48$). Eosinophils in bronchoalveolar lavage fluid were increased in asthmatic patients compared with controls and the percentage of eosinophils in broncho-

alveolar lavage fluid correlated with asthma symptom score. A relation was found between percentage of epithelial cells in bronchoalveolar lavage fluid and FEV₁ and methacholine PC₂₀.

Conclusion These results support the hypothesis that selective activation of memory CD4 T cells contributes to eosinophil accumulation, bronchial hyperresponsiveness, and symptoms in asthma.

(Thorax 1993;48:26-32)

Recent evidence has suggested that T lymphocyte activation and the recruitment and activation of eosinophils may be important in the development of airflow limitation in bronchial asthma. For example, peripheral blood CD4 T helper lymphocytes from patients with acute severe asthma expressed activation markers (interleukin-2 receptor (CD25), class II molecule (HLA DR), and the very late activation marker (VLA-1)) to a greater degree than those from controls. The degree of T lymphocyte activation was closely correlated with lung function and decreased with a time course that paralleled improvements in peak expiratory flow rates.¹ Electron microscopical studies of bronchial biopsy specimens from asthmatic patients demonstrated mucosal lymphocyte irregularities suggestive of activation.² Immunohistological studies of such specimens showed increased numbers of cells in the mucosa expressing IL-2 receptor as shown by positive staining with a monoclonal antibody directed against CD25.³ Flow cytometry allows simultaneous identification of phenotypic and activation markers on cells in suspension. This has recently been applied to bronchoalveolar lavage fluid and blood cells from asthmatic patients. The results showed an increase in numbers of activated CD4 and CD8 T cells in both bronchoalveolar lavage fluid and blood from asthmatic patients compared with normal healthy non-atopic control subjects.⁴ Our own studies⁵ and those of other groups⁶ have shown that eosinophil numbers and eosinophil cationic protein concentrations in bronchoalveolar lavage fluid and bronchial biopsy specimens correlated with severity of asthma as

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Date received
17 February 1992
Returned to Authors
24 June 1992
Revised received 7 July 1992
Accepted 15 July 1992

determined by symptom scores and the level of non-specific bronchial responsiveness. As well as the presence and activation of T lymphocytes and eosinophils, a characteristic feature of asthma is epithelial damage. Both the degree of epithelial denudation seen in bronchial biopsy specimen^{2,7} and the numbers of epithelial cells recovered in a small volume bronchial lavage have been shown to correlate with the degree of bronchial responsiveness in asthmatic patients.^{5,8}

Eosinophils may contribute to airway hyperresponsiveness in asthma through the actions of granule derived basic proteins (which damage bronchial epithelium in vitro), and the release of membrane derived lipid mediators such as leukotriene C₄ and platelet activating factor.^{9,10} It has been suggested that activation of T helper lymphocytes may account for eosinophil infiltration and activation in the airway in atopic asthma by the production of specific intercellular proteins (cytokines).^{11,12} Recently, by use of the technique of in situ hybridisation, we have shown that increased numbers of bronchoalveolar lavage cells from atopic asthmatic patients gave positive signals for messenger RNA (mRNA) for such cytokines when compared with bronchoalveolar lavage fluid cells from normal volunteer subjects.¹³ The cytokines interleukin-4 (IL-4) and IL-5 are particularly implicated in IgE synthesis and eosinophil activation respectively.^{14,15} and mRNA for these cytokines was shown to be predominantly present in T lymphocytes within bronchoalveolar lavage fluid from atopic asthmatic patients.¹³ These T lymphocytes would be expected to be of "memory" phenotype—that is, T cells that have previously responded to allergen. Memory T cells can be recognised by surface expression of the CD45RO subtype of the leucocyte common antigen,¹⁶ and these cells respond readily to recall antigens with rapid production of a wide range of cytokines.¹⁷ This is by contrast with the restricted response shown by "naive" T cells, which have not previously encountered antigens and have the CD45RA surface marker.¹⁸

The aim of this study was to compare lavage cells obtained by fibreoptic bronchoscopy of atopic asthmatic patients with those from normal healthy control subjects. T cell phenotype, memory state, and activation were assessed by flow cytometry with dual immunofluorescent staining. We also looked for associations between these findings and the severity of disease as reflected by current asthma symp-

toms and the degree of bronchial responsiveness as assessed by inhaled methacholine.

Methods

PATIENTS

Asthmatic patients (table 1) were recruited from the allergy clinic of the Royal Brompton National Heart and Lung Hospital, and normal volunteers from the staff of the National Heart and Lung Institute. All subjects gave written informed consent and the study was approved by the ethics committee. They were all non-smokers and none had received oral or inhaled corticosteroids in the three months preceding the study. The diagnosis of asthma was based on (1) a clinical history of variable wheeze, cough, or shortness of breath; (2) documented 15% variability in forced expiratory volume in one second (FEV₁) or peak expiratory flow rate (PEF); and (3) a methacholine PC₂₀ (provocative concentration causing a 20% fall in FEV₁ from baseline) less than 8 mg/ml (five of the seasonal asthmatic patients without symptoms had a PC₂₀ of at least 8 mg/ml when studied out of season). All asthmatic patients were atopic as defined by (1) positive skin prick tests to one or more extracts of the common aeroallergens: house dust (*Dermatophagoides pteronyssinus*), Timothy grass pollen, cat, dog, feathers, tree pollen, *Aspergillus fumigatus*, and (2) raised serum allergen specific IgE concentrations as determined by radioallergosorbent test (grade 2 or more on a scale of 1–4, Phadebas RAST, Pharmacia, Sweden) to house dust mite, cat, or grass pollen allergens. Nineteen asthmatic patients were symptomatic at the time of the study (defined by wheeze or chest tightness requiring the use of inhaled β_2 agonists more than twice a week). Ten asthmatic patients had strictly allergen related symptoms and had no symptoms when studied out of season or after allergen avoidance. To define the severity of asthma symptoms an Aas symptom score¹⁹ was allocated on the basis of a questionnaire administered by a clinician who was not involved in the laboratory measurements (AMB). The FEV₁ was measured with a dry bellows spirometer (Vitalograph, Buckingham, UK). Methacholine PC₂₀²⁰ was measured between 0800 and 0900 at least 48 hours before bronchoscopy. Normal volunteers were without symptoms and had negative skinprick responses, low total IgE, and no detectable serum allergen specific IgE to common aeroallergens.

FIBREOPTIC BRONCHOSCOPY

All fibreoptic bronchoscopies were performed by the same operator (SRD) between 0800 and 0900. Asthmatic patients and normal subjects were premedicated with 2.5 mg of nebulised salbutamol, intravenous atropine (0.5 mg), and midazolam as required for sedation (5–10 mg). Topical anaesthesia was with 2% lignocaine. Supplemental oxygen was given at 4 l/minute throughout. Arterial oxygen saturation was monitored by pulse oximeter (Ohmeda, Harlow, UK). The bronchoscope (Olympus p20, Olympus Corporation, Tokyo, Japan) was wedged at segmental or subsegmental level in

Table 1 Clinical data of subjects studied

	Asthmatic patients		Normal subjects
	Symptomatic	Without symptoms	
Age (mean, range)	24 (22–27)	26 (19–35)	25 (19–33)
Sex	14 W, 5 M	5 W, 5 M	7 W, 6 M
FEV ₁ (% predicted) (median, range)	82 (62–108)	103 (84–129)	111 (87–115)
PC ₂₀ (mg/ml) (median, range)	0.6 (0.24–3.2)	10.4 (2.2–19.9)	> 32

W, women; M, men.

the right middle lobe or lingula. An initial 60 ml of warmed, pH adjusted, isotonic saline was introduced. This was immediately collected and processed separately as the bronchial washing. Bronchoalveolar lavage was then performed with a further three 60 ml aliquots of saline, the return from which was pooled. Thirty ml of blood were collected into a heparinised container. After the procedure all asthmatic patients were given a further 2.5 ml salbutamol by nebuliser and all subjects were observed for at least four hours before discharge.

FLOW CYTOMETRY AND CYTOCENTRIFUGE PREPARATIONS

The total volume of bronchoalveolar lavage fluid recovered was noted. Any mucus was removed by filtration through sterile cotton gauze. Bronchoalveolar lavage fluid cells were centrifuged (300 *g* at 4°C) for seven minutes, washed in Roswell Park Memorial Institute medium (RPMI) 1640 (Flow Laboratories, Irvine, Scotland), and resuspended in RPMI 1640 with 25 mM HEPES, 0.5% BSA, and 0.1% sodium azide. A total cell count was performed on a Neubauer haemocytometer. Cytocentrifuge preparations were made with a Shandon 2 cytopsin (Shandon Southern Instruments, Runcorn, UK) at 800 rpm for five minutes for differential counts (stained with May-Grünwald Giemsa). Bronchial washings were not filtered but cells were pelleted as for bronchoalveolar lavage fluid and resuspended in RPMI 1640 before cytocentrifuge preparations were made. Peripheral blood mononuclear cells were separated on Ficoll-Paque (Pharmacia, Milton Keynes, UK) and resuspended in RPMI 1640. Aliquots of 5×10^5 cells were incubated for 15 minutes at 4°C with monoclonal antibodies directly conjugated to fluorescein isothiocyanate (FITC) or R-phycoerythrin (PE): CD3-PE, CD4-PE, CD8-PE, CD3-FITC, CD14-FITC, CD45RA-FITC, and IgG1-FITC and IgG2-PE isotype controls were from Becton Dickinson, Cowley, UK; CD25-FITC, HLA DR-FITC, and CD45RO-FITC were from Dako High Wycombe, UK; and VLA-1-FITC was from Laboratory Impex, Twickenham, UK. After two washes in medium, cells were resuspended

for analysis in Isoton II balanced electrolyte solution (Coulter Electronics, Luton, UK). A Becton-Dickinson FACS Analyser with FAC-Slite 488 nm Argon ion laser was used to collect 10 000 events in a gate defined by volume and 90° light scatter of peripheral blood lymphocytes. Cells positively stained for T cell phenotypic markers (CD4, CD8) were analysed for staining with activation or memory markers. Results were expressed as % positive relative to an isotype control antibody. One aliquot of cells was stained with CD3-PE and CD14-FITC to exclude monocytes or macrophages. One aliquot was also stained with CD4-PE and CD3-FITC to confirm that all CD4 cells analysed were lymphocytes.

Differential cell counts of bronchoalveolar lavage fluid and bronchial washings were performed blind by counting 600 cells on cytocentrifuge preparations stained by May-Grünwald Giemsa stain.

STATISTICAL ANALYSIS

Results for flow cytometry and cell counts for asthmatic patients and controls were compared with the Mann-Whitney U test and a standard computer software package (Minitab Inc, State College, Pennsylvania). Results of Mann-Whitney U tests are given as median values for each group, the estimated median difference, and the 95% confidence interval (95% CI) for this difference. Correlation coefficients were obtained by Spearman's rank method. Where multiple comparisons were made, Bonferroni's correction was applied. *p* values < 0.05 were considered significant.

Results

Fibreoptic bronchoscopy was well tolerated by all subjects. Occasional mild bronchospasm in eight of the subjects induced by the procedure was rapidly reversed by the administration of nebulised salbutamol.

There was a small but significant reduction in the total bronchoalveolar lavage fluid recovered from asthmatic patients compared with normal subjects (median 115 ml *v* 130 ml, estimated median difference -20.0, 95% CI for difference -35.0 to 0.01, *p* < 0.05). There was no difference in total cell recovery between the two

Table 2 Differential cell counts: percentage of cells on cytocentrifuge preparations of bronchoalveolar lavage fluid and bronchial washings from atopic asthmatic patients and controls

Cell type	Asthmatic patients (<i>n</i> = 29) Median (range)	Controls (<i>n</i> = 13) Median (range)	Estimated median difference (95% CI)	<i>p</i>
<i>Bronchoalveolar lavage</i>				
Macrophages	84.5 (50.3-90.2)	85.3 (77.8-94.0)	-2.4 (-7.3 to 2.3)	0.32
Lymphocytes	11.3 (7.2-23.5)	14.3 (4.5-22.2)	-2.6 (-6.0 to 1.40)	0.13
Eosinophils	1.8 (0.1-26.3)	0.4 (0.0-1.3)	1.4 (0.5 to 4.0)	0.0007
Neutrophils	0.5 (0.0-5.2)	0.4 (0.0-2.0)	0 (-0.3 to 0.3)	0.98
Epithelial cells	0.1 (0.0-10.0)	0.2 (0.0-0.8)	0 (-0.2 to 0.1)	0.83
<i>Bronchial washings*</i>				
Macrophages	71.5 (26.0-96.0)	73.8 (48.0-86.5)	4.3 (-8.1 to 14.0)	0.58
Lymphocytes	5.5 (7.2-23.5)	6.2 (4.5-22.2)	-0.1 (-2.3 to 2.2)	0.94
Eosinophils	3.8 (0.4-46.3)	0.2 (0.0-3.3)	3.2 (1.3 to 6.5)	0.0001
Neutrophils	1.3 (0.0-8.0)	7.5 (1.0-18.0)	-6.2 (-8.6 to 3.7)	0.0001
Epithelial cells	5.0 (0.0-57.7)	12.8 (0.7-43.4)	-6.8 (-12.9 to 1.1)	0.08

*Counts in bronchial washings from 25 asthmatic patients (four were too mucoid for analysis).

Table 3 Percentages of CD4 and CD8 T cells in bronchoalveolar lavage fluid from asthmatic patients and controls expressing activation and memory markers

Cell type	Asthmatic patients (n = 29) Median (range)	Controls (n = 13) Median (range)	Estimated median difference (95% CI)	p
CD4/CD25	16.8 (4.9–29.8)	8.7 (5.1–15.1)	6.7 (3.8 to 10.4)	0.0001
CD4/HLA DR	48.8 (22.3–78.0)	36.0 (11.3–66.0)	11.4 (–0.3 to 22.1)	0.061
CD4/VLA 1	23.1 (3.3–57.3)	16.0 (2.4–31.9)	6.4 (–4.0 to 16.2)	0.13
CD4/CD45RO	95.8 (51.5–99.5)	96.8 (73.3–99.2)	–0.5 (–2.8 to 2.1)	0.55
CD4/CD45RA	1.7 (0–13.0)	0.4 (0–1.9)	1.5 (0.4 to 3.2)	0.003
CD8/CD25	3.9 (0–15.3)	3.6 (0–10.9)	0.3 (–2.2 to 2.6)	0.78
CD8/HLA DR	48.2 (19.1–75.8)	42.9 (22.3–60.6)	5.4 (–2.6 to 15.2)	0.19
CD8/VLA 1	53.6 (32.3–83.8)	51.0 (37.7–73.8)	5.8 (–6.6 to 16.1)	0.26

groups (1.0×10^5 cells/ml in asthmatic patients and 0.9×10^5 cells/ml in controls).

DIFFERENTIAL CELL COUNTS

Bronchial washings from four of the asthmatic patients were too mucoid to allow processing and were excluded from analysis. There was a significant increase in cell numbers and percentage of eosinophils in both bronchial washings and bronchoalveolar lavage fluid from asthmatic patients compared with controls (table 2). A reduction in percentage of neutrophils was found in bronchial washings but not bronchoalveolar lavage fluid from asthmatic patients (table 2). Bronchial washings from both asthmatic patients and controls showed a reduced percentage of lymphocytes and increased neutrophils and epithelial cells when compared with cell counts in bronchoalveolar lavage fluid (table 2).

FLOW CYTOMETRY

Expression of CD25 by CD4 T cells in bronchoalveolar lavage fluid was significantly greater for asthmatic patients than for controls (table 3). The difference in expression of HLA DR by CD4 lavage T cells in the two groups did not reach significance and there was no significant difference in expression of VLA-1. There were no differences in expression of activation markers by CD8 T cells. Bronchoalveolar lavage fluid CD4 T cells were

CD45RO + CD45RA–, whereas both phenotypes were present in blood (table 3). There was a small but significant increase in CD45RA expression by CD4 cells in bronchoalveolar lavage fluid (1.7% v 0.4%), but no difference in CD45RO between asthmatic patients and controls.

ASTHMATIC PATIENTS WITH AND WITHOUT SYMPTOMS

There was a significant increase in CD25 expression by CD4 lavage T cells in symptomatic asthmatic patients compared with those without current symptoms, and with normal healthy control subjects (fig 1). Eosinophil numbers or percentages in bronchial washings or bronchoalveolar lavage fluid were not different between asthmatic patients with and without symptoms. There was a significantly higher percentage of epithelial cells, however, from symptomatic asthmatic patients compared with patients without symptoms in both bronchial washings (7.7% v 1.5, estimated median difference 6.3, 95% CI 1.5 to 37.0, $p < 0.05$) and bronchoalveolar lavage fluid (0.2% v 0.0, estimated median difference 0.2, 95% CI 0 to 0.5, $p < 0.05$).

PERIPHERAL BLOOD LYMPHOCYTES

Flow cytometric analysis of peripheral blood lymphocytes revealed increased expression of CD25 and HLA DR by CD4 cells from asthmatic patients compared with control subjects (15.3% positive v 8.7%, estimated median difference 6.3, 95% CI 2.6 to 10.2, $p < 0.001$ for CD25, 7.3% v 4.3%, estimated median difference 3.5, 95% CI 1.3 to 6.0, $p < 0.01$ for HLA DR). There was no significant difference in the expression of VLA-1 (1.2 v 1.3%), CD45RO (42.3% v 37.2%), or CD45RA (52.3% v 54.1%). There was increased expression of HLA DR by CD8 PBL from asthmatic patients compared with controls (19.6% v 8.9, estimated median difference 10.3, 95% CI 2.5 to 20, $p < 0.01$), but the difference in expression of CD25 was not significant (3.1% v 1.7%) and there was no difference in expression of VLA-1 (0.8% v 1.0%).

ASSOCIATION WITH ASTHMA SEVERITY

Associations were assessed among the level of CD25 expression by CD4 cells in bronchoalveolar lavage fluid, severity of asthma, and the degree of bronchoalveolar lavage fluid eosinophilia within the asthmatic patients (fig 2).

Figure 1 Flow cytometry of bronchoalveolar lavage fluid from asthmatic patients: expression of activation marker CD25 on CD4 lymphocytes in bronchoalveolar lavage fluid (% positive) for symptomatic asthmatic patients (n = 19), asthmatic patients without symptoms (n = 10), and normal controls (n = 13).

Figure 2 Relation between the degree of bronchoalveolar lavage (BAL) T cell activation and (A) eosinophilia ($r = 0.48$, $p < 0.01$), (B) asthma severity as assessed by FEV₁ ($r = -0.39$, $p < 0.05$), (C) airway responsiveness ($r = -0.43$, $p < 0.05$), and (D) asthma symptoms (Aas score) ($r = 0.75$, $p < 0.001$).

Significant correlations were found between bronchoalveolar lavage fluid CD4 CD25 expression and clinical asthma score ($r_s = 0.75$, $p < 0.0001$), baseline lung function (FEV₁, $r_s = -0.39$, $p < 0.05$), and the level of airway responsiveness to methacholine (log PC₂₀, $r_s = 0.43$, $p < 0.05$). There was also a relation between CD4 T cell CD25 expression and the percentage of eosinophils in bronchoalveolar lavage fluid.

The percentage eosinophils in bronchoalveolar lavage fluid correlated with Aas symptom score ($r_s = 0.44$, $p < 0.05$). A relation was also found between epithelial cells in bronchial washings and log PC₂₀ ($r_s = -0.46$, $p < 0.05$), FEV₁ ($r_s = 0.52$, $p < 0.01$), and between epithelial cells in bronchoalveolar lavage fluid and CD25 expression by bronchoalveolar lavage fluid CD4 cells ($r_s = 0.57$, $p < 0.001$) and FEV₁ ($r_s = 0.48$, $p < 0.005$).

Discussion

In this study, which was based on fiberoptic bronchoscopy of a large group of atopic asthmatic patients with a broad range of clinical disease, we have shown that T lymphocytes obtained by bronchoalveolar lavage are activated as reflected by an increase in expression of CD25. This increase was confined to CD4 T cells that were of memory phenotype. The degree of T cell activation in bronchoalveolar lavage fluid was closely related to severity of asthma as judged by an asthma symptom score as well as baseline lung function and the degree of airway responsiveness. Associations were also found between bronchoalveolar lavage fluid eosinophilia and symptoms of asthma, and between numbers of activated T cells, eosinophils, and epithelial

cells. This suggests that T cell activation, eosinophil recruitment, and epithelial shedding may possibly be linked and represent a final common pathway in the development of airway narrowing, hyperresponsiveness, and symptoms in atopic allergic asthma.

Bronchoalveolar lavage has been used widely in the investigation of asthma and is safe and well tolerated provided agreed guidelines are followed.^{21,22} It has been suggested that small volume lavage gives a more proximal sample of airway cells than does conventional bronchoalveolar lavage,²³ and may be more appropriate to the study of airway disease. Labelling studies have shown, however, that bronchoalveolar lavage does sample small airways,²⁴ and this may be important given their contribution to abnormalities of lung function in asthma.²⁵ We did not study T cell activation in bronchial washings because of the small numbers of cells recovered, their low viability relative to bronchoalveolar lavage fluid, and the potential problems from mucus in flow cytometry, but have confirmed that cellular changes in asthma are broadly similar in bronchial washings and bronchoalveolar lavage fluid.^{5,26} Previous studies have found increases in eosinophils and epithelial cells in both bronchial washings and bronchoalveolar lavage fluid from asthmatic patients compared with control subjects and have indicated a correlation between numbers of these cell types and the amount of bronchial responsiveness.^{5,8} The detection of increased concentrations of major basic protein and eosinophil cationic protein in bronchoalveolar lavage fluid from asthmatic patients, together with the potential of these eosinophil granule proteins to damage respiratory epithelium *in vitro*,²⁷ supports the hypothesis that eosinophil mediated epithelial injury may contribute to airway lability in asthma. Because epithelial cells (and neutrophils) were also prominent, however, in bronchial washings from some normal control subjects caution must be exercised in attributing epithelial cell loss to asthma. Infiltration of eosinophils and subsequent activation in the bronchial mucosa may occur, at least in part, as a consequence of T lymphocyte activation with the production of cytokines that recruit and modify eosinophil function.

The finding of increased expression of CD25 by cells in bronchoalveolar lavage fluid from asthmatic patients is in agreement with a recent report which showed relations among CD25 expression, lung function (FEV₁), airway methacholine responsiveness, and bronchoalveolar lavage fluid eosinophilia.⁴ These investigators found increased expression of HLA DR and VLA-1 by CD4 bronchoalveolar lavage fluid lymphocytes and increased expression of activation markers by CD8 T cells in bronchoalveolar lavage fluid. As in some, but not all, previous reports they described an increase in lymphocytes in bronchoalveolar lavage fluid from asthmatic patients. We did not find increased expression of the late activation markers (HLA DR, VLA 1) by bronchoalveolar lavage CD4 T cells, or activation of

CD8 cells. Although these differences may reflect our slightly younger study group there were no obvious differences in our subjects for severity of asthma or atopic state. Peripheral blood lymphocytes from patients with acute severe asthma showed increased expression of activation markers (CD25, HLA DR and VLA-1) by CD4 but not CD8 cells when compared with control subjects and patients with chronic obstructive airways disease.¹ Activation of T cells was also suggested by the increased numbers of cells staining for CD25 in bronchial biopsy specimens from asthmatic patients,³ and double immunostaining has recently confirmed that most of the increased CD25 expression in such biopsy specimens is indeed on CD3 T lymphocytes rather than alternative cells such as macrophages.²⁸

The explanation for increased expression of late activation markers by lymphocytes in bronchoalveolar lavage fluid compared with those from peripheral blood may lie in the finding that lavage CD4 T cells were of memory phenotype (CD45RO + CD45RA -). This is in agreement with previous studies of bronchoalveolar lavage fluid from healthy volunteers and patients with interstitial lung disease.²⁹ The small increase in percentage of CD4 cells in bronchoalveolar lavage fluid expressing CD45RA in asthmatic patients compared with controls is of doubtful biological significance. Previous activation leading to memory status may explain continued expression of late activation markers; indeed the β_1 chain of VLA-1 (CD29) has been used as a marker of memory T cells.³⁰ Airway T cells might be expected to encounter antigens frequently, and the transient expression of CD25, rather than the more prolonged expression of late activation markers, might more accurately reflect ongoing allergenic T cell stimulation in asthma.

It is possible that the increased level of T lymphocyte activation and eosinophilia that we found in our atopic asthmatic patients may reflect the atopic state rather than asthma itself. Further studies including a group of non-asthmatic subjects would be required to exclude this possibility. The correlation with severity of asthma shown here, however, together with findings from studies of peripheral blood¹ and bronchial mucosal biopsy samples from both extrinsic² and intrinsic asthmatic patients,³¹ strongly supports the role of CD4 T cell activation in asthma, rather than simply the atopic state.

The Aas symptom score that we employed uses five categories of asthma based on severity and duration of attacks, resting lung function, and treatment requirements.¹⁹ No grading system of asthma is widely accepted as satisfactory, but this system does allow stratification of patients and our findings concur with a previous report of significant correlations between the Aas symptom score and bronchoalveolar lavage fluid eosinophilia and concentrations of eosinophil cationic protein in bronchoalveolar lavage supernatant fluid.⁶

In conclusion we have shown increased expression of an early activation marker by

memory CD4 T cells in bronchoalveolar lavage fluid from atopic asthmatic patients, and relationships among bronchoalveolar lavage fluid CD4/CD25 expression, eosinophilia, numbers of epithelial cells, and severity of asthma. Taken together these support the hypothesis that T cell activation may contribute to local eosinophilia and airway epithelial damage. In turn the associations, although not necessarily causally related, support the view that these events may contribute to airway narrowing, increased responsiveness, and severity of symptoms in bronchial asthma.

This work was supported by the Medical Research Council of Great Britain. We thank Julia Barkans and Janet North for expert assistance.

- Corrigan CJ, Hartnell A, Kay AB. T lymphocyte activation in acute severe asthma. *Lancet* 1988;i:1129-31.
- Jeffrey PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. *Am Rev Respir Dis* 1989; 140:1745-53.
- Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles GK, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990;142:1407-13.
- Walker C, Kaegi MK, Braun P, Blaser K. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 1991;88:935-42.
- Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. *Am Rev Respir Dis* 1988; 137:62-9.
- Bousquet J, Chanez P, Lacoste J-Y, Barneon G, Ghavanian N, Enander I., et al. Eosinophilic inflammation in asthma. *New Engl J Med* 1990;323:1033-9.
- Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis* 1985;131: 599-606.
- Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989;139:806-17.
- Gleich GJ. The eosinophil and bronchial asthma: current understanding. *J Allergy Clin Immunol* 1990;85:422-36.
- Weller PF. The immunobiology of eosinophils. *N Engl J Med* 1991;324:1110-9.
- Mosmann TR, Coffman RL. Two types of mouse helper T-cell clone. Implications for immune regulation. *Immunology Today* 1987;8:223-7.
- Bottomley K. A functional dichotomy in CD4+ T lymphocytes. *Immunology Today* 1988;9:268-74.
- Robinson DS, Hamid Q, Sun Ying, Tsicopoulos A, Barkans J, Bentley AM, et al. Evidence for a predominant "Th2-type" bronchoalveolar lavage T-lymphocyte population in atopic asthma. *New Engl J Med* 1992;326:298-304.
- Del Prete G, Maggi E, Parronchi P, Chretien I, Tiri A, Macchia D, 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;140:4193-8.
- Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin-5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-24.
- Akbar AN, Terry L, Timms A, Beverley PCL, Janossy G. Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. *J Immunol* 1988;140:2171-8.
- Ehlers S, Smith KA. Differentiation of T cell lymphokine gene expression: the in vitro acquisition of T cell memory. *J Exp Med* 1991;173:25-36.
- Merkenschlager M, Terry L, Edwards R, Beverley PCL. Limiting dilution analysis of proliferative responses in human lymphocyte populations defined by the monoclonal antibody UCHL1: implications for differential CD45 expression in T cell memory formation. *Eur J Immunol* 1988;18:1653-61.
- Aas K. Heterogeneity of bronchial asthma: subpopulations, or different stages of disease? *Allergy* 1981;36:3-14.
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-43.
- NHBLI workshop summary. Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1985;132:180-2.
- Djukanovic R, Wilson JW, Lai KW, Holgate ST, Howarth PH. The safety aspects of fiberoptic bronchoscopy,

- bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am Rev Respir Dis* 1991;143:772-7.
- 23 Rennard SI, Ghafouri M, Thompson AB, Linder J, Vaughan W, Jones K, *et al.* Fractional processing of sequential bronchoalveolar lavage to separate bronchial and alveolar samples. *Am Rev Respir Dis* 1990;141:208-17.
 - 24 Kelly CA, Kotre JC, Ward C, Hendrick DJ, Walters EH. Anatomical distribution of fluid at bronchoalveolar lavage. *Thorax* 1987;42:625-9.
 - 25 Wagner EM, Liu MC, Weinmann GG, Permutt S, Bleeker E. Peripheral lung resistance in normal and asthmatic subjects. *Am Rev Respir Dis* 1990;141:584-8.
 - 26 Lam S, LeRiche C, Kijek K, Phillips D. Effect of bronchial lavage volume on cellular and protein recovery. *Chest* 1986;89:477-83.
 - 27 Gleich GJ, Frigas E, Loegering DA, Wassom DL, Steinmuller D. Cytotoxic properties of the eosinophil major basic protein. *J Immunol* 1979;123:2925-7.
 - 28 Hamid Q, Barkans J, Robinson DS, Durham SR, Kay AB. Co-expression of CD25 and CD3 in atopic allergy and asthma. *Immunology* 1992;75:659-63.
 - 29 Saltini C, Kirby B, Trapnell BC, Tamura N, Crystal RG. Biased accumulation of T lymphocytes with "memory"-type CD45 leukocyte common antigen gene expression on the epithelial surface of the human lung. *J Exp Med* 1990;171:1123-40.
 - 30 Dominique S, Bouchonnet F, Smiejan J-M, Hance A. Expression of surface antigens distinguishing "naive" and previously activated lymphocytes in bronchoalveolar lavage fluid. *Thorax* 1990;45:391-6.
 - 31 Bentley AM, Menz G, Storz C, Robinson DS, Bradley BL, Jeffery PK, *et al.* Identification of T-lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma: relationship to symptoms and bronchial hyperresponsiveness. *Am Rev Respir Dis* 1993 (in press).

Adventitia

It is a matter of conjecture whether my spell at the Army chest centre in 1957-8 helped to shape my future career. It was certainly time well spent. I was posted from an army unit to the Connaught Hospital, near Hindhead, Surrey, as the first epidemic of the "Asian" flu was waning. The effects of the epidemic had been quite dramatic and we had been very busy, with barrack rooms doubling as sick bays where the few not affected and those convalescing from the acute phase of the illness helped to care for those laid low by the virus. It was as much as a regimental medical officer could do to get round the unit to examine any soldier whose illness or progress was out of the ordinary, and thus it was that one picked up the patients with staphylococcal pneumonia. My experience with this disease and its complications was soon to be consolidated at the Army chest centre, where we saw many cases, and the lessons learned stood one in good stead for years to come.

The striking opportunism of the staphylococcus as a cause of pneumonia during influenza epidemics is less evident today, and indeed in recent years staphylococcal pneumonia has presented itself as a sporadic and infrequent event. Now we seem to be much more concerned with *Pneumocystis carinii* pneumonia and other infections that are also opportunistic, but for different reasons. It surprised me 35 years ago, and still does, that a necrotising pneumonia that so often led to the formation of multiple cavities within the lung was not more often a cause of life threatening haemorrhage. Although I have seen this happen it has been a rare event. I recall one patient, an extremely ill young woman, whose haemoptysis ceased when the blood throughout her left bronchial tree clotted to form a perfect bronchial cast, resulting in the collapse of that lung. The bronchial thrombus could be removed only by piecemeal extraction at bronchoscopy. I remember also that she went on to suffer widespread staphylococcal osteitis but eventually made a complete recovery.

As at any chest unit in the 1950s, tuberculosis was a major item. Primary pulmonary tuberculosis and pleural effusions were commonplace and there was a wealth of experience with post-primary disease, which was often extensive at

the time of diagnosis. Happily, treatment regimens were well established and effective. Resectional surgery was still in vogue for selected patients in whom gross residual disease persisted after adequate chemotherapy, in case the disease was quiescent but not cured and might later relapse. Besides the surgery that was offered to regular British soldiers there was in progress a programme of such treatment for Gurkhas, who were transferred from Malaya for operation after completing their medical care at the British Military Hospital in Kinrara. The presence of these cheerful men was a notable feature of life in the hospital at that time. It was rumoured that while being prepared for their proposed operation some of the Gurkhas had been given to understand that this was needed in the fight against their enemy within, and that the resulting chest wound would represent an honourable battle scar and a successful outcome. Although this is no doubt apocryphal it does illustrate the paternalism that pervaded our profession at that time.

We were fortunate to see a wide variety of chest diseases, some of them uncommon, such as pulmonary arteriovenous aneurysms, sequestered segments, and the Hamman-Rich syndrome (as it was then called, still "in its infancy" and apparently rare). Competence was gained in the investigative techniques of the day and in interpretation of chest radiographs, tomograms, and bronchograms. We became quite adept at bronchography, and it was surprising how often underlying bronchiectasis was identified in our population of patients, who until their recent acute illness had been "fighting fit" and gave no previous history of lung disease.

A sense of humour prevailed. On the occasion of the arrival of a new commanding officer an addendum mysteriously appeared overnight in large block capitals beneath the name of the hospital on the signboard facing the main road:

UNDER NEW MANAGEMENT,
OLD BOY!

I know of no instance where any of the recently appointed NHS trust hospital managers has been similarly greeted. Such was the spirit of the times.—T B STRETTON