

IL-4 and IL-5 mRNA expression in induced sputum of asthmatic subjects: Comparison with bronchial wash

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Background: The local production of T_H2 -type cytokines is thought to orchestrate the ongoing eosinophilic inflammation and contribute to the pathophysiologic features of allergic asthma. Previous studies investigating cytokine expression in asthmatic individuals have used invasive fiberoptic bronchoscopy techniques. To date, there have been no reports of cytokine mRNA expression in induced sputum as a means of quantifying local inflammatory events.

Objectives: We examined whether IL-4, IL-5, and IFN- γ mRNA expression could be detected in cells from induced sputum in subjects with mild asthma and normal control subjects. In addition, we compared the profile of inflammatory cells and cytokine mRNA in sputum and bronchial wash fluid.

Methods: Cells positive for IL-4, IL-5, and IFN- γ mRNA were determined by using in situ hybridization on cytospun aliquots of sputum induced by successive inhalations of hypertonic saline. Inflammatory cells were quantified by using immunologic cell surface markers and immunocytochemistry.

Results: IL-4 and IL-5 mRNA were detected in the sputum of all asthmatic subjects, and the number of cells expressing these cytokines was significantly higher than that found in control subjects. Colocalization studies showed CD3-positive T cells were the major sources of IL-4 and IL-5 mRNA.

Conclusions: This study demonstrates that induced sputum can be used to detect mRNA for T_H2 -type cytokines in bronchial asthma and that the increase in IL-4 and IL-5 mRNA expression is similar to that seen with more invasive techniques. The qualitative differences in inflammatory cell numbers between sputum induction and bronchial wash are consistent with their sampling of different airway compartments. (*J Allergy Clin Immunol* 1999;103:238-45.)

Key words: Sputum, IL-4, IL-5, bronchial wash, airways inflammation, lymphocytes, eosinophils

Cytokines are important immunomodulatory agents that regulate the inflammatory reaction occurring within

Abbreviations used

APAAP:	Alkaline phosphatase anti-alkaline phosphatase
BAL:	Bronchoalveolar lavage
ICC:	Immunocytochemistry
ISH:	In situ hybridization
MBP:	Major basic protein

the asthmatic airway.^{1,2} T_H2 -type cytokines, including IL-4 and IL-5, have been implicated in the pathogenesis of asthma by virtue of their ability to promote IgE synthesis³ and to direct the differentiation, recruitment, and activation of eosinophils.⁴ It has previously been established that there is an elevated expression of T_H2 -type cytokines (mRNA and immunoreactivity) in inflammatory cells from bronchial wash and bronchoalveolar lavage (BAL) fluid and in bronchial biopsy specimens from asthmatic individuals.⁵⁻⁷ However, these techniques are relatively invasive because of the requirement for intubation of the airways. Thus sputum induction by hypertonic saline⁷⁻¹⁰ has been introduced as a means of facilitating investigations into the histopathology of lung inflammation.

Studies examining various inflammatory cells (particularly eosinophils) recovered from induced sputum have demonstrated that this methodology samples a distinct compartment of the airways when compared with bronchial wash, BAL, and bronchial biopsy.^{7,11} Although IL-5 mRNA and its immunoreactive protein have been detected in induced sputum by using PCR¹² and an enzyme-linked immunoassay,^{10,13} it is unclear whether inflammatory cells recovered from sputum induction of asthmatic subjects have a higher expression of T_H2 -type cytokines compared with those recovered from nonasthmatic subjects. Therefore the principal objective of this study was to determine whether expression of cytokine mRNA could be measured in induced sputum of asthmatic subjects by using in situ hybridization (ISH) to detect cell-associated mRNA sequences. Immunohistochemistry for cytokine-immunoreactive protein was performed to ensure translation of the mRNA. We also compared the expression of cytokine mRNA in induced sputum with that of bronchial wash fluid to determine whether induced sputum could be substituted for bronchial wash fluid to monitor cytokine expression in asthmatic airways. Bronchial wash was used as a comparison rather than BAL because it has been found that the constituents of induced sputum more closely resemble those in bronchial

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Supported by the J. T. Costello Memorial Research Fund, the Montreal Chest Research Institute, and Inspiraplex. Dr Hamid is a recipient of a Chercheur-Boursier award from the Fonds de la Recherche en Sante du Quebec. Dr Eleanor Minshall is a recipient of a Medical Research Council/Canadian Lung Association Fellowship.

Received for publication July 24, 1998; revised Sept 15, 1998; accepted for publication Sept 15, 1998.

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0091-6749/99 \$8.00 + 0 1/1/94552

wash fluid than in BAL fluid, likely because of less admixture with alveolar-lining fluid.^{7,14}

METHODS

Subjects

Thirteen patients fulfilling the American Thoracic Society criteria for asthma,¹⁵ with documented airways reversibility to inhaled β_2 -agonists and an increased airways responsiveness to methacholine ($PC_{20} < 8$ mg/mL), were recruited from the Asthma Clinic at the Montreal Chest Hospital, McGill University. These patients were classified as having mild-to-moderate asthma and had their symptoms controlled by regular use of inhaled selective β_2 -agonists. All patients were atopic on the basis of skin test reactivity to a series of common allergens, and their clinical characteristics are given in Table I. Subjects were nonsmokers or exsmokers who had stopped smoking for at least 12 months and had smoked less than 5 pack-years in their lifetime. Subjects using inhaled or oral corticosteroids, nonsteroidal anti-inflammatory medications (cromolyn, ketotifen), theophylline, long-acting β_2 -agonists, leukotriene antagonists, or anti-histamines within the 3 months before the study were excluded. None of the subjects had a history of respiratory tract infection within the previous 6 weeks or immunotherapy within the previous 12 months. As control subjects, 10 nonasthmatic nonatopic individuals volunteered to participate in the study. None of these control subjects were current smokers or had any evidence of a recent respiratory tract infection.

Study design

The study was approved by the Ethics Committee of the Montreal Chest Hospital, and all subjects gave written informed consent. The patients and control subjects were screened by a questionnaire, spirometry, and a methacholine inhalation test¹⁶ (if $FEV_1 > 70\%$). Allergy skin prick tests were performed, and atopy was defined as a greater than 3-mm skin wheal response to 1 or more of 13 common allergens. Blood was drawn for complete blood count and differential, total serum IgE, and coagulation studies. Sputum was induced in the laboratory at least 48 hours after methacholine bronchoprovocation.

In 6 asthmatic subjects (4 men; mean percent predicted FEV_1 , 84.2%) sputum induction and fiberoptic bronchoscopy were performed in randomized order at least 96 hours, but not more than 7 days, apart. Methacholine inhalation tests preceded randomization by at least 48 hours. The difference in FEV_1 on the 2 test days did not exceed 10%.

Fiberoptic bronchoscopy

Fiberoptic bronchoscopy was performed according to recommended guidelines.¹⁷ Subjects were premedicated with nebulized salbutamol (2.5 mg) 10 to 15 minutes before the procedure. Bronchial wash was performed by gently wedging the bronchoscope in the right middle lobe, rapidly instilling 50 mL of saline into a single subsegment, and then aspirating a mean volume of 30 mL. The bronchial wash fluid was transferred into sterile polypropylene tubes and centrifuged at 300g for 7 minutes at 4°C. The cell pellet was then suspended in RPMI 1640 medium at a concentration of 1×10^6 cells/mL, and cytospin preparations of the bronchial wash fluid (100 μ L aliquots) were made. The cells were cytospun onto either poly-L-lysine-coated (for ISH) or untreated slides (for immunocytochemistry [ICC]). For ISH, the cytopins were fixed in 4% freshly prepared paraformaldehyde, washed twice in diethyl pyrocarbonate-treated PBS, baked overnight at 37°C, and stored at -80°C until further use. For ICC, the cytopins were briefly fixed in a solution of acetone:methanol (40:60), air dried, and stored at -80°C.

TABLE I. Clinical characteristics of asthmatic patients and control subjects used in this study

	Asthmatic subjects	Normal control subjects
N	13	10
Age (y)		
Mean	31.2	33.8
Range	19-60	24-52
Sex (M:F)	6:7	4:6
FEV_1 (% predicted)		
Mean	95	103.2
Range	70-128	93-119
PC_{20} (mg/mL)		
Median	2.0	>16
Range	0.16-6.0	—
Total serum IgE (U/mL)		
Median	69.2	36.2
Range	<32.7-4240	<32.7-98.5
Eosinophils ($\times 10^6$ cells/L)		
Mean	216	70
Range	45-570	42-128

Sputum induction

Sputum induction in normal and asthmatic individuals was performed with an aerosol of hypertonic saline and a modification of the method of Pin et al.⁹ Salbutamol (200 μ g) was inhaled to inhibit possible bronchoconstriction during sputum induction. Increasing concentrations (3%, 4%, and 5%) of hypertonic saline generated by a DeVilbiss ultrasonic nebulizer (Ultra-Neb 99; Sunrise Medical, Somerset, Pa) were administered. The procedure was interrupted every 2 minutes to measure PEF. Subjects were asked to rinse the mouth and blow the nose to minimize contamination with saliva and postnasal drip. They were also instructed to cough sputum into a sterile container when they felt that sputum might be present. These procedures were repeated sequentially for 8-minute periods at each concentration unless a fall in PEF of greater than 10% occurred, in which case the procedure was terminated.

Sputum examination

The volumes of the sputum samples were recorded. All portions of sputum that were macroscopically free of salivary contamination were selected¹⁸ to minimize squamous cell contamination and processed within 2 hours by a modification of the technique described by Pizzichini et al.¹⁰ Briefly, sputum was treated by adding 2 mL of HBSS containing 0.1% dithiothreitol, vortexed for 2 to 3 minutes to homogenize the sample, and then 2 mL of PBS was added to stop the action of dithiothreitol. The suspension was centrifuged at 300g for 10 minutes. The cell pellet was resuspended in PBS, and a total cell count of leukocytes and cell viability were determined. For ISH, the cell suspension was placed into cups of a Shandon II cytocentrifuge (Shandon Somerset, Cheshire, England), and coded cytopins were air dried and fixed in 4% paraformaldehyde for 30 minutes, washed twice for 5 minutes with PBS, kept at 37°C overnight, and then stored at -80°C until further use. For ICC, the cytopins were briefly fixed in a solution of acetone:methanol (40:60), air dried, and stored at -80°C.

ISH

To detect IFN- γ , IL-4, and IL-5 mRNA in cytopins of induced sputum and cells from bronchial wash fluid, radiolabeled comple-

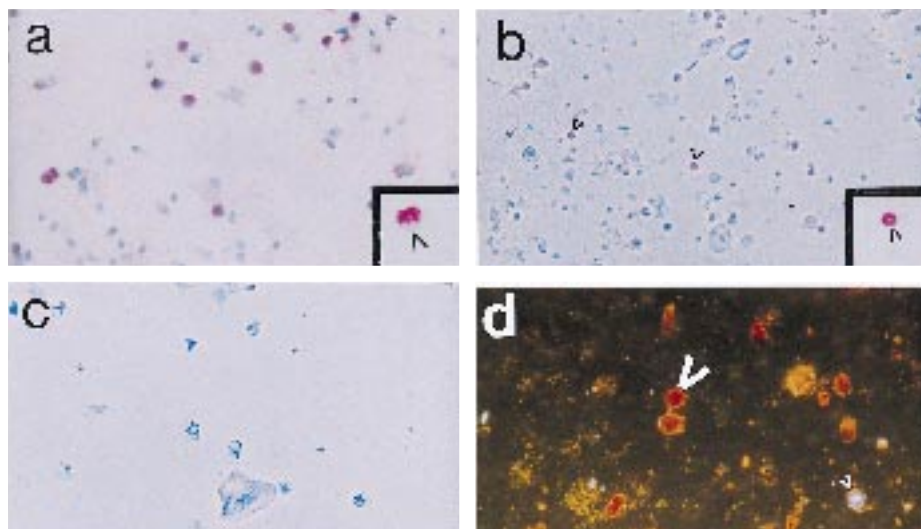


FIG 1. Representative examples of radiolabeled ISH and APAAP ICC of cells recovered by induced sputum. Red cytoplasmic staining in panels **A** and **B** indicates immunolocalization of MBP- and CD3-positive cells, respectively, in an asthmatic subject (*arrows*). *Inserts* at a higher magnification show the typical morphology of eosinophils and lymphocytes, respectively. **C** demonstrates the paucity of MBP-positive cells in induced sputum from a normal control subject. Also shown in panel **D** are the results of combined ISH and ICC for IL-5 mRNA and CD3 immunoreactivity in cells recovered by sputum induction in an asthmatic patient. By using dark field illumination, cells positive for only CD3 were observed as having dense silver grains (*small arrow*), whereas those CD3-positive cells expressing IL-5 mRNA are seen as collections of yellow granules overlaid by red cytoplasmic staining (*large arrow*).

mentary riboprobes (cRNA antisense) were used. The cDNA sequences for IFN- γ , IL-4, and IL-5 were inserted into pGEM vectors, grown in *Escherichia coli*, and linearized with the appropriate enzymes. Before ISH, *in vitro* transcription to generate sense and antisense probes was performed in the presence of ^{35}S -uridine triphosphate (UTP) and the appropriate T7 or SP6 polymerases as previously reported.¹⁹ Cytospins were permeabilized, treated with acetic anhydride and triethanolamine to reduce the nonspecific binding, and prehybridized in formamide at 42°C. ^{35}S -labeled antisense probes were used (10^6 cpm/section), followed by high-stringency posthybridization washings. Unhybridized single-stranded RNA was removed by treating the preparations with a solution containing RNase. After posthybridization, the slides were immersed in emulsion fluid and exposed for 10 to 14 days. The autoradiograms were developed, fixed, and counterstained with hematoxylin. To ensure ISH specificity, sections and cytopins were hybridized with the sense probe or treated with an RNase solution. The specificity of all probes had already been established in previous experiments with Northern blot analysis and ISH.

ICC

To detect cytokine immunoreactivity recovered by sputum induction and bronchial wash, we used specific primary mAbs for IL-4 (Genzyme, Cambridge, Mass) and IL-5 (5A5; a kind gift of Dr Jan Tanvernier, Ghent, Belgium). To ascertain the phenotype of inflammatory cells, immunohistochemical markers for eosinophils (major basic protein [MBP]), T lymphocytes (CD3), B lymphocytes (CD20), neutrophils (elastase), and macrophages (CD68) were used. ICC was performed by using a modified alkaline phosphatase anti-alkaline phosphatase (APAAP) method, on which we have previously reported.²⁰ Briefly, cytopins were incubated with the specific antibodies overnight at 4°C. After washing in tris-buffered saline, the secondary antibodies were applied and the slides were washed in tris-buffered saline and incubated with the APAAP complex. The reaction was visualized by alkaline-phosphatase substrate

added to fast red. All the preparations were counterstained with hematoxylin and mounted before examination.

Combined ICC/ISH

To detect the phenotype of cells expressing IL-4 and IL-5 mRNA in cytopins from induced sputum and bronchial wash fluid, the technique of combined ICC/ISH was used as previously described.²¹ Briefly, cytopins on poly-L-lysine-coated slides were immunostained with mAbs directed against T lymphocytes or eosinophils. After visualization of the positive immunostaining with the APAAP technique, the cytopins underwent ISH for IL-4 and IL-5 mRNA detection.

Data analysis

Sputum samples containing less than 20% of contaminating squamous cells were considered suitable for analysis. The numbers of positive cells expressing IL-4 or IL-5 mRNA or markers of cellular phenotype were counted per 1000 total cells, excluding squamous cells, with the help of a phase-contrast lens. Cells recovered during the bronchial wash were similarly analyzed. To avoid observer bias, slides were coded before analysis and read in a blind fashion. At least 2 cytopins were counted for each cytokine mRNA or immunocytochemical marker, and the mean value of these slides was reported. Significant differences were detected by ANOVA and Wilcoxon signed-rank test. Significance was accepted at the level of 95%. Correlation coefficients were determined by using Pearson's product-moment analysis and subsequent post hoc Bonferroni multiple comparison tests to determine whether the correlation was statistically significant.

RESULTS

Fig 1, A and B show representative examples of APAAP immunostaining for MBP and CD3 in an asth-

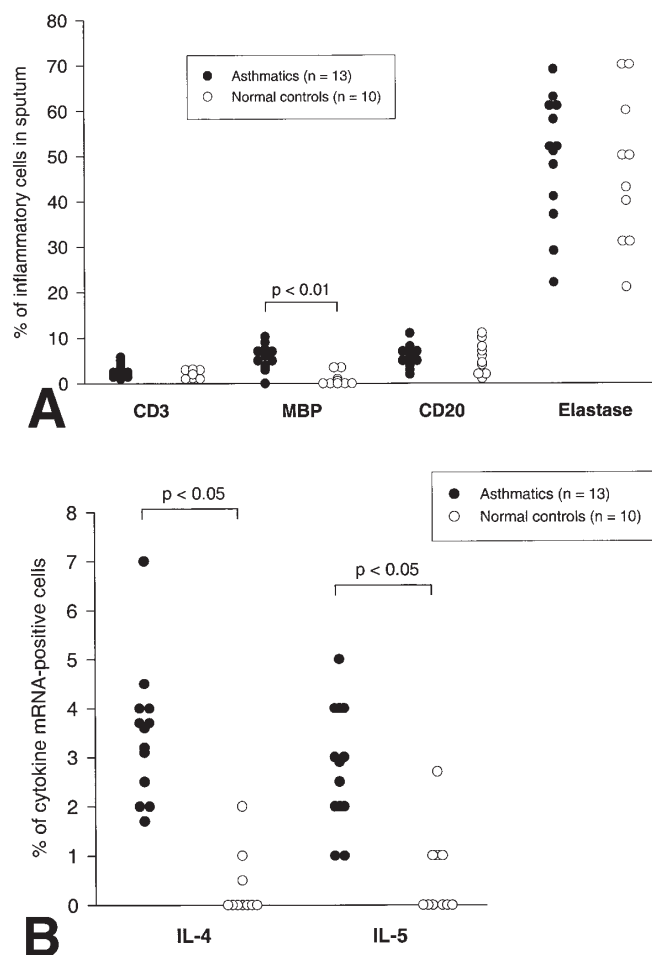


FIG 2. Percentage of cells expressing cytokine mRNA and inflammatory cell markers in induced sputum from asthmatic and control individuals. **A** shows the percentage of T lymphocytes (*CD3*), eosinophils (*MBP*), B lymphocytes (*CD20*), and neutrophils (*elastase*). There was a significant increase in the percentage of MBP-positive cells in sputum from asthmatic subjects compared with normal control subjects ($P < .05$). **B** shows increased numbers of IL-4 and IL-5 mRNA cells in asthmatic subjects compared with normal control subjects ($P < .05$).

matic individual. By using fast red to visualize the signal, positive immunoreactivity can be seen as red cytoplasmic staining, which is more evident on higher magnification (inserts in Fig 1, A and B). Occasionally, MBP-positive cells were seen in some control subjects (Fig 1, C). There was no specific immunostaining when the primary antibody was omitted or when an irrelevant secondary antibody was used. By using the technique of ISH, positive signals for IL-4 and IL-5 mRNA were observed as specific deposits of silver grains in emulsion fluid overlying individual cells. Under conditions of combined ISH and ICC, these silver grains appeared yellow because of the concomitant positive immunocytochemical signals (Fig 1, D). There were no significant hybridization signals observed when sections were treated with RNase before hybridization or when sections were treated with a sense probe identical to the cytokine mRNA.

Quantification of inflammatory cells in induced sputum

Of the cell types investigated with immunocytochemical markers, neutrophils (elastase positive) comprised the majority of cells (approximately 50% of the total) recovered by sputum induction in both asthmatic and nonasthmatic subjects. However, significant differences between these 2 groups were only observed in the percentages of MBP-positive cells (mean $\pm$ SEM, asthmatic subjects, $5.67\% \pm 0.65\%$; control subjects, $0.58\% \pm 0.32\%$; $P < .01$; Fig 2, A). There was no difference between asthmatic subjects and normal control subjects in the percentage of CD3-, CD20-, or elastase-positive cells ($P > .05$). There was also no significant difference between the percentages of CD68-positive cells in asthmatic subjects versus nonasthmatic control subjects (asthmatic subjects, $31.5\% \pm 3.1\%$; control subjects, $36.1\% \pm 3.6\%$; $P = .37$).

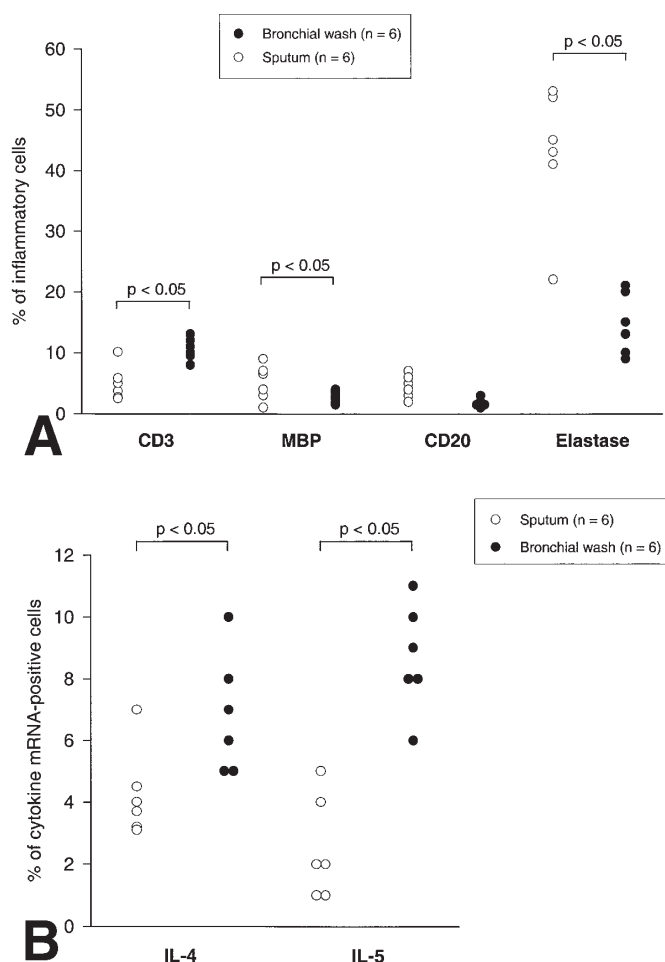


FIG 3. Percentage of cells expressing cytokine mRNA and inflammatory cell markers in induced sputum and bronchial wash fluid from 6 asthmatic individuals. Percentage of T lymphocytes (*CD3*), eosinophils (*MBP*), B lymphocytes (*CD20*), and neutrophils (*elastase*) is shown in **A**. There was a significant increase in the percentage of MBP-positive cells in sputum from asthmatic subjects compared with bronchial wash fluid ($P < .05$). Numbers of CD3+ T cells and neutrophils were significantly increased in bronchial wash fluid of asthmatic airways compared with induced sputum ($P < .05$). **B** shows increased numbers of IL-4 and IL-5 mRNA cells in bronchial wash fluid compared with induced sputum ($P < .05$).

Cytokine mRNA in induced sputum

By using ISH, cells expressing IL-4 and IL-5 mRNA were detected in all samples of induced sputum from asthmatic subjects but only in approximately 50% of induced sputum samples from control subjects (Fig 2, *B*). There was a significant increase in the numbers of IL-4 mRNA-positive cells in the induced sputum of asthmatic subjects compared with nonasthmatic control subjects (asthmatic subjects, $3.46\% \pm 0.38\%$; control subjects, $0.35\% \pm 0.21\%$; $P < .05$). Similarly, there was a significant increase in the percentage of cells expressing IL-5 mRNA in asthmatic subjects compared with control subjects (asthmatic subjects, $2.8\% \pm 0.34\%$; control subjects, $0.42\% \pm 0.27\%$; $P < .05$). There were very few cells expressing IFN- γ mRNA in the sputum samples of asthmatic subjects and nonasthmatic control subjects, and

there was no significant difference in cell numbers between the 2 groups (asthmatic subjects, $0.05\% \pm 0.03\%$; control subjects, $0.04\% \pm 0.02\%$; $P = .98$). There was a significant correlation between the percentage of CD3-positive cells and the percentage of cells expressing IL-4 mRNA ($r = 0.75$, $P < .01$). A positive correlation between the percentage of cells expressing MBP and the percentage of cells expressing IL-5 mRNA in induced sputum was also found, but it did not reach statistical significance ($r = 0.52$, $P = .067$).

Cytokine immunoreactivity in induced sputum

Immunoreactivity for IL-4 and IL-5 was present in all asthmatic sputum samples examined ($n = 2$ to 3) and in 2 of the 3 nonasthmatic subjects studied. The number of cytokine-immunoreactive cells was generally lower than that observed with ISH, with approximately 3% of all

cells expressing IL-4 or IL-5 immunoreactivity in asthmatic subjects and 0.5% in control subjects (mean [range]; IL-4: asthmatic subjects, 2.8% [2.7% to 2.9%]; control subjects, 0.3% [0% to 1%]; IL-5: asthmatic subjects, 3.2% [2.5% to 3.5%]; control subjects, 0.5% [0% to 1%]).

Colocalization of cytokine mRNA expression in induced sputum

By using simultaneous ISH and ICC, 80% of IL-5 mRNA-positive cells in induced sputum from asthmatic subjects were CD3+ T lymphocytes. Of those cells expressing IL-4 mRNA, 92% were found to express the CD3+ T-lymphocyte cell marker. Only 15% of IL-5 mRNA-positive cells and 5% of IL-4 mRNA-positive cells were positive for MBP-immunoreactivity.

Comparison of inflammatory indices in induced sputum and bronchial wash

As shown in Fig 3, A, the numbers of elastase-positive and MBP-positive cells were significantly greater in induced sputum compared with bronchial wash fluid ($P < .05$). However, CD3+ lymphocytes were present in significantly higher numbers in bronchial wash fluid compared with induced sputum ($P < .05$). There was no difference, however, in the numbers of CD20-positive cells between the 2 techniques ($P > .05$).

Comparison of cytokine mRNA in induced sputum and bronchial wash fluid

IL-4 and IL-5 mRNA was detected in all samples of induced sputum and bronchial wash fluid from the 6 asthmatic individuals (Fig 3, B). The percentage of cells expressing IL-4 mRNA was significantly higher in the bronchial wash fluid compared with induced sputum in asthmatic subjects (bronchial wash, $6.8\% \pm 0.8\%$; sputum, $4.2\% \pm 0.6\%$; $P < .05$). A similar significant increase in IL-5 mRNA was also observed in the bronchial wash compared with sputum induction (bronchial wash, $8.7\% \pm 0.7\%$; sputum, $2.5\% \pm 0.7\%$; $P < .01$). There was no significant correlation between IL-4 or IL-5 mRNA expression in bronchial wash fluid compared with induced sputum.

DISCUSSION

In this study we have investigated the inflammatory characteristics of induced sputum in asthmatic subjects and have demonstrated the feasibility of detecting cytokine mRNA in inflammatory cells recovered from induced sputum with ISH. Our results in asthmatic subjects indicate that there are an increased number of eosinophils and IL-4 and IL-5 mRNA-positive cells in the cellular component of induced sputum compared with that of control subjects. By using immunohistochemistry, we also confirmed that this cytokine mRNA was translated into immunoreactive protein. The majority of these IL-4 and IL-5 mRNA-positive cells were CD3+ T lymphocytes, and their expression correlated with the presence of T cells and eosinophils, respective-

ly. When we compared bronchial wash and induced sputum as techniques to sample airways inflammation, IL-4 and IL-5 mRNA-positive cells were preferentially recovered in the bronchial wash fluid. However, there was no correlation between cytokine mRNA expression in induced sputum and bronchial wash, suggesting that these techniques sample distinct compartments of the respiratory tract.

Although previous studies have detected both IL-4 and IL-5 mRNA in bronchial biopsy specimens^{6,22} and BAL fluid^{5,23,24} of asthmatic subjects by using ISH, to date no studies have used this technique to examine cytokine mRNA expression in sputum. Nevertheless, IL-5 mRNA has been detected in sputum by virtue of the PCR.¹² However, the results of this PCR study indicated that IL-5 mRNA could only be detected in a subgroup of subjects with mild asthma and that IL-4 mRNA could not be detected at all. Cytokine immunoreactivity has also been measured in the fluid phase of induced sputum from asthmatic subjects by using an enzyme-linked immunoassay.¹⁰ Again, IL-5 was not detectable within the induced sputum in all asthmatic subjects, and furthermore, there were difficulties with the reproducibility of the IL-5 immunoreactivity measurements. Thus ISH appears to be a more sensitive technique for detection of mRNA expression in induced sputum.

One problem associated with the technique of sputum induction is that it is difficult to exclude a possible contribution of the upper respiratory tract to the presence of cytokine mRNA and immunoreactive protein in the sample. Although our methodology for examining induced sputum has been modified to minimize salivary cellular contamination,^{18,25} a variable within-subject dilutional effect on different test days caused by salivary contamination has been demonstrated in the fluid phase of the induced sputum.²⁶ Because the vast majority of cells recovered in the saliva are squamous cells,²⁷ we are reasonably confident that the cell-associated cytokine mRNA and inflammatory cell markers measured in this study are accurately reflecting the composition of sputum within the large airways.

The expression of IL-4 mRNA in induced sputum correlated with the percentage of T lymphocytes in the sputum and was colocalized mainly to CD3-positive cells in keeping with previous results from bronchial biopsies⁶ and BAL in atopic asthmatic subjects.^{5,6} Similarly, the vast majority of the IL-5 mRNA was associated with CD3+ T lymphocytes. Compared with these previous reports,^{5,6} there were actually slightly greater numbers of IL-4 and IL-5 mRNA-positive cell phenotypes for T lymphocytes in sputum, although the percentages of these cytokines expressed by eosinophils were very comparable. It is likely that these T cells produce a T_H2 -type profile of cytokine expression (IL-4 and IL-5) because there were very few IFN- γ mRNA-positive cells. Although we found a weak correlation between the numbers of IL-5 mRNA-positive cells in induced sputum and MBP-immunoreactive positive cells, a much stronger relationship has been previously reported between these

2 variables.¹⁰ One possible explanation for this is that the higher correlation reported in that study was applicable only to the 8 of 19 asthmatic subjects in whom IL-5 was detected in the sputum. It is not clear from that study why IL-5 was not detectable from induced sputum in the other asthmatic subjects despite the presence of eosinophils. A strong correlation between eosinophil numbers and IL-5 mRNA-positive cells in bronchial biopsy specimens of asthmatic subjects has been previously noted in selected individuals with severe asthma.²⁸ Nevertheless, when this relationship was examined in the BAL fluid of a variety of subjects with symptomatic and nonsymptomatic asthma, no such significant association was observed.²⁹ Thus it is possible that a number of factors in addition to IL-5 are responsible for the presence of eosinophils within the airways of atopic asthmatic subjects but that this cytokine may become more important in acute exacerbations or in more severely affected individuals.

Although it has been reported that there is a paucity of B lymphocytes within the lungs of asthmatic and normal individuals,³⁰ we detected relatively large numbers of CD20-positive B cells in the induced sputum of both groups. Large numbers of B lymphocytes are found within the upper respiratory tract, and this finding could represent contamination of our sputum samples. However, our data agree with the results of previous studies examining the expression of CD19-positive lymphocytes (B cells) in sputum.^{31,32} The presence of B lymphocytes in the upper airways may be responsible for the local production of IgA,³² and they are possibly one of the target cells for the increased IL-4 expression observed in our study. Further studies are required to address the role of B lymphocytes within the large airways of asthmatic individuals and particularly the local switching of these cells to produce allergen-specific IgE under the influence of IL-4.

We found a significant increase in the expression of IL-4 and IL-5 mRNA detected in the bronchial wash fluid compared with induced sputum. These findings may reflect the increased number of T cells found in the bronchial wash fluid that are likely to be the cellular sources of these cytokines. Although it would be attractive to substitute the technique of induced sputum for bronchial wash to evaluate cytokine expression in asthmatic subjects, we found that there was no significant correlation in cytokine mRNA expression between these 2 methodologies. The differences in the number of IL-4 and IL-5 mRNA-positive cells found between induced sputum and bronchial wash fluid are consistent with previous studies that have examined inflammatory cell numbers between these 2 techniques.^{7,33-35} Although the distinct compartments of the lungs sampled by these techniques have not been identified with certainty to date, the distribution of the bronchial wash fluid has previously been investigated with digital subtraction radiography.³⁶ These researchers found that the first aliquot of saline stayed close to the end of the bronchoscope and probably sampled the proximal airways in this vicinity. Those airways sampled by sputum induction are likely to reflect

the distribution of all the mucus-secreting cells (epithelial goblet, serous, Clara cells, and cells of the submucosal glands). Although it is assumed that the submucosal glands of the large cartilaginous airways secrete most of the mucus in human airways,³⁷ the contribution of the goblet cells found throughout the respiratory tree remains to be assessed. Our data thus support the hypothesis that specimens derived by bronchial wash and sputum induction reflect different compartments within the airways and that the sputum preferentially samples the larger airways.

In conclusion, ISH can be used to detect the expression of IL-4 and IL-5 mRNA in induced sputum of asthmatic subjects in a more consistent fashion than has been previously demonstrated with PCR. The expression of T_H2-type cytokines correlated with inflammatory cells provides further evidence supporting the relationship of IL-4 and IL-5 to airway inflammation in asthma. However, the data from our study suggests induced sputum and bronchial wash sample distinct compartments of the airways. It does not support the use of induced sputum as a direct substitute for the more invasive technique of bronchoscopy to measure cytokine expression within the airways.

We thank the Glaxo Institute for Molecular Biology, Geneva, Switzerland and Dr Colin Sanderson for their generous gifts of human cDNA for the human IL-4 and IL-5. We also thank Ms Elsa Schotman, Ms Zivart Yasrael, and the nurses at the Montreal Chest Institute for their technical assistance, and Ms Linda Karpowicz for her help in the preparation of this manuscript.

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