Allergen-induced Proliferation and Interleukin-5 Production by Bronchoalveolar Lavage and Blood T Cells after Segmental Allergen Challenge

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In order to detect and characterize allergen-specific T cells in the airways of atopic asthmatics, we measured proliferation and cytokine production by bronchoalveolar lavage (BAL) T cells isolated from *Dermatophagoides pteronyssinus* (*Der p*)-sensitive asthmatics and nonatopic control subjects, and compared the results with those generated using peripheral blood (PB) T cells. BAL and PB mononuclear cells were collected 24 h after segmental allergen challenge by fibreoptic bronchoscopy and venepuncture, respectively. T cells purified from BAL and PB were stimulated with autologous, irradiated antigen-presenting cells and *D. pteronyssinus* extract or a control, nonallergen antigen (*M. tuberculosis* purified protein derivative [PPD]). IL-5 and IFN-γ concentrations were measured in culture supernatants by ELISA, and T-cell proliferation by ^{3}H-thymidine uptake. *D. pteronyssinus*-induced proliferation of T cells derived from both BAL and PB was elevated in asthmatics when compared with control subjects ($p < 0.05$), whereas PPD-induced proliferation was equivalent in both compartments. In the asthmatics, *D. pteronyssinus*-induced proliferative responses of equivalent numbers of BAL and PB T cells obtained after allergen challenge were statistically equivalent. Nevertheless, BAL T cells stimulated with *D. pteronyssinus* produced significantly greater amounts of IL-5 than did PB T cells ($p < 0.05$). Allergen-induced proliferation and IL-5 production by BAL T cells in the asthmatics after segmental allergen challenge correlated with the percentages of eosinophils in the BAL fluid ($p < 0.01$). Further, BAL T cells from asthmatic patients produced significantly higher amounts of IL-5 than did the same number of cells from nonatopic control subjects ($p < 0.05$). We conclude that, in *D. pteronyssinus*-sensitive asthmatics, allergen-specific T cells can be detected in the bronchial lumen after allergen challenge and that allergen-induced proliferation and IL-5 production by these cells correlates with local eosinophil influx. Although bronchial luminal T cells show an equivalent proliferative response to allergen stimulation as compared with PB T cells, they do produce more IL-5, consistent with the hypothesis that local differentiation or priming of these cells within the bronchial mucosal environment results in upregulation of allergen-induced IL-5 secretion. **Till SJ, Durham SR, Rajakulasingam K, Humbert M, Huston D, Dickason R, Kay AB, Corrigan CJ. 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;158:404–411.

T-cell–derived cytokines are considered to play a key role in the process of selective eosinophil accumulation and activation in the bronchial mucosa of atopic asthmatics. In particular, the cytokine interleukin-5 (IL-5) acts on eosinophils and their committed precursors selectively to promote maturation, endothelial adhesion, activation, and survival (1–3). Furthermore, IL-5 stimulates eosinophil release into the bloodstream circulation from bone marrow (4) and primes these cells for enhanced chemotactic responses to C-C chemokines such as RANTES (5). T cells from sensitized atopic subjects, particularly allergen-specific cells, are characterized by elevated secretion of cytokines such as IL-5 after specific activation as compared with cells from nonatopic subjects (6–8).

Despite these observations, there are some fundamental questions regarding the role of allergen-specific T cells in the pathogenesis of asthma that remain unanswered. One question relates to the possible role of allergen-specific T cells within the airways in the pathogenesis of allergen-induced airways inflammation. Allergen bronchial challenge of atopic asthmatics, which may cause late-phase bronchoconstriction, is associated with elevated local IL-5 synthesis, principally by

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T cells (9) in the bronchial mucosa (10) or lumen (11). This implies (and indeed it is often assumed) that at least some of these T cells are allergen-specific, but this has never been demonstrated directly. Although allergen-specific T-cell clones have been propagated from bronchial biopsies of asthmatics (12), such clones can also be propagated from non-atopic healthy subjects (6, 13, 14), and so this observation alone does not implicate allergen-specific T cells in the pathogenesis of allergen-induced airways inflammation. Secondly, there exists the possibility that, if allergen-specific T cells do populate the airways of asthmatics, they differ functionally from those in the peripheral blood, perhaps reflecting selective recruitment of certain subpopulations and/or the influences of the bronchial mucosal environment after recruitment. In this regard, it has been shown that elevated percentages of T cells in bronchoalveolar lavage fluid show phenotypic characteristics suggestive of previous activation (15, 16) and are more prone to apoptosis (17) as compared with T cells in the peripheral blood. Of particular interest is the possibility that allergen-specific T cells within the airways secrete elevated quantities of asthma-relevant cytokines such as IL-5, as compared with peripheral blood cells. Again, studies on allergen-specific T-cell clones, which require prolonged culture of the cells *in vitro* often in the presence of unphysiologic stimuli, cannot address this question.

Our aim in this study, therefore, was to compare allergen-induced proliferation and IL-5 production by T cells isolated freshly and simultaneously from the bronchial lumen and peripheral blood of a group of sensitized, atopic asthmatics and nonatopic normal control subjects in an allergen challenge setting (24 h after local segmental allergen challenge). We hypothesized that, after allergen challenge of sensitized, atopic asthmatics, T cells isolated from the bronchial lumen as well as the peripheral blood show a proliferative response to allergen *in vitro*, which is greater than that shown by equivalent numbers of cells isolated from the same compartments from allergen-challenged nonatopic control subjects. We further hypothesized that T cells isolated from the bronchial lumen after allergen challenge of atopic asthmatics are characterized by elevated allergen-induced IL-5 production *in vitro* as compared with equivalent numbers of those in the peripheral blood and those in both compartments in non-atopic control subjects. To establish the antigen- and cytokine-specificity of these phenomena, allergen-specific responses were compared with responses to the nonallergen ubiquitous antigen *M. tuberculosis* PPD, and IL-5 production was compared with that of IFN-γ.

METHODS

Subjects

The clinical characteristics of the study subjects are shown in Table 1. Because of the nature of the study we were constrained to the investigation of asthmatic patients with mild disease. Asthmatics, defined by the standard ATS criteria, were required to have $FEV_1 > 80\%$ predicted and histamine PC_{20} (defined as that concentration of inhaled histamine resulting in a 20% reduction in baseline FEV_1) of > 1 mg/ml, but < 16 mg/ml. In addition, all subjects were required to have ⩾ 3 mm diameter wheal at 15 min after skin prick testing with *D. pteronyssinus* ("Soluprick"; ALK, Horsholm, Denmark) in the presence of negative diluent and positive histamine controls, and elevated concentrations of serum IgE antibodies specific for *D. pteronyssinus* (RAST > 0.70 IU/ml, CAP system; Pharmacia Diagnostics, Uppsala, Sweden). Control subjects were required to have a lifelong absence of any symptoms indicative of allergic disease, histamine $PC_{20} > 32$ mg/ml, serum IgE concentration within the laboratory normal range (0 to 150 IU/ml), and negative skin prick tests and RAST to a range of 12 common aeroallergens including *D. pteronyssinus.* All subjects participating in this study were non-smokers. Inhaled glucocorticoid therapy was withheld 2 wk prior to bronchoscopy, and none of the subjects had received oral glucocorticoids for at least 6 mo prior to the study. The study was approved by the Ethics Committee of the Royal Brompton Hospital, London, and all subjects gave written, informed consent.

Fiberoptic Bronchoscopy, Endobronchial Allergen Challenge, and Bronchoalveolar Lavage

All subjects were premedicated with 2.5 mg nebulized albuterol, and 0.6 mg atropine and 5–10 mg midazolam administered intravenously. Local anaesthesia of the vocal cords and trachea was induced with 2 to 4% lidocaine. After inspection of the bronchial tree, the tip of the bronchoscope (an Olympus BFP20; Olympus Corp., London, UK)

TABLE 1

CLINICAL DETAILS OF ASTHMATIC AND NONATOPIC CONTROL SUBJECTS

Subject No.	Age (*yr*)	Sex	Histamine PC_{20} (*mg/ml*)	Total Serum IgE (*IU/ml*)	*Der p* RAST (*IU/ml*)	Skin Prick Test*	FEV_1 (*L*) (*% pred*)	Use of Inhaled β_2-agonist*	Inhaled Glucocorticoid† ($\mu g/d$)
Asthmatic subjects									
1	26	M	3.7	78	12.5	Dp,G,C,D	3.7 (86)	400 μg/d	200
2	29	F	4.2	71	13.1	Dp,G,C	3.25 (90)	< 200 μg/d	—
3	36	F	3.6	62	9.4	Dp,G,D	3.2 (> 100)	< 200 μg/wk	—
4	34	F	1.3	309	> 100	Dp,G,C,D	3.45 (> 100)	200 μg/d	100
5	25	M	2.0	210	47.3	Dp,G	4.8 (> 100)	> 200 μg/wk	—
6	21	F	14.0	169	83.3	Dp,C	4.2 (> 100)	< 200 μg/wk	—
7	25	F	1.9	1,009	2.3	Dp,G,C	3.1 (90)	< 200 μg/wk	200
8	24	M	6.5	2,878	14.4	Dp,G,C	3.9 (84)	< 200 μg/wk	—
9	23	F	1.4	121	29.0	Dp,G,C	4.6 (> 100)	800 μg/d	800
10	25	M	15.4	260	69.4	Dp	3.8 (82)	200 μg/d	100
Median	25	NA	5.4	190	21.7	NA	3.8 (95)	NA	NA
Control subjects									
11	25	M	> 32	20	< 0.35	Negative	4.88 (> 100)	—	—
12	42	F	> 32	47	< 0.35	Negative	2.5 (> 100)	—	—
13	25	M	> 32	70	< 0.35	Negative	3.82 (> 100)	—	—
14	21	F	> 32	< 4	< 0.35	Negative	4.0 (> 100)	—	—
15	21	M	> 32	< 4	< 0.35	Negative	4.5 (> 100)	—	—
16	19	M	> 32	46	< 0.35	Negative	4.0 (> 100)	—	—
Median	23	NA	> 32	33	< 0.35	NA	4.0 (> 100)	NA	NA

Definition of abbreviations: *Der p* = *Dermatophagoides pteronyssinus*; G = mixed grass pollen; C = cat dander; D = dog dander; NA = not applicable.

* Albuterol metered-dose inhaler.

† Beclomethasone dipropionate metered-dose inhaler (all glucocorticoids were discontinued at least 2 wk prior to study commencement).

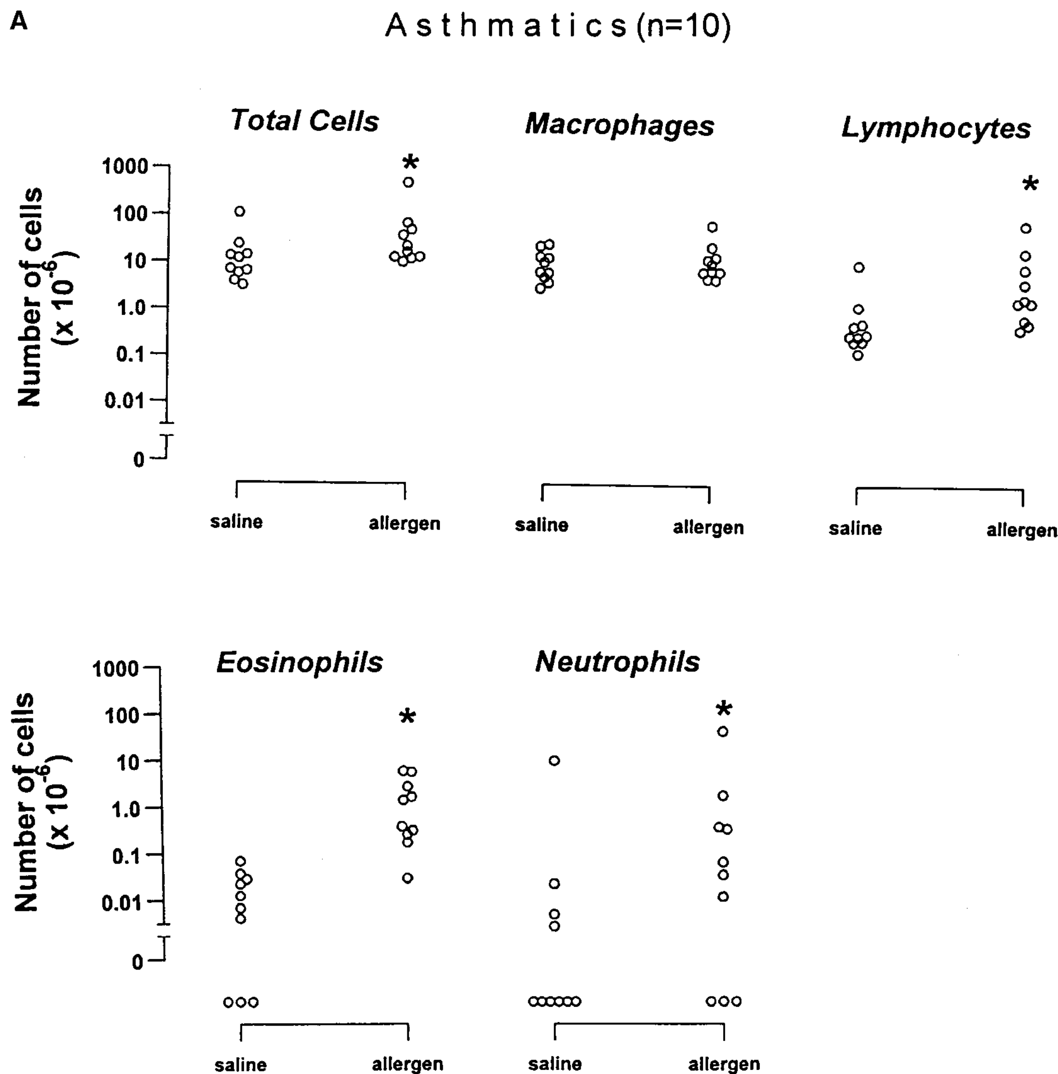

Figure 1. Absolute total and differential cell counts in BAL fluid 24 h after challenge of atopic asthmatics (*A*) and normal control subjects (*B*) with 500 BU *D. pteronyssinus* or saline control in remote bronchial segments. *Asterisks* indicate $p < 0.05$ (allergen versus saline).

was wedged at random in a segmental bronchus of the left lingula or right middle lobe, and allergen challenge was performed by instilling 100 BU of *D. pteronyssinus* ("Aquagen" extract, kindly provided by ALK) made up in 5 ml of sterile saline. The challenge site was observed for a further 5 min, and in the absence of excessive local bronchoconstriction, a further 400 BU of allergen were introduced in 5 ml of saline. For the saline control challenges, two 5-ml aliquots of saline were instilled at 5-min intervals into a segmental bronchus of the left lingula or right middle lobe (on the side contralateral to that which had received the allergen challenge). All subjects were subsequently detained in hospital overnight for observation. During this period, nebulized bronchodilator (albuterol 5 mg) was administered as necessary to the asthmatics to maintain $FEV_1 > 80\%$ of the predicted value. Similarly, standardized dosages were administered to the control subjects. A second bronchoscopy was repeated after 24 h. Just prior to premedication for the second bronchoscopy, a sample of peripheral venous blood was collected in a sterile heparinized syringe. Bronchoalveolar lavage was then performed of both the saline and allergen-challenged segments by sequentially instilling two 60-ml aliquots of sterile warmed saline followed by gentle aspiration into a sterile glass bottle ("saline" and "allergen challenge" BAL). To minimize cross contamination of samples collected from each segment, the bronchoscope was flushed with 10 ml of sterile saline between lavages.

Cell Preparation

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples by density gradient centrifugation over Ficoll-Hypaque (Pharmacia), washed twice in HEPES-buffered RPMI (Chester Beatty Laboratories, London, UK) and resuspended in RPMI (Gibco, Paisley, Scotland) supplemented with 5% human AB serum (Sigma, Poole, UK), 100 IU/ml penicillin/streptomycin (Gibco) and 2 mM L-glutamine (Gibco). This supplemented medium was used for all cell culture experiments. BAL fluid was passed through two layers of sterile gauze to remove mucus and washed twice in HEPES-buffered RPMI. A differential cell count was performed on a cytospin of BAL cells using May Grünwald Giemsa stain. Mononuclear cells were similarly isolated from BAL cells by density gradient centrifugation over Ficoll-Hypaque. T lymphocytes were then purified from peripheral blood or BAL mononuclear cells by passage of cells through a T-cell enrichment column containing anti-human Ig coated glass beads (R&D Systems, Abingdon, UK). Lymphocytes constituted > 90% of such preparations as judged by morphology, and showed good viability (> 90%), as determined by trypan blue exclusion.

Cell Culture

Antigen-specific T-cell proliferation was measured in 96 well round-bottomed plates (Nunc, Roskilde, Denmark) in a minimum of tripli-

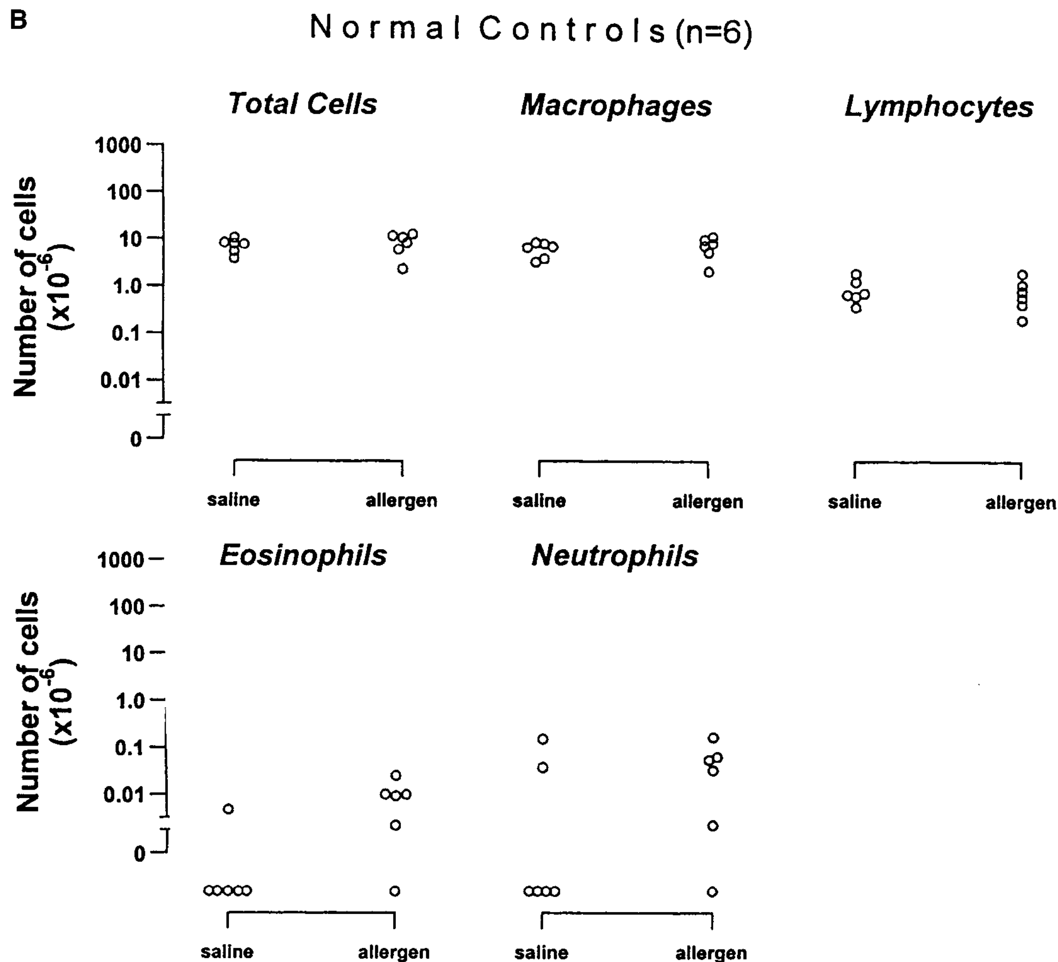

Figure 1. (*Continued*).

cate, by adding 10^5 irradiated (3,000 rads) autologous PBMC as antigen-presenting cells (APC) to 2×10^4 BAL or PB lymphocytes and culturing (200 μl total volume) in the presence of 10 μg/ml *D. pteronyssinus* ("Aquagen" extract), 10 μg/ml *M. tuberculosis* purified protein derivative (PPD; Evans Medical Ltd, Leatherhead, UK) or medium control. In all cases, control cultures were performed with APC and antigen only to confirm the absence of background proliferation in the irradiated PBMC population. Then 100 μl of culture supernatant were removed from each microculture well on Day 6, and cellular proliferation was determined on Day 7 by adding 0.5 μCi of tritiated methyl-thymidine to each well for the last 16 h of culture, and measuring label incorporation into cellular DNA by β-spectrometry.

Measurement of Cytokine Concentrations

IL-5 concentrations in T-cell culture supernatants were measured in duplicate using a specific sandwich ELISA as previously described (18). IFN-γ concentrations were measured by ELISA (CMB, Leiden, The Netherlands) sensitive above 0.5 pg/ml. Allergen- or PPD-induced cytokine production was calculated by subtracting cytokine concentrations in control cultures (T cells and APC in the absence of antigen) from concentrations measured in *D. pteronyssinus*- or PPD-stimulated cultures.

Statistics

All statistical comparisons were made using Student's *t* test. Correlations were evaluated using Pearson's test. All tests were performed with the aid of a commercial software package (Minitab, State College, PA) with $p < 0.05$ being considered significant in all cases.

RESULTS

Cellular Recruitment After Allergen Challenge

Absolute total and differential cell counts in BAL fluid 24 h after challenge of asthmatics and normal control subjects with allergen or saline control in remote bronchial segments are

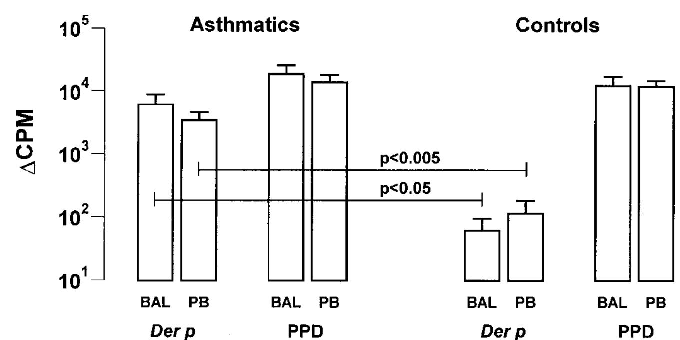

Figure 2. Proliferative responses of bronchoalveolar lavage (BAL) and peripheral blood (PB) T cells harvested 24 h after segmental allergen challenge of atopic asthmatics and nonatopic control subjects to 10 μg/ml *D. pteronyssinus* (*Der p*) and 10 μg/ml *M. tuberculosis* purified protein derivative (PPD). Δcpm is specific antigen-induced proliferation (counts per minute of cultures containing antigen minus counts per minute of cultures without antigen [background]).

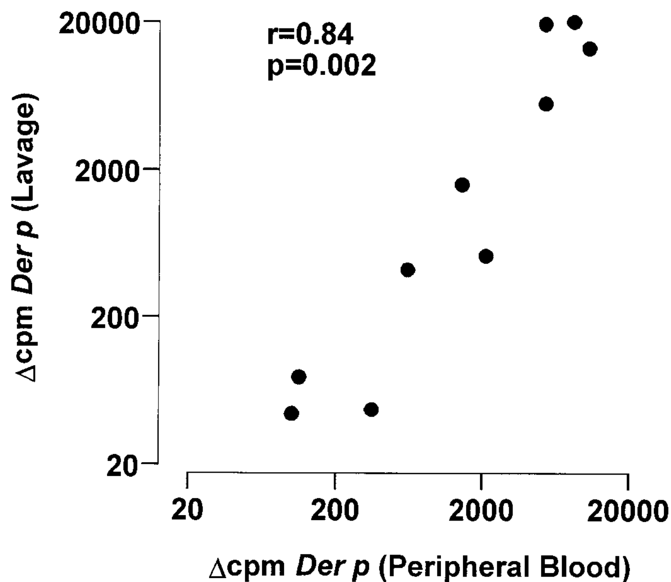

Figure 3. Correlation (Pearson's) between the proliferative responses of BAL and peripheral blood T-cells (obtained 24 h after allergen challenge) to 10 μg/ml *D. pteronyssinus* in the asthmatic subjects.

shown in Figure 1. Allergen, as compared with saline control challenge of the asthmatics, was associated with significant elevations of the total numbers of cells recovered, and the absolute numbers of lymphocytes, eosinophils, and neutrophils ($p < 0.05$ in each case). In contrast, challenge of the nonatopic control subjects with allergen as compared with saline was not associated with significant changes in total or differential absolute cell numbers in BAL fluid.

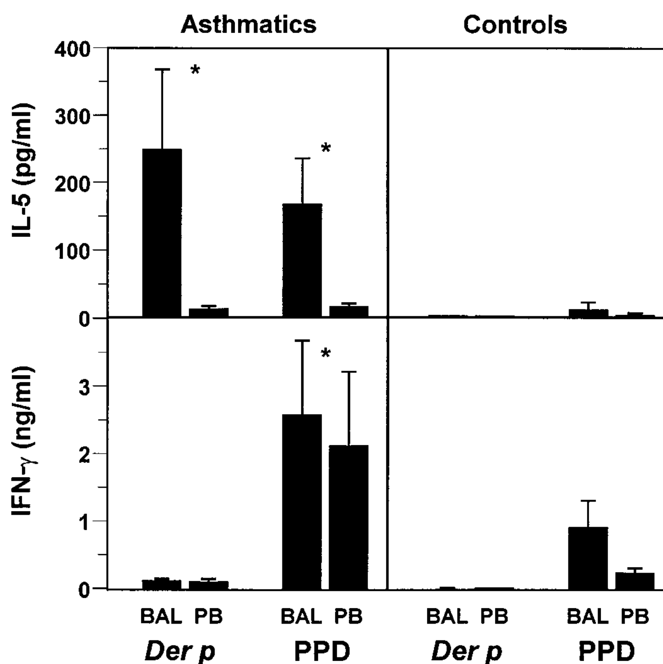

Figure 4. D. pteronyssinus (*Der p*) and PPD-induced IL-5 and IFN-γ production (mean, SEM) by fixed numbers of BAL and PB T cells harvested 24 h after segmental allergen challenge of atopic asthmatics (n = 7) and nonatopic control subjects (n = 5). *Asterisks* indicate $p < 0.05$.

Allergen-induced, but not PPD-induced, Proliferation of Bronchoalveolar Lavage and Peripheral Blood T-cells Is Elevated in Asthmatics as Compared with Control Subjects

D. pteronyssinus-induced proliferation of BAL T cells harvested 24 h after bronchial segmental allergen challenge was significantly elevated in asthmatics as compared with equivalent numbers of cells from nonatopic control subjects (mean ± SEM Δcpm proliferation in asthmatics and control subjects was 6,127 ± 2,627 and 61.5 ± 34, respectively, $p < 0.05$) (Figure 2). Similarly, allergen-induced proliferation of equivalent numbers of peripheral blood T cells obtained simultaneously was elevated in asthmatics as compared with control subjects (3,436 ± 1,192 and 112 ± 67, respectively, $p < 0.005$) (Figure 2). In contrast, PPD-induced proliferation of equivalent numbers of BAL and peripheral blood T cells (Figure 2) was not statistically significantly different in the asthmatic subjects (Δcpm = 18,657 ± 7,053 and 13,752 ± 4,362, respectively) and the nonatopic control subjects (Δcpm = 12,294 ± 4,819 and 11,900 ± 2,839, respectively). In the asthmatic subjects, the *D. pteronyssinus*-induced proliferative responses of equivalent numbers of T cells obtained from the bronchial lumen and the peripheral blood after allergen challenge were statistically equivalent ($p = 0.16$) and, furthermore, showed a tight positive linear correlation ($r = 0.84$, $p = 0.002$) (Figure 3).

IL-5 and IFN-γ Concentrations in *D. pteronyssinus*- and PPD-stimulated BAL and PB T-cell Culture Supernatants

IL-5 and IFN-γ concentrations were measured in culture supernatants of equivalent numbers of BAL and PB T cells isolated simultaneously 24 h after allergen challenge of seven asthmatics and five control subjects, and stimulated with *D. pteronyssinus* or PPD (10 μg/ml in each case). In four of the subjects (three asthmatics and one control), T cell numbers recovered from the BAL fluid were insufficient to allow these studies to be performed. In the asthmatics, mean allergen-induced IL-5 production by BAL T cells was elevated approximately 10-fold compared with that of PB T cells ($p < 0.05$) (Figure 4) despite the fact that allergen-induced proliferation in each compartment was statistically equivalent (Figure 2). In the normal control subjects, allergen-induced IL-5 production by both BAL and PB T cells was close to or below the limit of detection of the assay. Relatively little IFN-γ was produced by allergen-stimulated BAL or PB T cells from both the asthmatics and

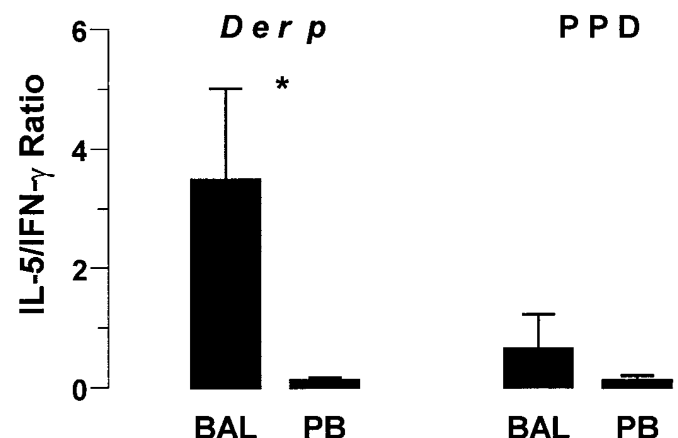

Figure 5. Ratio of IL-5/IFN-γ production (mean, SEM) by fixed numbers of BAL and PB T cells harvested 24 h after segmental allergen challenge of atopic asthmatics (n = 7) in response to *D. pteronyssinus* (*Der p*) and PPD stimulation. *Asterisk* indicates $p < 0.05$.

TABLE 2

CORRELATIONS (PEARSON'S CORRELATION COEFFICIENT AND PROBABILITY) BETWEEN *Der p*- AND PPD-INDUCED T-CELL PROLIFERATION, IL-5 PRODUCTION AND EOSINOPHIL RECRUITMENT AFTER SEGMENTAL ALLERGEN CHALLENGE OF ATOPIC ASTHMATICS

	Antigen	Proliferation versus BAL Eosinophils (%)	IL-5 Production versus BAL Eosinophils* (%)
BAL T cells	*Der p*	$p = 0.005$ ($r = 0.80$)	$p = 0.01$ ($r = 0.87$)
PB T cells	*Der p*	$p = 0.002$ ($r = 0.84$)	NS
BAL T cells	PPD	NS	NS
PB T cells	PPD	NS	NS

* Similar correlations were not possible in the control subjects since IL-5 production was low or undetectable. NS = not significant.

the control subjects (Figure 4). In the asthmatics, the mean ratio of IL-5/IFN-γ production in response to allergen stimulation was elevated approximately 30-fold ($p < 0.05$) (Figure 5) in BAL, as compared with PB T cells. With PPD stimulation, IL-5 production was also significantly elevated in BAL, as compared with PB T cells ($p < 0.05$) in the asthmatics but negligible in both compartments in the control subjects (Figure 4). In contrast to the situation with allergen stimulation, however, this IL-5 production was seen in the context of elevated IFN-γ production, abundantly produced by BAL and PB T cells from both the asthmatics and the control subjects (Figure 4), resulting in a low mean ratio of IL-5/IFN-γ production (Figure 5).

Correlations between T-cell Activation and Inflammatory Cell Recruitment

In order to determine whether BAL T-cell proliferation and IL-5 production in response to *D. pteronyssinus* in the asthmatics could be related to the *in vivo* response to allergen challenge, possible relationships between these measurements and inflammatory cell recruitment were examined. In the asthmatics, the percentages of eosinophils present in BAL fluid 24 h after allergen challenge correlated positively with *D. pteronyssinus*-induced proliferation of, and IL-5 production by, BAL T-cells ($r = 0.80$, $p = 0.005$; $r = 0.87$, $p = 0.01$, respectively) (Table 2). Concordant with the statistically equivalent proliferation of, but disparate production of IL-5 by, allergen-stimulated BAL and PB T cells in the asthmatics, PB proliferation, but not IL-5 production, also correlated with the percentages of eosinophils in BAL fluid after allergen challenge (Table 2). In contrast, PPD-induced proliferation and IL-5 production in BAL T cells did not correlate with eosinophil recruitment (Table 2). Significant correlation between T-cell proliferation and IL-5 production and recruitment of other cell types was not observed.

DISCUSSION

This study represents the first attempt to characterize the functional properties of allergen-specific T cells freshly isolated from human asthmatic airways, and the first demonstration that allergen-specific T cells can be identified in the bronchial lumen of atopic asthmatics in association with allergen challenge. Although previous studies have addressed the properties of T-cell clones and lines expanded from bronchial biopsies or BAL (12, 19–21), these all involved nonspecific expansion and selection steps that make it difficult to relate the characteristics of these cells, particularly in terms of cytokine production, to those of the starting population. In contrast, we have been able to show that it is possible to perform functional studies on relatively small numbers of BAL T-cells without prior expansion or artificial stimulation *in vitro*.

Segmental bronchial challenge of sensitized atopic asthmatics, but not control subjects, with allergen, as compared with diluent control was associated with a significant influx, 24 h later, of lymphocytes, eosinophils and neutrophils into the lumen of the airways (Figure 1). We compared the allergen-induced proliferative responses of fixed numbers of T cells harvested simultaneously from the bronchial lumen (i.e., the site of the disease) and the peripheral blood 24 h after allergen challenge in the asthmatics and control subjects. It was originally our intention to extend and further refine these studies by measuring, using limiting dilution analysis, the frequencies of allergen-specific T cells in the bronchial lumen and peripheral blood of the asthmatics after both allergen and saline challenge, but the limited recovery of T cells from the bronchial lumen, particularly after saline challenge, precluded such comparisons being made in a significant number of the subjects.

Our data did, nevertheless, clearly demonstrate significantly elevated allergen-induced proliferation of T cells from both the bronchial lumen and the peripheral blood of sensitized atopic asthmatics as compared with nonatopic control subjects (Figure 2). This was not a generalized property of T cells in asthmatic subjects since proliferation of T cells in response to the nonallergen recall antigen *M. tuberculosis* PPD was statistically equivalent in both compartments in both the asthmatics and the control subjects. Even without the added benefit of limiting dilution analysis, the most obvious interpretation of this elevated T-cell proliferative response to allergen is that it reflects an increased frequency of allergen-specific T cells. If interpreted in this way, these data confirm previous reports suggesting an elevated frequency of allergen-specific cells in the peripheral blood of asthmatics as compared with normal control subjects (22, 23) but uniquely and importantly extend these observations to show that this is also true of the target organ. This observation lends support to the hypothesis that at least some of the T cells in the bronchial lumen responsible for elevated IL-5 synthesis in association with allergen challenge (9–11) are indeed allergen-specific. The physiologic significance of these data is further underlined by the fact that, in the asthmatics, allergen-induced proliferation and IL-5 production by bronchial luminal T cells recovered after allergen challenge correlated with eosinophil influx into the airways (Table 2). One interpretation of these data is that products of allergen-activated T cells, including IL-5, may be implicated in eosinophil recruitment to the airways after allergen exposure. This is in accord with previous, less direct studies (22, 24) showing that the magnitude of late-phase bronchoconstrictor responses after aerosolized allergen challenge of the entire bronchial tree can be correlated with peripheral blood T-cell allergen reactivity. These global physiologic processes are more difficult to interpret in a segmental endobronchial challenge model such as that employed the present study. Although some of the asthmatics did show global bronchoconstriction after segmental allergen challenge (as measured by depression of FEV_1 in the 24 h period after challenge), the regular administration of inhaled β_2-agonists to both the asthmatics and the control subjects after challenge precluded any systematic analysis of the magnitude of these responses. This is a general limitation of all segmental allergen challenge studies. Finally, although segmental allergen challenge of the asthmatics was associated with an influx of T cells into the bronchial lumen, we found no evidence for elevated allergen-specific proliferation by these bronchial luminal cells as compared with simultaneously isolated peripheral blood T cells. Indeed, these

two measurements correlated closely in the asthmatics (Figure 3). Although again these observations might have been further clarified by limiting dilution analysis, they do not appear to support the hypothesis that allergen bronchial challenge is associated with selective accumulation of allergen-specific T cells in the airways.

We lastly showed that allergen-driven IL-5 production by T cells isolated from the bronchial lumen of atopic asthmatics 24 h after allergen challenge was significantly elevated as compared with equivalent numbers of T cells isolated simultaneously from the peripheral blood (Figure 4). This difference was approximately 10-fold and is very unlikely to have arisen from any significant variability in the CD4/8 T-cell subset ratios in the respective compartments (25). Paucity of cells precluded systematic phenotypic analysis. Because the proliferative responses of T cells from each of these compartments to allergen stimulation were statistically equivalent, the most likely interpretation of these data is that allergen-specific T cells within the bronchial mucosa are predisposed, as a population, to produce elevated quantities of IL-5 as compared with those in the peripheral blood. We similarly reported elevated IL-5 production by both CD4 and CD8 T-cell lines isolated from the BAL fluid of atopic asthmatics using a polyclonal (anti-CD3) stimulus as compared with both atopic and nonatopic nonasthmatic control subjects (19). We propose two hypotheses to explain this observation that are not mutually exclusive. First, allergen-specific T cells may be "primed" for enhanced IL-5 production during their passage from the peripheral blood into the bronchial lumen. Cytokines such as IL-4, which promote "Th2-type" development of T cells and which may be released locally, for example, by mast cells (26, 27) or basophils (28) in an allergen challenge situation, may be at least partly responsible for this. Second, elevated IL-5 production by allergen-specific T cells within the bronchial lumen might reflect selective ingress of these cells into the lumen according to criteria other than their antigen specificity. Consistent with this, in a recent study it was reported that in atopic asthmatics, elevated percentages of T cells expressing intracellular IFN-γ were found in BAL versus PB after PMA/ionomycin stimulation *in vitro* (29). Further experiments would be necessary to investigate these hypotheses systematically. It is of interest that PPD stimulation of BAL T cells in the asthmatics resulted in the production of IL-5, although in contrast to allergen this was in the context of much greater IFN-γ production (compare IL-5/IFN-γ ratios in Figure 5). We speculate that this occurred as a result of nonspecific bystander effects exerted on activated allergen-specific T cells: the ability of IL-2 to induce IL-5 secretion by activated T cells has previously been described (30), and therefore it seems plausible that endogenous IL-2 produced by PPD-specific T cells may have induced IL-5 secretion by CD25+ *D. pteronyssinus*-stimulated BAL T cells within the same culture. It should be noted that the absolute quantities of IL-5 and IFN-γ produced in response to allergen and PPD stimulation are not directly comparable since the relative frequencies of T cells responsive to these particular antigens in individual subjects are unknown.

In this study, five of the 10 asthmatics had been receiving inhaled glucocorticoids, which were discontinued a minimum of 2 wk prior to its commencement. To investigate possible prolonged effects of this therapy on the allergen-challenge process, we compared allergen-induced BAL eosinophil recruitment and BAL T-cell proliferation and IL-5 production in the asthmatics who had and had not been receiving inhaled glucocorticoids, but we were unable to demonstrate statistically significant differences in any of these measurements in the two groups. With the proviso, therefore, that the numbers of patients involved were small we conclude that prior inhaled glucocorticoid therapy was not a confounding factor in this study.

Because in this study we have compared atopic asthmatics with nonatopic control subjects, the possibility arises that some of our observed phenomena reflect the atopic diathesis per se rather than the atopic asthma specifically. We have previously shown that peripheral blood T cells from asymptomatic atopic subjects sensitized to *D. pteronyssinus* exhibit low allergen-induced proliferation and IL-5 production that is no higher than that observed in nonatopic control subjects, whereas both measurements are clearly elevated in atopic asthmatics (31). We have also shown that the numbers of cells expressing IL-5 messenger RNA in the bronchial mucosa of both atopic and nonatopic, nonasthmatic subjects are low and statistically equivalent, whereas again IL-5 expression is clearly elevated in both atopic and nonatopic asthmatics (32). In view of these observations, and the close correspondence of allergen-induced proliferation of PB and BAL T cells at least in the asthmatics in the present study, we would hypothesize that PB and BAL T cells from atopic, nonasthmatic subjects show baseline and allergen-induced IL-5 expression equivalent to that of nonatopic control subjects. Nevertheless, this cannot be assumed, and in the absence of additional data relating to allergen-induced IL-5 production by PB and BAL T cells from atopic nonasthmatics, we cannot exclude the possibility that our findings relate to atopy rather than specifically to atopic asthma. Further studies are warranted to explore the role of the atopic diathesis in contributing to allergen-induced bronchial inflammation.

In summary, these observations provide fundamental evidence for a role for allergen-specific T cells and their cytokine products in the pathogenesis of allergen-induced bronchial inflammation in asthma.

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