

T_H2 cytokine expression in bronchoalveolar lavage fluid T lymphocytes and bronchial submucosa is a feature of asthma and eosinophilic bronchitis

Christopher E. Brightling, MRCP, Fiona A. Symon, PhD, Surinder S. Biring, MRCP, Peter Bradding, DM, MRCP, Ian D. Pavord, DM, FRCP,* and Andrew J. Wardlaw, PhD, FRCP* *Leicester, United Kingdom*

Background: Asthma is characterized by variable airflow obstruction and airway hyperresponsiveness in association with airway inflammation under the influence of T_H2 cytokines. Eosinophilic bronchitis has similar immunopathology to asthma but without disordered airway physiology. Whether eosinophilic bronchitis is associated with increased expression of T_H2 cytokines is unknown.

Objective: We sought to assess the expression of T_H2 cytokines in eosinophilic bronchitis.

Methods: Expression of activation markers and chemokine receptors from blood and bronchoalveolar lavage (BAL) fluid T cells and the T_H2 cytokine expression from these T cells and bronchial mucosa biopsy specimens were assessed from subjects with eosinophilic bronchitis, subjects with asthma, and healthy control subjects.

Results: The proportion of resting (stimulated) CD4 BAL fluid T cells expressing intracellular IL-4 was significantly higher in the subjects with eosinophilic bronchitis 7.2% (11.4%) and subjects with asthma 5.3% (5.5%) than in healthy control subjects 2.8% (3.9%) ($P = .03$). The number of IL-4⁺ ($P < .001$) and IL-5⁺ ($P = .003$) cells per square millimeter of bronchial submucosa was significantly higher in the disease groups than in the healthy control subjects. Expression of intracellular IFN- γ was significantly higher in stimulated blood CD8 T cells from subjects with eosinophilic bronchitis (24%) and asthma (17%) than in the healthy control subjects (5%; $P = .003$). There were no between-group differences in expression of IFN- γ in the BAL fluid T cells or in the bronchial submucosa and no differences in expression of activation markers or chemokine receptors.

Conclusion: These findings support the concept of asthma as a disease associated with activation of T_H2 lymphocytes in the airway and provide evidence that these cytokines play a role in the development of airway inflammation in eosinophilic bronchitis but suggest that the release of T_H2 cytokines is not sufficient for the elaboration of disordered airway physiology in asthma. (*J Allergy Clin Immunol* 2002;110:899-905.)

Key words: T_H2 cells, asthma, eosinophilic bronchitis, eosinophils

Asthma is a condition characterized by variable airflow obstruction and airway hyperresponsiveness in association with airway inflammation. Pathologically, there is accumulation of eosinophils and CD4 lymphocytes, mucus hypersecretion, thickening of the sub-epithelial collagen layer, mast cell degranulation, and smooth muscle hypertrophy and hyperplasia.^{1,2}

There is increasing evidence that the development and maintenance of the airway inflammation in asthma is due to T-lymphocyte activation with the production of T_H2 cytokines, such as IL-4, IL-5, and IL-13.³ These T_H2 cytokines cause B-cell switching to IgE, increased expression of vascular cell adhesion molecule 1 and P-selectin leading to selective eosinophil recruitment,⁴ and mucus hypersecretion.⁵ However, the extent to which T_H2 cytokine expression, eosinophilic airway inflammation, and airway hyperresponsiveness are related is unclear.

A dissociation between airway inflammation and airway hyperresponsiveness is observed in subjects with eosinophilic bronchitis, a condition characterized by corticosteroid-responsive cough and the presence of sputum eosinophilia without airway hyperresponsiveness.⁶⁻⁸ Studies suggest that the nature and state of activation of lower airway inflammation in asthma and eosinophilic bronchitis appear similar.⁸⁻¹⁰ Whether the airway inflammation in eosinophilic bronchitis is associated with activation of lymphocytes expressing T_H2 cytokines or with expression of T_H2 cytokines by other cells in the airway submucosa is unknown.

To address this question, we have undertaken a comparative immunopathologic study of the activation and cytokine expression of bronchoalveolar lavage (BAL)

From the Division of Respiratory Medicine, Institute for Lung Health, Leicester-Warwick Medical School and University Hospitals of Leicester, Leicester.

Supported by a grant from The National Asthma Campaign (UK). S.S.B. is funded by a University Hospitals of Leicester Clinical Fellowship, and P.B. is a Wellcome Advanced Fellow.

*I.D.P. and A.J.W. were joint senior investigators.

Received for publication May 8, 2002; revised August 22, 2002; accepted for publication September 5, 2002.

Reprint requests: Andrew J. Wardlaw, PhD, FRCP, Division of Respiratory Medicine, Institute for Lung Health, Clinical Sciences Wing, University Hospitals of Leicester, Groby Rd, Leicester, LE3 9QP, United Kingdom.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/83/129698

doi:10.1067/mai.2002.129698

TABLE I. Subjects' clinical characteristics

	Subjects with eosinophilic bronchitis	Asthmatic subjects	Healthy control subjects
No.	16	12	14
Age*	48 (3)	47 (5)	42 (5)
Male	10	6	8
Atopy	10	8	4
PC ₂₀ FEV ₁ (mg/mL)†	94 (18-128)	1.2 (0.16-4.6)	64 (16-128)
FEV ₁ % predicted*	100 (2.6)	100 (3.1)	100 (3.6)
FEV ₁ /FVC %*	80 (1.4)	72 (2.3)	80 (1.9)

FVC, Forced vital capacity.

*Mean (SEM).

†Median (range).

Abbreviations used

BAL: Bronchoalveolar lavage

PMA: Phorbol 12-myristate 13-acetate

fluid T lymphocytes and T_H2 cytokine expression from bronchial mucosa biopsy specimens from subjects with eosinophilic bronchitis, subjects with symptomatic asthma, and healthy control subjects.

METHODS

Subjects

Sixteen subjects with eosinophilic bronchitis, 12 subjects with asthma, and 14 healthy control subjects were recruited from Glenfield Hospital outpatients and staff and local advertising. The subjects' clinical characteristics are shown in Table I. Subjects with asthma provided a consistent history and had objective evidence of asthma, as indicated by one or more of the following: (1) methacholine airway hyperresponsiveness (PC₂₀FEV₁ <8 mg/mL); (2) greater than 15% improvement in FEV₁ 10 minutes after administration of 200 µg of inhaled salbutamol; or (3) greater than 20% of maximum within-day amplitude from twice daily peak expiratory flow measurements over 14 days. The subjects with eosinophilic bronchitis had an isolated cough, no symptoms suggesting variable airflow obstruction, normal spirometric values, normal peak expiratory flow variability over 2 weeks, a methacholine PC₂₀ value of greater than 16 mg/mL, a normal chest radiograph, and sputum eosinophilia (>3% nonsquamous cell). Healthy control subjects were asymptomatic and had no evidence of variable airflow obstruction or airway hyperresponsiveness and had less than 3% sputum eosinophil count. The subjects with asthma were taking short-acting β₂-agonists as required. All were current nonsmokers for at least 6 months with a past smoking history of less than 10 pack-years. None of the subjects had taken inhaled or oral corticosteroids or antihistamines for at least 6 weeks before the study. The study was approved by the Leicestershire Ethics Committee, and all subjects provided written informed consent.

Protocol and clinical measurements

Subjects attended on 2 occasions. At the first visit, we measured spirometry, allergen skin prick test responses, and methacholine airway responsiveness. None of the subjects had taken bronchodilator therapy for at least 12 hours before spirometry and challenge testing. This was followed, after recovery, by a sputum induction. Spirometry was performed by using a dry bellows spirometer (Vitalograph), with the FEV₁ recorded as the best of successive readings within 100 mL. Allergen skin prick tests were performed to *Der-*

matophagoides pteronyssinus, cat fur, grass pollen, and *Aspergillus fumigatus* solutions, with normal saline and histamine controls (Bencard). A positive response to an allergen on the skin prick tests was recorded in the presence of a wheal of greater than 2 mm larger than that produced by the negative control. The methacholine challenge was performed by using the tidal breathing method with doubling concentrations of methacholine (0.03-128 mg/mL) nebulized with a Wright nebulizer.¹¹ Sputum was induced and processed as previously described.¹²

At the second visit, the subjects underwent bronchoscopy with an Olympus fiberoptic bronchoscope (Olympus Company) in line with the most recent British Thoracic Society guidelines.¹³ Bronchial mucosa biopsy specimens were taken from the right middle and lower lobe carinae. PBMCs from a 20-mL venous blood sample and cells recovered from a 180-mL BAL fluid sample of prewarmed normal saline placed into the middle lobe were analyzed from a subgroup of 10 subjects with eosinophilic bronchitis, 9 subjects with asthma, and 10 healthy control subjects. All subjects received 2.5 mg of nebulized salbutamol 20 minutes before bronchoscopy and had appropriate sedation, as required, of 0 to 5 mg of intravenous midazolam. Lignocaine (1%-4%) was used for local anesthesia and continuous oxygen administered through nasal cannulae throughout the procedure.

Mucosal biopsy specimens were immediately transferred into ice-cooled acetone containing the protease inhibitors iodoacetamide (20 mmol/L) and phenylmethylsulfonyl fluoride (2 mmol/L) for fixation, stored at -20°C for 24 hours, and then processed into water-soluble resin glycolmethacrylate (Polysciences) for embedding.

Immunohistochemistry

Two-micrometer sections were cut, floated on a 0.2% ammonia solution in water for 1 minute, and dried at room temperature for 1 to 4 hours. The following mouse IgG₁ mAbs were used: IL-4 3H4 (AMS Biotechnology), IL-4 4D9 (AMS Biotechnology), IL-5 (a gift from GlaxoSmithKline), and IFN-γ (R&D Systems). The technique of immunostaining applied to glycolmethacrylate-embedded tissue has been described previously.¹⁴

Assessment and quantification of immunohistochemical staining

The subepithelial mucosa was identified morphologically, and the area was calculated by using a computer analysis system (Scion Image). Nucleated immunostained cells present in coded sections were enumerated in the submucosa, and numbers of cells were expressed as the number per square millimeter of submucosa by an experienced blinded observer. Data on mast cell, eosinophil, and T-cell counts from the bronchial biopsy specimens of 11 of the subjects with eosinophilic bronchitis, 8 of the subjects with asthma, and 7 of the healthy control subjects has been previously reported.¹⁵

Flow cytometry of surface receptor expression and intracellular cytokines

The PBMC fraction was obtained by means of centrifugation on Ficoll. After washing with FACS buffer (PBS and 0.5% BSA), PBMCs and BAL fluid cells were divided for analysis of surface receptor expression and intracellular cytokine staining.

For surface receptor expression, cells were resuspended in FACS buffer at a concentration of between 0.5 and 1 × 10⁶/mL, depending on the number of cells available. Nonspecific antibody binding was blocked by using mouse IgG (Sigma). BAL cells and PBMCs were stained with directly conjugated mAbs against CD3-RPE (Dako, Ltd), CD4-PerCP (BD), and CD8 (Dako, Ltd); the activation markers CD25 (Dako, Ltd), HLA-DR (Dako, Ltd), and CD49a (Serotec); and the chemokine receptors CCR3 (gift from Millenium), CCR5

(BD), CCR6, and C-X-CR3 (R&D Systems) indirectly labeled with FITC with appropriate isotypic controls. Lymphocytes were gated for CD3 expression and then further subdivided by CD4 or CD8 expression and were analyzed by means of 3-color flow cytometry on a FACScan (BD).

The remaining cells were either stimulated with phorbol 12-myristate 13-acetate (PMA; 5 ng/mL, Sigma), calcium ionophore (250 ng/mL, Sigma), and brefeldin A (10 µg/mL, Sigma) or incubated in culture medium alone (resting) for 4 hours at 37°C. The cells were fixed in 4% paraformaldehyde (Sigma) and stored overnight at 4°C in PBS and 0.5% BSA. The following day, the cells were washed and incubated with CD3-FITC/RPE (Dako, Ltd) and CD8-PerCP (BD) because CD4 becomes internalized after stimulation, permeabilized in 4% paraformaldehyde and 0.1% saponin (Sigma) for 15 minutes on ice, labeled with IL-4-RPE (BD) and IFN-γ-FITC (BD) or isotypic controls, and analyzed by means of 3-color flow cytometry, as for the surface receptor expression.

Statistical analysis

Subject characteristics were described by using descriptive statistics. The number of submucosal cells expressing cytokines and the proportion of PBMCs and BAL fluid lymphocytes expressing chemokine receptors, activation markers, and intracellular cytokines were expressed as medians and ranges. Comparisons across the 3 groups were undertaken by using the Kruskal-Wallis test and between groups by using the Mann-Whitney test. A *P* value of less than .05 was taken as being statistically significant.

RESULTS

The individual values for the number of cells expressing IL-4 (3H4 and 4D9), IL-5, and IFN-γ in the submucosa are as shown in Fig 1. The median number of cells per square millimeter of submucosa was significantly higher in the subjects with eosinophilic bronchitis and asthma than in healthy control subjects for IL-4 3H4+ (6.5, 7.6, and 0.5, respectively; *P* < .001), IL-4 4D9+ (8.2, 9.6, and 1.5, respectively; *P* < .001), and IL-5+ (6.3, 6.4, and 1.1, respectively; *P* = .003) cells. There were no differences seen in IL-4 and IL-5 expression between those subjects with asthma or eosinophilic bronchitis. There were no differences in the median IFN-γ+ cells per square millimeter of submucosa in the subjects with eosinophilic bronchitis (3.9), subjects with asthma (1.9), and healthy control subjects (2.4, *P* = .8). Counts in atopic and nonatopic subjects within groups were similar.

PBMC expression of intracellular IFN-γ was increased in those subjects with eosinophilic bronchitis and asthma compared with that seen in healthy control subjects in stimulated CD3+CD8+ (*P* = .003) and CD3+CD8- (*P* = .024) cells (Table II). There were no differences in IFN-γ expression between the disease groups in resting cells and no between-group differences in IL-4 expression in resting or stimulated PBMCs (Table II). Example dot-plots illustrating expression in resting and stimulated cells of IL-4 in BAL fluid cells for each subject group are shown Fig 2.

The proportion of CD3+CD8- BAL fluid cells expressing intracellular IL-4 was increased in subjects with eosinophilic bronchitis and asthma compared with that seen in healthy control subjects in both resting cells (median of 7.2%, 5.3%, and 2.8%, respectively; *P* = .03)

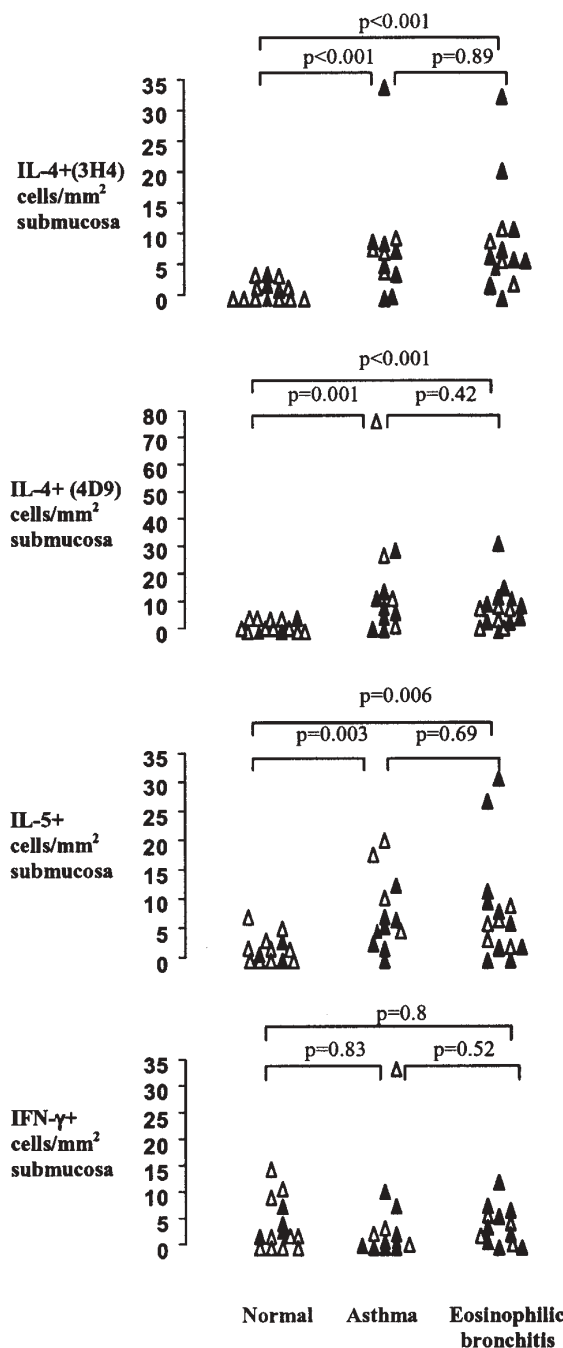


FIG 1. Submucosal cell counts per square millimeter expressing IL-4 (3H4 and 4D9), IL-5, and IFN-γ in subjects with asthma, subjects with eosinophilic bronchitis, and healthy control subjects. Filled triangles, Atopic subjects; open triangles, nonatopic subjects. *P* values are given for comparison between groups by using the Mann-Whitney test.

and stimulated cells (median of 11.4%, 5.5%, and 3.9%, respectively; *P* = .03; Fig 3). The proportion of CD3+CD8+ BAL fluid cells expressing intracellular IL-4 was significantly higher in subjects with eosinophilic bronchitis and asthma than in healthy control subjects in resting cells (median [range] of 6.1% [1.2%-13%], 5.3%

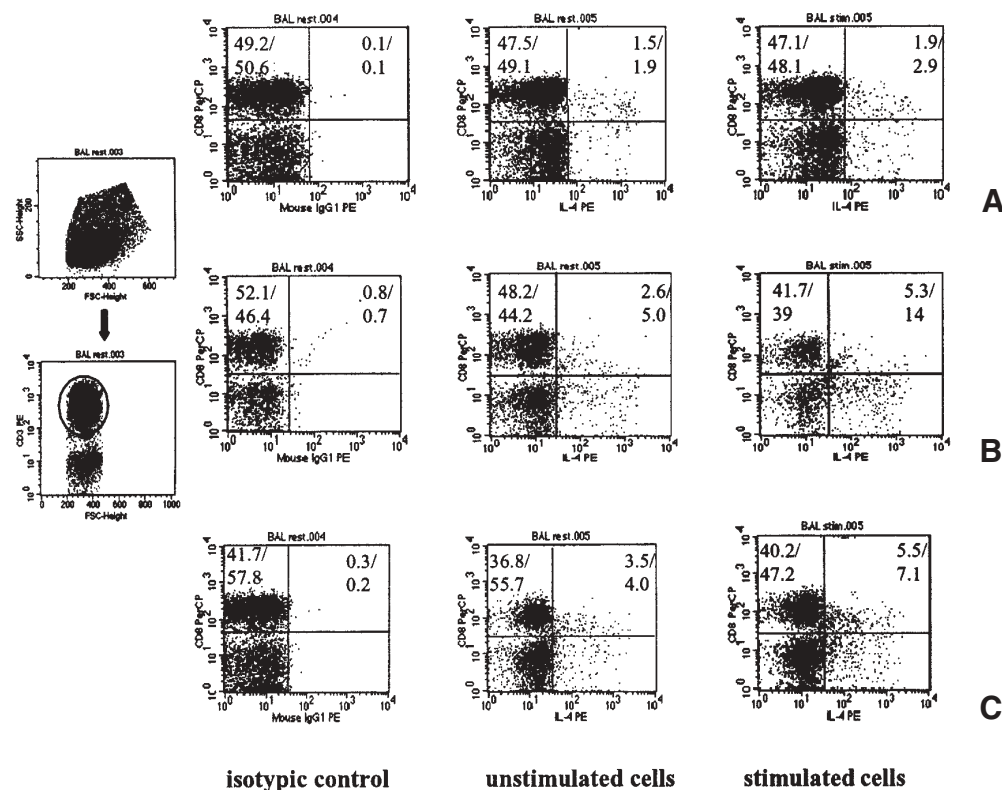


FIG 2. Example dot-plots from an individual healthy control subject (A), a subject with asthma (B), and a subject with eosinophilic bronchitis (C) for IL-4 intracellular cytokine staining and isotypic control in resting and stimulated BAL fluid T lymphocytes. The figures in the upper quadrant boxes show the percentage of cells within the upper-lower quadrants.

TABLE II. Proportion of CD4⁺ (CD3⁺CD8⁻) and CD8⁺ (CD3⁺CD8⁺) peripheral blood T lymphocytes expressing intracellular cytokine staining

	Subjects with eosinophilic bronchitis		Asthmatic subjects		Control subjects	
	CD4 ⁺ (%)	CD8 ⁺ (%)	CD4 ⁺ (%)	CD8 ⁺ (%)	CD4 ⁺ (%)	CD8 ⁺ (%)
IL-4 R	1.7 (0.8-5)	1.3 (0.6-5)	2 (0-4.6)	2 (0-4.6)	0.7 (0-3.6)	0.7 (0-3.6)
IL-4 S	3.1 (0.9-9)	3.1 (0-9)	4 (0-17)	3.4 (0-17)	2.2 (0-4)	1.8 (0-3.8)
IFN- γ R	0.9 (0-5.8)	1.5 (0-6)	1.3 (0.2-3.8)	1.3 (0.2-3.8)	0.7 (0.3-2.5)	0.7 (0.1-6)
IFN- γ S	14 (2.8-52)*	24 (5-52)*	15 (7-30)*	17 (10-57)*	3.9 (0.1-12)	5 (0.1-12)

Values are given as medians (ranges).

R, Resting cells; S, stimulated cells.

* $P < .05$ for comparison between disease group and control group by using the Mann-Whitney test.

[1.5%-12.8%], and 2.3% [0%-5%], respectively; $P = .017$) and stimulated cells (8% [3%-16%], 7.8% [3.6%-17%], and 3.8% [0%-18%], respectively; $P = .05$). Intracellular IFN- γ expression in CD3⁺ BAL fluid cells markedly increased after stimulation compared with that seen in resting cells, but there were no between-group differences in the proportion of BAL fluid cells expressing intracellular IFN- γ . Counts in atopic and nonatopic subjects within groups were similar (Fig 3).

The proportion of CD3⁺ BAL fluid cells expressing CD4 was similar in subjects with asthma (median [range] of 57.5% [45%-77%]), subjects with eosinophilic bron-

chitis (55% [20%-76%]), and healthy control subjects (55.5% [36%-78%]; $P = .9$). No differences were seen in the proportion of CD3⁺CD4⁺ or CD3⁺CD4⁻ cells expressing activation or chemokine receptors between the groups in PBMCs (data not shown) and BAL fluid cells (Table III).

DISCUSSION

This study has made 3 important and novel observations. First, we have demonstrated that there is increased constitutive expression of IL-4 protein, the classical T_H2

cytokine, in T cells from the airways of asthmatic subjects, supporting the concept of asthma as a disease associated with activation of T_H2 lymphocytes. Second, we have shown that eosinophilic bronchitis is a disease characterized by increased expression of T_H2 cytokines, demonstrating a dissociation between T-cell activation and the abnormalities in airway physiology that characterize asthma. Third, we have shown that both asthma and eosinophilic bronchitis are characterized by priming of peripheral blood T cells for cytokine release after stimulation with PMA and ionomycin, suggesting there is a systemic T-cell abnormality in these diseases.

The concept of asthma as a disease associated with activation of T_H2 cells was proposed in the early 1990s with the observation that human, as well as mouse, memory T-cell clones could be committed toward cells releasing IL-4 and IL-5 (T_H2) or IFN- γ and IL-2 (T_H1).¹⁶ Of particular relevance to asthma, IL-4 promotes IgE synthesis and eosinophil recruitment,^{17,18} whereas IL-5 is the major eosinophilopoietic cytokine.¹ This hypothesis was supported by the observation, made by using in situ hybridization, that up to 50% of BAL fluid T cells from asthmatic subjects but very few from healthy control subjects expressed mRNA for IL-4, with no difference between asthmatic and nonasthmatic subjects in mRNA expression for IFN- γ .¹⁹ Although the concept of asthma as a T_H2 disease is now widely supported, there are surprisingly little data showing that T cells in asthma are producing increased amounts of T_H2 cytokines. Indeed, Krug et al,²⁰ using intracellular flow cytometry, found an increase in IFN- γ but not IL-5 or IL-4 expression by BAL fluid T cells from asthmatic subjects after stimulation with PMA or ionomycin. They observed that only a mean of about 2% of cells in this study and a median of 8% in a later report²¹ expressed IL-4, even after stimulation, raising the possibility that the increase in mRNA expression seen by means of in situ hybridization was not being translated into protein. Similarly, in a study using flow cytometry to investigate cytokine expression in peripheral blood, an increase in IL-4 expression was related to atopy, but IFN- γ production, mainly in the CD8 population of T cells, was more closely allied to the asthma phenotype.²² Although Till et al²³ observed increased production of IL-5 by BAL fluid T cells from asthmatic subjects after allergen challenge, this finding is inconsistent because Krug et al²¹ found no change in IL-4 or IL-5 after segmental allergen challenge. However, Krug et al²¹ did observe an increase in IL-13 in subjects with asthma compared with that seen in control subjects in a subset of T cells ($\gamma\delta^+$ T cells) and observed that after allergen challenge, the proportion of T cells expressing IFN- γ decreased in asthmatic subjects but not in control subjects, providing evidence for a change in the T_H1 versus T_H2 balance in favor of a T_H2 bias. Increased amounts of IL-4 and IL-5 expression have been found by means of immunohistochemistry in subjects with asthma, but this is mainly present in mast cells and eosinophils.²⁴⁻²⁶ The current evidence for asthma being a T_H2 disease is therefore limited. Our study provides

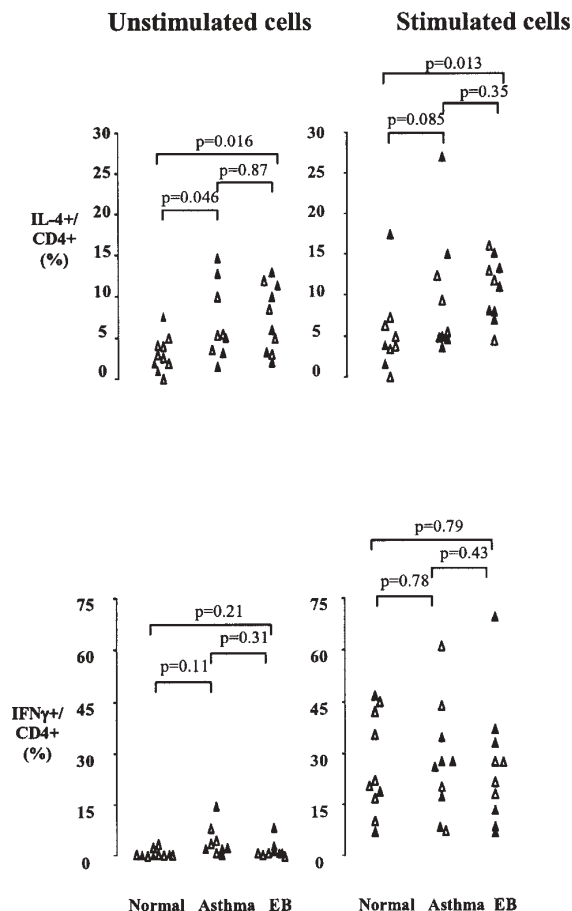


FIG 3. Individual data for the proportion (percentage) of CD4 (CD3+CD8⁻)–expressing intracellular IL-4 and IFN- γ cells in resting and stimulated BAL fluid T lymphocytes. Filled triangles, Atopic subjects; open triangles, nonatopic subjects; EB, eosinophilic bronchitis. P values are given for comparisons between groups by using the Mann-Whitney test.

direct support for this hypothesis by demonstrating constitutive expression of IL-4 by a small percentage of both CD4 and CD8 cells in the airway lumen. This represented at most about 10% of the total CD3 population. Virtually no cells were constitutively expressing IFN- γ in any of the groups, although IFN- γ -producing cells were easily the most frequent type of T cell after polyclonal stimulation in all 3 groups, with no significant difference between them. Although at odds with the levels of mRNA expression seen in earlier studies, it seems plausible that T_H1 and T_C1 cells, presumably directed against respiratory viruses and bacteria, will make up the majority population of T cells in the adult lung and that allergen-specific cells making T_H2 cytokines will be in the minority. The crucial difference appears to be that in asthma the T_H2 cells are actively secreting mediators, whereas the T_H1 cells are quiescent. It is of interest that we found increased numbers of IL-4-secreting T cells in nonatopic, as well as atopic, subjects, supporting the idea of intrinsic asthma as a T_H2 -associated condition.²⁷ Sim-

TABLE III. Proportion of CD4⁺ (CD3⁺CD4⁺) and CD8⁺ (CD3⁺CD4⁻) T cells that express chemokine receptors and activation markers in BAL fluid in subjects with eosinophilic bronchitis, subjects with asthma, and control subjects

	Subjects with eosinophilic bronchitis		Asthmatic subjects		Control subjects	
	CD4 (%)	CD8 (%)	CD4 (%)	CD8 (%)	CD4 (%)	CD8 (%)
CD25	36 (22-60)	20 (16-36)	38 (19-49)	21 (12-58)	50 (29-67)	25 (12-53)
CD49a	54 (30-78)	75 (60-86)	59 (42-95)	72 (60-90)	52 (12)	76 (23)
HLA-DR	87 (74-95)	81 (63-90)	79 (50-95)	71 (43-88)	72 (26)	66 (20)
CCR3	7 (3-43)	6 (5-48)	16 (3-54)	13 (3-43)	10 (3-23)	6 (3-20)
CCR5	94 (80-98)	93 (88-98)	94 (86-99)	94 (81-97)	92 (76-93)	94 (88-96)
CCR6	66 (50-78)	30 (25-46)	58 (38-94)	49 (14-89)	64 (30-86)	34 (16-46)
C-X-CR3	93 (78-98)	96 (92-98)	91 (53-98)	87 (17-99)	87 (43-94)	95 (80-98)

Values are given as medians (ranges).

ilar to previous studies, we found increased expression of IL-4 and IL-5 but not IFN- γ in the airway mucosa, but we also found that the majority of these cells were mast cells and eosinophils (data not shown).

Eosinophilic bronchitis, like asthma, is characterized by eosinophilic airway inflammation, but unlike asthma, there is no airway hyperresponsiveness or bronchoconstriction. The observation that T_H2 cells are also present in eosinophilic bronchitis strongly suggests that this disordered immunology, although plausibly causing airway eosinophilia, cough, and mucus hypersecretion, is not directly responsible for airway hyperresponsiveness and bronchoconstriction. In this and other recent reports, it has been shown that the immunopathology of asthma and eosinophilic bronchitis are almost identical^{8,9} apart from one fundamental difference, which is that in asthma but not eosinophilic bronchitis mast cells infiltrate the airway smooth muscle.¹⁵ This points toward a mast cell–smooth muscle myositis being responsible for the disordered airway function seen in subjects with asthma and not T_H2-mediated inflammation.

Another intriguing finding of our study was the increased expression of IFN- γ in stimulated peripheral blood T cells in both asthma and eosinophilic bronchitis. This has been previously noted by Magnan et al,²² where it was seen principally in the CD8 population. In contrast, in our study it occurred in both CD4 and CD8 cells. At first sight, there appears to be a discrepancy between constitutive activation of T_H2 cells in the airway in subjects with asthma and eosinophilic bronchitis and increased numbers of T cells expressing IFN- γ in the blood. However, in the blood the difference between subjects with airway inflammation and healthy control subjects was seen only after stimulation, whereas in the airway we saw evidence of T cells constitutively producing IL-4. This suggests that increased IFN- γ production is due to a priming effect on peripheral blood T cells and is perhaps consistent with a systemic inflammatory process, which is present in subjects with mild disease and no obvious systemic features.

Against the concept of there being primed T cells in the blood of asthmatic subjects, we found no difference in the expression of activation markers on T cells among

the 3 groups either in the blood or airway. One possible explanation is that these markers are not very sensitive. We also found no difference in the expression of chemokine receptors among the 3 groups. Indeed, as we reported previously,²⁸ high levels of expression of the putative T_H1 chemokine receptors CCR5 and C-X-CR3 and low levels of expression of the putative T_H2 chemokine receptor CCR3 were seen in the asthmatic subjects, as well as in the subjects with eosinophilic bronchitis. However, because the T_H2 cells only make up about 10% of the BAL fluid T-cell population, it is still possible that there are differences in chemokine receptor expression between the 2 T-cell phenotypes, which our techniques are not sufficiently discriminating to detect.

In summary, we have shown that eosinophilic bronchitis, like asthma, is a disease characterized by activation in the airways of T_H2 lymphocytes. However, it seems that although T_H2-mediated cytokines are closely linked to eosinophilic inflammation, they are not directly associated with the hallmarks of the asthmatic phenotype, airway hyperresponsiveness and variable airflow obstruction.

We thank the subjects who participated in the study, Mrs D. Parker and Mr S. Barlow for technical assistance in the laboratory, and Dr R. Green, Mrs S. McKenna, and Mrs B. Hargadon for assistance in the clinical characterization of some of the subjects.

REFERENCES

- Wardlaw AJ, Brightling C, Green R, Woltmann G, Pavord ID. Eosinophils in asthma and other allergic diseases. *Br Med Bull* 2000;56:985-1003.
- Kay AB. Pathology of mild, severe, and fatal asthma. *Am J Respir Crit Care Med* 1996;154:S66-9.
- Robinson DS. Th-2 cytokines in allergic disease. *Br Med Bull* 2000;56:956-68.
- Woltmann G, McNulty CA, Dewson G, Symon FA, Wardlaw AJ. Interleukin-13 induces PSGL-1/P-selectin-dependent adhesion of eosinophils, but not neutrophils, to human umbilical vein endothelial cells under flow. *Blood* 2000;95:3146-52.
- Chung KF, Barnes PJ. Cytokines in asthma. *Thorax* 1999;54:825-57.
- Gibson PG, Dolovich J, Denburg J, Ramsdale EH, Hargreave FE. Chronic cough: eosinophilic bronchitis without asthma. *Lancet* 1989;1:1346-8.
- Brightling CE, Ward R, Goh KL, Wardlaw AJ, Pavord ID. Eosinophilic bronchitis is an important cause of chronic cough. *Am J Respir Crit Care Med* 1999;160:406-10.

8. Brightling CE, Ward R, Woltmann G, Bradding P, Sheller JR, Dworski R, et al. Induced sputum inflammatory mediator concentrations in eosinophilic bronchitis and asthma. *Am J Respir Crit Care Med* 2000;162:878-82.
9. Gibson PG, Zlatić K, Scott J, Sewell W, Woolley K, Saltos N. Chronic cough resembles asthma with IL-5 and granulocyte-macrophage colony-stimulating factor gene expression in bronchoalveolar cells. *J Allergy Clin Immunol* 1998;101:320-6.
10. Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. *J Allergy Clin Immunol* 2000;106:638-44.
11. Juniper EF, Cockcroft DW, Hargreave FE. Histamine and methacholine inhalation tests: a laboratory tidal breathing protocol. Lund, Sweden: Astra Draco AB; 1994.
12. Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax* 1997;52:498-501.
13. British Thoracic Society. British Thoracic Society guidelines on diagnostic flexible bronchoscopy. *Thorax* 2001;56(suppl 1):1-21.
14. Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosa biopsies. *Biotech Histochem* 1993;68:271-80.
15. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. A comparison of the immunopathology of asthma and eosinophilic bronchitis: evidence that mast cell infiltration of airway smooth muscle characterises the asthmatic phenotype. *N Engl J Med* 2002;346:1699-705.
16. Romagnani S. The Th1/Th2 paradigm. *Immunol Today* 1997;18:263-6.
17. Vercelli D, Jabara HH, Lee BW, Woodland N, Geha RS, Leung DY. Human recombinant interleukin 4 induces Fc epsilon R2/CD23 on normal human monocytes. *J Exp Med* 1988;167:1406-16.
18. Wardlaw AJ. Molecular basis for selective eosinophil trafficking in asthma: a multistep paradigm. *J Allergy Clin Immunol* 1999;104:917-26.
19. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
20. Krug N, Madden J, Redington AE, Lackie P, Djukanovic R, Schauer U, et al. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996;14:319-26.
21. Krug N, Erpenbeck VJ, Balke K, Petschallies J, Tschernig T, Hohlfeld JM, et al. Cytokine profile of bronchoalveolar lavage-derived CD4(+), CD8(+), and gammadelta T cells in people with asthma after segmental allergen challenge. *Am J Respir Cell Mol Biol* 2001;25:125-31.
22. Magnan AO, Mely LG, Camilla CA, Badier MM, Montero-Julian FA, Guillot CM, et al. Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma. *Am J Respir Crit Care Med* 2000;161:1790-6.
23. Till SJ, Durham SR, Rajakulasingam K, Humbert M, Huston D, Dickson R, et al. Allergen-induced proliferation and interleukin-5 production by bronchoalveolar lavage and blood T-cells after segmental allergen challenge. *Am J Respir Crit Care Med* 1998;158:404-11.
24. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994;10:471-80.
25. Moller GM, de Jong TA, van der Kwast TH, Overbeek SE, Wiereng Wolf AF, Thepen T, et al. Immunolocalisation of interleukin-4 in eosinophils in the bronchial mucosa of atopic asthmatics. *Am J Respir Cell Mol Biol* 1996;14:439-43.
26. Nonaka M, Nonaka R, Woolley K, Adelroth E, Minra K, Okhawara Glibetic M, et al. Distinct immunohistochemical localisation of IL-4 in human inflamed airway tissues. IL-4 is localised to eosinophils in vivo and is released by peripheral blood eosinophils. *J Immunol* 1995;155:3234-44.
27. Humbert M, Durham S, Ying S, Kimmitt P, Barkans J, Assoufi B, et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against "intrinsic" asthma being a distinct immunopathological entity. *Am J Respir Crit Care Med* 1996;154:1497-504.
28. Campbell JJ, Brightling CE, Symon FA, Qin S, Murphy KE, Hodge M, et al. Expression of chemokine receptors by lung T cells from normal and asthmatic subjects. *J Immunol* 2001;166:2842-8.

Availability of Journal back issues

As a service to our subscribers, copies of back issues of *The Journal of Allergy and Clinical Immunology* for the preceding 5 years are maintained and are available for purchase until inventory is depleted. Please write to Mosby Subscription Customer Service, 6277 Sea Harbor Dr, Orlando, FL 32887, or call (800) 654-2452 or (407) 345-4000 for information on availability of particular issues and prices.