

Gene expression of the GATA-3 transcription factor is increased in atopic asthma

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Background: High expression of IL-5 by T cells in the airways of asthmatic individuals is believed to play a fundamental role in the eosinophilia associated with this disease. Recently, the transcription factor GATA-3 was shown to be critical for IL-5 gene expression in T_{H2} cells in vitro.

Objective: Our aim was to examine the expression of GATA-3 mRNA and its colocalization within the airways of asthmatic and nonasthmatic individuals.

Methods: We investigated the association between GATA-3 gene expression, airway inflammatory cells, and IL-5 gene expression in bronchoalveolar lavage fluid and bronchial biopsy specimens from atopic asthmatic subjects (n = 10) and normal control subjects (n = 10).

Results: We report that GATA-3 mRNA expression is significantly increased in the airways of asthmatic subjects compared with those of normal control subjects ($P < .001$). Numbers of cells expressing GATA-3 transcripts correlated significantly with reduced airway caliber ($P < .05$) and airways hyperresponsiveness ($P < .05$) in asthmatic subjects. Colocalization studies showed that the majority (approximately 60% to 90%) of GATA-3 mRNA⁺ cells in asthmatic airways were CD3⁺ T cells, with smaller contributions from major basic protein⁺ eosinophils and tryptase⁺ mast cells. The density of GATA-3 mRNA⁺ cells correlated significantly with the numbers of cells expressing IL-5 mRNA ($P < .001$, $r = 0.879$ for bronchoalveolar lavage fluid; $P < .05$, $r = 0.721$ for biopsy specimens). Furthermore, double in situ hybridization demonstrated that approximately 76% of GATA-3 mRNA⁺ cells coexpressed IL-5 mRNA and that 91% of IL-5 mRNA⁺ cells coexpressed GATA-3 mRNA.

Conclusion: The results of this study provide the first evidence of increased GATA-3 gene expression in association with IL-5 mRNA⁺ cells in asthmatic airways. These findings support a causal association between augmented GATA-3 expression and dysregulated IL-5 expression in atopic asthma. (*J Allergy Clin Immunol* 1999;103:215-22.)

Key words: Asthma, GATA-3, T lymphocytes, IL-5, eosinophils

Asthma is a chronic disease associated with reversible airway obstruction, bronchial hyperreactivity, and airway eosinophilia.¹ Infiltration and activation of eosinophils in the airway tissue has been linked to events considered to be central to the pathophysiology of asthma, including bronchial hyperresponsiveness, airway smooth muscle constriction, epithelial alterations, and pulmonary remodeling.²⁻⁵ Contrasting with the putative effector function of eosinophils, clinical and experimental evidence suggests that CD4⁺ T cells elicit an indirect, albeit critical, role in airway inflammation and the associated lung dysfunction by regulating the recruitment and activation of eosinophils.⁶⁻⁸

Activated CD4⁺ T cells may be subdivided into at least 2 subsets on the basis of the cytokines they produce. T_{H1} cells produce IL-2 and IFN- γ and promote cellular immune responses, whereas T_{H2} cells produce IL-4 and IL-5, which are associated with allergic diseases including asthma.⁹⁻¹² The regulatory function of CD4⁺ T cells on tissue eosinophilia appears to be delivered in large part through the expression of IL-5,¹³ a cytokine with multiple effects on the biology of eosinophils, including differentiation, activation, and survival.¹⁴⁻¹⁶ The central role of IL-5 in asthma has been demonstrated in animal models of the disease; for example, mice rendered deficient in IL-5 by gene targeting or infusion with anti-IL-5 antibodies do not exhibit the eosinophilia, airway damage, and airway hyperresponsiveness normally resulting from aeroallergen challenge in these models.^{17,18} Studies of bronchoalveolar lavage (BAL) fluid³ and bronchial biopsy specimens from asthmatic subjects have demonstrated highly significant correlations in the increased numbers of IL-5 and IL-5 receptor mRNA⁺ cells with eosinophil numbers and pulmonary function tests.^{19,20} Moreover, we and others have shown that the steroid-

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Abbreviations used

BAL:	Bronchoalveolar lavage
MBP:	Major basic protein
NF- κ B:	Nuclear factor- κ B
SSC:	Standard saline citrate

dependent remission of asthma symptoms is associated with an abrogation of IL-5 gene expression and a resolution of airway eosinophilia.²¹ Collectively, these studies suggest that the mechanisms governing IL-5 gene expression in asthma are of fundamental importance in the induction and/or maintenance of the disease.

The expression of IL-5 is highly regulated and cell specific; it is expressed primarily in T cells and, to a lesser extent, in mast cells and eosinophils.²²⁻²⁵ Regulation of the IL-5 gene, like most cytokine genes, is exerted in large part through the presence of regulatory elements in the promoter region.²⁶ Studies to date have suggested the requirement of at least 2 specific positive regulatory elements for IL-5 promoter activation in humans and mice: an AP-1 site and an upstream GATA site.²⁷⁻³¹ The AP-1 site binds JunB and JunD, which are present in both T_{H1} and T_{H2} extracts, whereas the GATA site was shown to bind the transcription factor GATA-3, which is selectively expressed in T_{H2}-type cytokine-expressing T cells.^{28,32} Recent studies have shown that GATA-3 is crucial for IL-5 gene expression in bona fide T_{H2} cells and that ectopic expression of GATA-3 alone results in IL-5 promoter activation in a non-IL-5-producing cell line.²⁸ These findings suggest that GATA-3 gene expression may be relevant in the pathogenesis of atopic asthma.

The objective of this study was to examine GATA-3 gene expression in vivo in the airways of patients with asthma compared with normal control subjects by in situ hybridization. The phenotype of cells expressing GATA-3 was determined by combined in situ hybridization; immunocytochemistry and GATA-3 gene expression was colocalized to cells expressing IL-5 mRNA by double in situ hybridization.

METHODS

Subjects

The study was approved by the Ethics Committee of the Montreal Chest Institute of the Royal Victoria Hospital (McGill University, Montreal, Canada). Twenty subjects were included in this study, and informed written consent was obtained from each. Subjects with atopic asthma and healthy nonallergic normal subjects were carefully defined according to the criteria of the American Thoracic Society.³³ In brief, asthma was defined on the basis of (1) a clear clinical history with current clinical symptoms (episodic wheezing, chest tightness, and dyspnea) and (2) 15% or greater reversibility of FEV₁ spontaneously or after 200 μ g salbutamol and/or a methacholine provocation test result with a PC₂₀ of less than 8 mg/mL in the previous 2 weeks. Atopy was defined by (1) a positive skin prick test response with extracts of 1 or more common aeroallergens and (2) a positive RAST response of greater than 0.70 IU/mL to 1 or more of these allergens. None of the patients were taking inhaled steroids. Normal subjects had negative skin prick test responses to a wide

range of local aeroallergens in the presence of a positive histamine control. The nonatopic control subjects also had negative RAST responses to 25 common aeroallergens. All subjects were nonsmokers or exsmokers who had stopped smoking for greater than 12 months and had smoked less than 5 pack-years in their lifetime. Asthmatic subjects were treated with β_2 -agonists as required only; inhaled corticosteroids (<1000 μ g/day) or nonsteroidal anti-inflammatory medications were discontinued at least 30 days before the study. Treatment with greater than 1000 μ g/day inhaled steroids, oral steroids, theophylline, long-acting β_2 -agonists, leukotriene antagonists, or antihistamines was discontinued at least 3 months before the study. Exclusion criteria included age less than 18 years or greater than 65 years, FEV₁ less than 60% of the predicted value on the proposed bronchoscopy day, and evidence of acute or chronic infection, pregnancy, breast-feeding, or any chronic medical illness other than asthma. To examine GATA-3 in T_{H1}-mediated pulmonary disease, cytospin preparations from 6 patients with active pulmonary tuberculosis were used in this study. The clinical characteristics of these patients were described previously.³⁴

Fiberoptic bronchoscopy and sample processing

The technique of fiberoptic bronchoscopy for obtaining BAL fluid and bronchial biopsy specimens in asthmatic and normal subjects was performed as described previously.^{7,13,19,20,22} For each subject, baseline spirometry was recorded, and a methacholine inhalation test was performed. Nebulized salbutamol was given to all subjects (asthmatic and control subjects) before the bronchoscopy procedure. Bronchial biopsy specimens were obtained from the subsegmental airways, and BAL was performed by instillation of four 60-mL aliquots of warmed, pH-balanced sterile saline into the right middle lobe or lingula with immediate gentle aspiration into a siliconized glass bottle as detailed before. Total volume of BAL fluid recovered from asthmatic and control subjects did not differ significantly (mean values: 120 mL and 130 mL, respectively; $P > .05$). The biopsy specimens were immediately fixed in 4% paraformaldehyde, washed in 15% sucrose/PBS, and frozen in isopentane cooled in liquid nitrogen.^{7,19,20,22} Eight-micrometer cryostat sections were mounted on poly-L-lysine-coated slides and stored at -80°C until use. Cells from BAL fluid were filtered through a double layer of sterile gauze and centrifuged at 400g for 7 minutes at 4°C and then resuspended in 1.5 mL RPMI medium (Gibco-BRL). Cells were adjusted to a concentration of $5 \times 10^5/\text{mL}$, and cytospin preparations were made on poly-L-lysine-coated slides as previously described.^{13,19} Samples were air-dried for 10 minutes, fixed in 4% paraformaldehyde, and washed in 15% sucrose/PBS. Cytospin samples were incubated at 37°C overnight and stored at -80°C until use.

Preparation of riboprobes

Riboprobes were prepared from cDNA for GATA-3 and IL-5 as previously described.^{35,36} Briefly, cDNA was inserted into pGEM vectors and linearized with the appropriate enzymes before in vitro transcription. Transcription was performed in the presence of ³⁵S-UTP and the appropriate RNA polymerases to generate antisense and sense probes.

In situ hybridization

In situ hybridization was performed as previously described.^{13,35,36} Briefly, slides were first permeabilized by immersion in 0.3% Triton X-100 in PBS for 10 minutes followed by exposure to proteinase K solution (1 mg/mL in 20 mmol/L Tris-HCl and 1 mmol/L EDTA, pH 7.2) for 30 minutes at 37°C . The slides were then prehybridized with 50% formamide in 2 $\times$ SSC for 15 minutes

at 37°C. Hybridization was performed by using ^{35}S -UTP-labeled riboprobes (either antisense or sense) for 16 hours at 42°C. Posthybridization washings were carried out by using SSC solutions ($4\times$ SSC to $0.1\times$ SSC) followed by RNase treatment to remove unhybridized single stranded RNA. The preparations were dehydrated, immersed in emulsion fluid, and then subjected to autoradiography for 14 days. The autoradiograms were developed and subsequently counterstained with hematoxylin. As a negative control, sections were hybridized with the sense probe or were pretreated with RNase before hybridization with the antisense probe.

Immunocytochemistry and combined in situ hybridization-immunocytochemistry

Immunocytochemistry was used to determine the profile of inflammatory cells in BAL fluid and biopsy specimens. To identify the phenotype of GATA-3 mRNA-positive cells, colocalization studies were performed on preparations by using combined in situ hybridization-immunocytochemistry. These techniques have been described elsewhere in detail.^{5,22,37} The specimens were immunostained by using the alkaline phosphatase antialkaline phosphatase technique to phenotype cells with mAbs directed against human T-cell receptor (anti-CD3; Becton Dickinson, Mississauga, Ontario, Canada), human mast cell tryptase (antitryptase; Chemicon International Inc, Temecula, Calif), human major basic protein (MBP) (anti-MBP; a kind gift from Dr Redwan Moqbel, University of Alberta, Canada), or human CD68 (anti-CD68; Becton Dickinson). For combined in situ hybridization-immunocytochemistry, all solutions used were made up in 0.1% diethylpyrocarbonate-treated double-distilled water to avoid RNase contamination, and specimens were processed for in situ hybridization with a radiolabeled antisense riboprobe specific for GATA-3 after immunocytochemical staining. Cell counts were expressed as the percentage of GATA-3 mRNA⁺ cells that coexpressed immunoreactivity for CD3, tryptase, MBP, or CD68.²²

Double in situ hybridization

To colocalize GATA-3 and IL-5 mRNA, double in situ hybridization was performed. The GATA-3 antisense riboprobe was labeled with ^{35}S -UTP, and the IL-5 probe was labeled with biotin-11-UTP (Enzo Diagnostic) as previously described.^{38,39} Sections were treated similarly to single in situ hybridization but hybridized simultaneously with radiolabeled GATA-3 and nonradiolabeled IL-5. Biotin-labeled cRNA-mRNA hybrids were visualized by using an avidin-biotin complex and diaminobenzidine (brown stain). Sections were then dried and covered with emulsion fluid to develop the radiolabeled cRNA-mRNA hybrid. Double-hybridization signals were demonstrated by the presence of silver grains in brown-staining cells.

Quantification

Slides were coded and positive cells counted blindly by using $200\times$ magnification with an eyepiece graticule. In the airways subepithelium, positively stained cells were counted to a depth of 115 μm below the basement membrane, and the individual results were expressed as the mean number of positive cells per millimeter of basement membrane $\pm$ SD.^{13,20} In BAL fluid, at least 1000 cells were counted, and the results were presented as the number of positive cells per 1000 cells.^{7,19}

Statistics

The numbers of cells expressing GATA-3 and IL-5 mRNA were compared by using the Mann-Whitney *U* test (Systat v5.0; Systat Inc, Evanston, Ill). The coefficients of determination (*r*) and significance values were calculated by using linear regression analysis. Results were considered statistically significant for *P* values less than .05.

RESULTS

Subjects and airway cellular profile

Airway specimens were obtained from 10 atopic asthmatic patients and 10 nonasthmatic control subjects (BAL fluid and bronchial biopsy specimens). Demographics, allergen reactivity, and indices of lung function for each group are shown in Table I. Total cell recovery in BAL fluid did not differ significantly between asthmatic patients and normal control subjects (data not shown). The numbers of cells expressing the phenotypic markers CD3, MBP, tryptase, and CD68 by immunocytochemistry in bronchial biopsy specimens and BAL was determined. Whereas the absolute numbers of MBP⁺ eosinophils were significantly elevated in the asthmatic patients as compared with the control subjects ($P < .001$), numbers of CD3⁺, tryptase⁺, and CD68⁺ cells did not differ significantly. Similarly, in BAL fluid only the percentage of MBP⁺ eosinophils were significantly higher in asthmatic patients (4.5%) compared with normal control subjects (0.5%).

GATA-3 gene expression in the airways of asthmatic and control subjects

GATA-3 gene expression was investigated by in situ hybridization with radiolabeled antisense riboprobes. Numbers of cells expressing GATA-3 mRNA were significantly increased in BAL cells from asthmatic patients compared with those from normal control subjects (Fig 1, A; $P < .001$) and patients with tuberculosis ($P < .001$) and in biopsy specimens from asthmatic subjects compared with those from normal control subjects (Fig 1, B). Positive GATA-3 mRNA signals localized predominantly to infiltrating inflammatory cells, with no signal appearing in airway structural cells (epithelial cells and endothelial cells; Fig 2). Treatment of sections with the antisense probe for GATA-3 subsequent to RNase treatment or with the sense probe for GATA-3 resulted in an absence of hybridization signal. In asthmatic subjects the numbers of cells expressing GATA-3 mRNA correlated inversely with airway caliber (FEV_1) ($P < .01$, $r = -0.81$) and directly with bronchial responsiveness ($P < .05$, $r = 0.60$).

Phenotype of GATA-3 mRNA⁺ cells in the airways

To definitively phenotype the cells expressing GATA-3 mRNA, a combination of immunocytochemistry and radioactive in situ hybridization with ^{35}S -labeled riboprobes was used on BAL specimens from asthmatic subjects. As shown in Fig 2, double-positive cells exhibited both a red color (immunocytochemistry) and overlying silver grains (in situ hybridization). In BAL cells from asthmatic patients, the majority of cells expressing GATA-3 mRNA were CD3⁺ cells ($73.7\% \pm 10.5\%$). Smaller percentages of GATA-3 mRNA-expressing cells were accounted for by MBP⁺ eosinophils ($12.4\% \pm 3.9\%$) and tryptase⁺ mast cells ($7.8\% \pm 6.3\%$), but CD68⁺ cells showed no hybridization with the GATA-3 antisense probe (Table II). The specificity of the signals

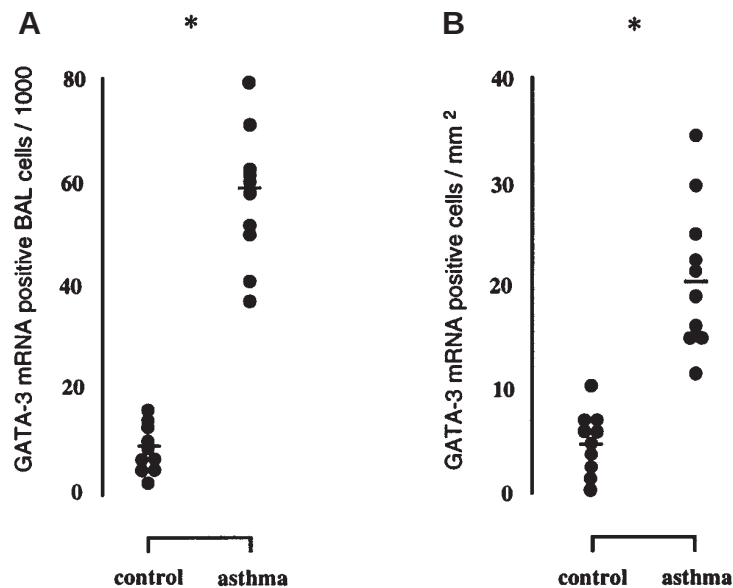


FIG 1. GATA-3 gene expression in BAL fluid (**A**) and bronchial biopsy specimens (**B**) from subjects with atopic asthma and nonasthmatic control subjects. Numbers of GATA-3 mRNA⁺ cells were determined by in situ hybridization with ³⁵S-labeled antisense riboprobes transcribed from GATA-3 cDNA followed by autoradiographic development of the signal as described in the Methods section. **P* < .001.

TABLE I. Demographics of asthmatic patients and normal control subjects

	Asthmatic patients	Normal control subjects
Age (y)	25-50	20-33
Sex (M/F)	6/4	7/3
FEV ₁ (% predicted)		
Mean	95	103.2
Range	70-128	93-119
Positive skin test responses	10/10	0/10
Total IgE (IU/mL)	920 ± 302	95 ± 37
Methacholine PC ₂₀ (mg/mL)	2.64 ± 1.22	>16

was confirmed by a series of controls involving substitution of the primary antibody with an irrelevant species and isotype-matched antibody and/or treatment with the GATA-3 sense riboprobe.

Colocalization of GATA-3 mRNA to cells expressing IL-5 transcripts in the airways

Because GATA-3 has been shown to be crucial for IL-5 gene expression in vitro, we examined the expression of IL-5 in the same asthmatic and control subjects. As shown in Fig 3, numbers of IL-5 mRNA⁺ cells were substantially increased in BAL fluid from atopic asthmatic subjects compared with BAL fluid from normal control subjects and in bronchial biopsy specimens from asthmatic subjects compared with biopsy specimens from control subjects. Numbers of cells expressing GATA-3 mRNA correlated significantly with IL-5 mRNA⁺ cell

TABLE II. Percentages of GATA-3 mRNA⁺ cells coexpressing CD3, MBP, tryptase, and CD68 immunoreactivity in BAL fluid from atopic asthmatic subjects*

Subject	%GATA-3+/CD3 ⁺	%GATA-3+/MBP ⁺	%GATA-3+/tryptase ⁺	%GATA-3+/CD68 ⁺
1	79	10	5	0
2	72	11	6	0
3	75	9	4	0
4	83	10	3	0
5	62	17	7	0
6	89	10	1	0
7	85	9	4	0
8	56	21	17	0
9	69	13	11	0
10	67	14	20	0
Mean ± SD	73.7 ± 10.5	12.4 ± 3.9	7.8 ± 6.3	0

*Results of combined in situ hybridization-immunocytochemistry of BAL fluid obtained from atopic asthmatic subjects.

numbers in BAL fluid and biopsy specimens from asthmatic subjects (*P* < .001, *r* = 0.879 in BAL fluid; *P* < .05, *r* = 0.721 in biopsy specimens) (Fig 4). To determine whether GATA-3 and IL-5 transcripts are coexpressed by the same cells, double in situ hybridization analyses were performed on BAL cells from asthmatic patients by using a radiolabeled GATA-3 probe and a nonradiolabeled IL-5 probe. The mean percentage of IL-5 mRNA-positive cells coexpressing GATA-3 mRNA was 76%. In contrast, 91% (mean percentage) of the GATA-3 mRNA-positive cells expressed IL-5 transcripts in BAL fluid (Fig 2).

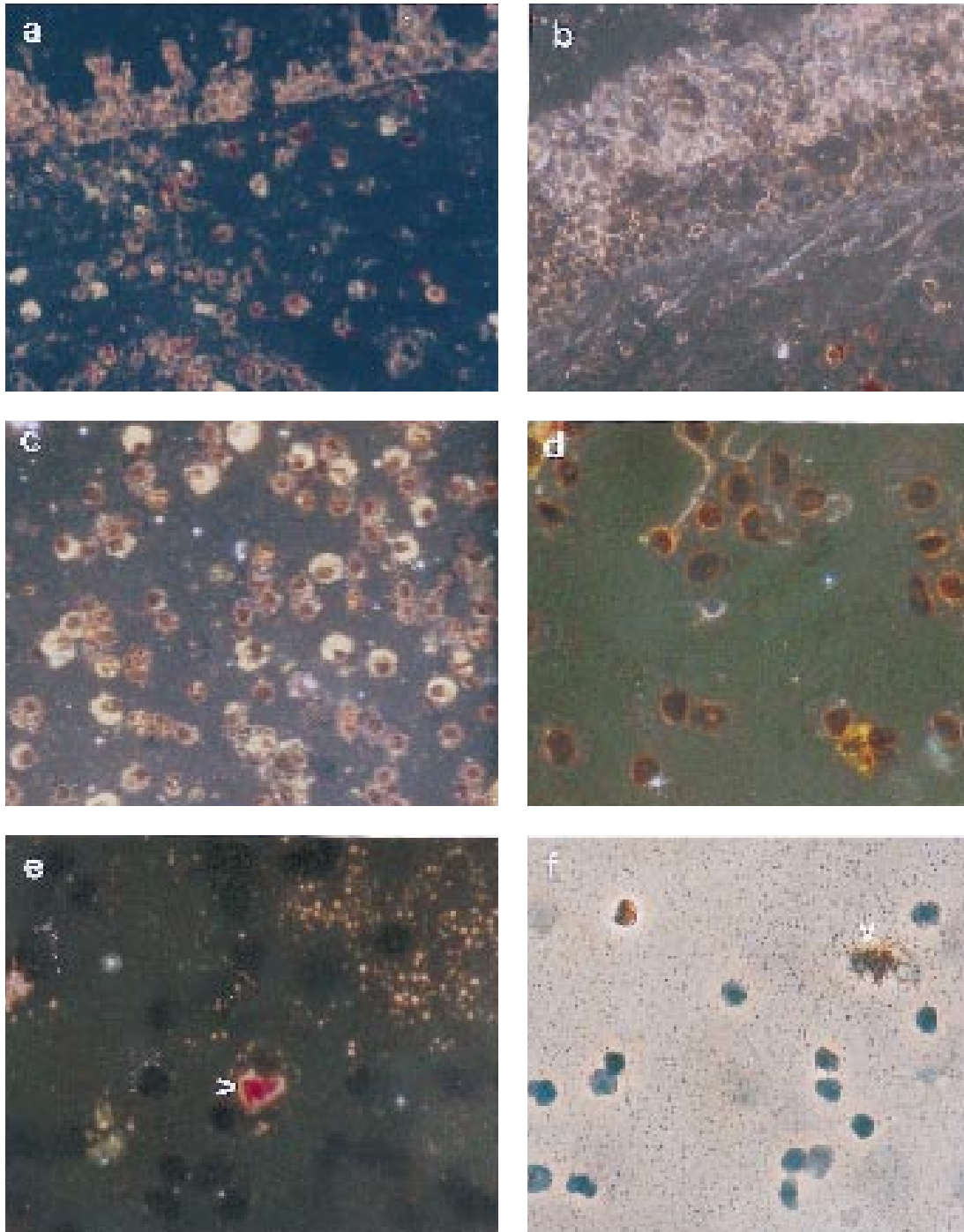


FIG 2. Representative examples of in situ hybridization of GATA-3 mRNA in bronchial biopsy specimens and BAL preparations from atopic asthmatic subjects and normal control subjects by using complementary RNA probes. **a**, In situ hybridization (dark field illumination) of GATA-3 mRNA in a bronchial biopsy specimen from an asthmatic subject. Note the presence of positive signals within infiltrating cells but not in the epithelial or endothelial cells. **b**, GATA-3 mRNA expression under dark field illumination in a bronchial biopsy specimen from a normal control subject. **c**, In situ hybridization for GATA-3 in BAL cells from a subject with asthma and **(d)** a normal control subject. **e**, Combined in situ hybridization-immunocytochemistry localizing GATA-3 mRNA to CD3-immunoreactive cells in BAL fluid from an asthmatic subject. A GATA-3 mRNA⁺/CD3⁺ cell is indicated (*arrowhead*). **f**, Coexpression of GATA-3 mRNA and IL-5 mRNA in BAL cells from an asthmatic subject by double in situ hybridization. GATA-3 probes were labeled with ³⁵S, and IL-5 probes were labeled with biotin. Signals were developed by autoradiography and diaminobenzidine, respectively, as described in the Methods section. An example of a GATA-3 mRNA⁺/IL-5 mRNA⁺ cell is shown (*arrowhead*).

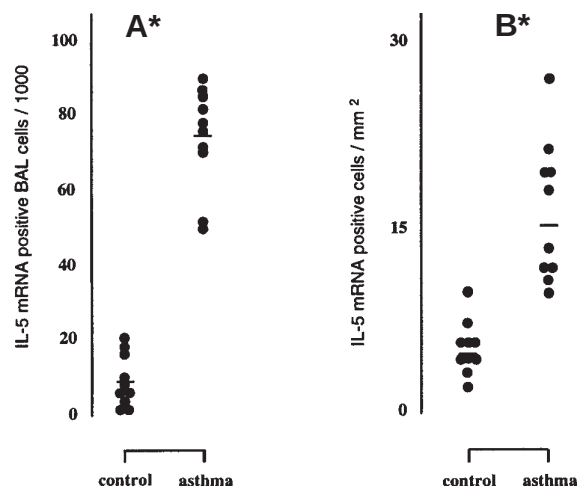


FIG 3. Expression of IL-5 mRNA in BAL fluid (**A**) and bronchial biopsy specimens (**B**) from subjects with atopic asthma and normal control subjects. IL-5 mRNA⁺ cells were enumerated in BAL fluid and biopsy specimens by in situ hybridization as detailed in the Methods section. * $P < .001$.

DISCUSSION

This study shows that gene expression of the GATA-3 transcription factor is significantly increased in the airways of asthmatic subjects compared with normal control subjects. The majority of GATA-3 mRNA⁺ cells were phenotypically identified as T lymphocytes, with lesser contributions from eosinophils and mast cells. Consistent with the in vitro activity of GATA-3 as a transactivator of IL-5 gene transcription, a large proportion of cells expressing IL-5 transcripts were found to coexpress GATA-3 mRNA in asthmatic airways. Taken together, the results of this study provide the first evidence implicating a specific transcription factor in dysregulated IL-5 gene expression in vivo in asthmatic airways.

In agreement with previous studies of mild asthma, numbers of T lymphocytes in the bronchial mucosa did not differ significantly between asthmatic and normal subjects.²² Augmented GATA-3 expression therefore does not appear to be a consequence of T-cell recruitment. However, GATA-3 mRNA was found to be expressed in small numbers of cells in BAL fluid and biopsy specimens from normal control subjects in parallel with the low basal expression of IL-5 transcripts. Although the majority of GATA-3 was localized to T cells, eosinophils, and mast cells, a small number of GATA-3 mRNA-positive signals in asthmatic airways did not colocalize with any of the cells identified by immunocytochemistry. Basophils, which are present in asthmatic airways but lack a reliable cell surface marker, have been shown to express GATA-3 and may therefore comprise some of the unidentified GATA-3 mRNA⁺ cells.⁴⁰

The numbers of cells expressing GATA-3 mRNA correlated significantly with reduced airway caliber and bronchial hyperresponsiveness. The biologic function of

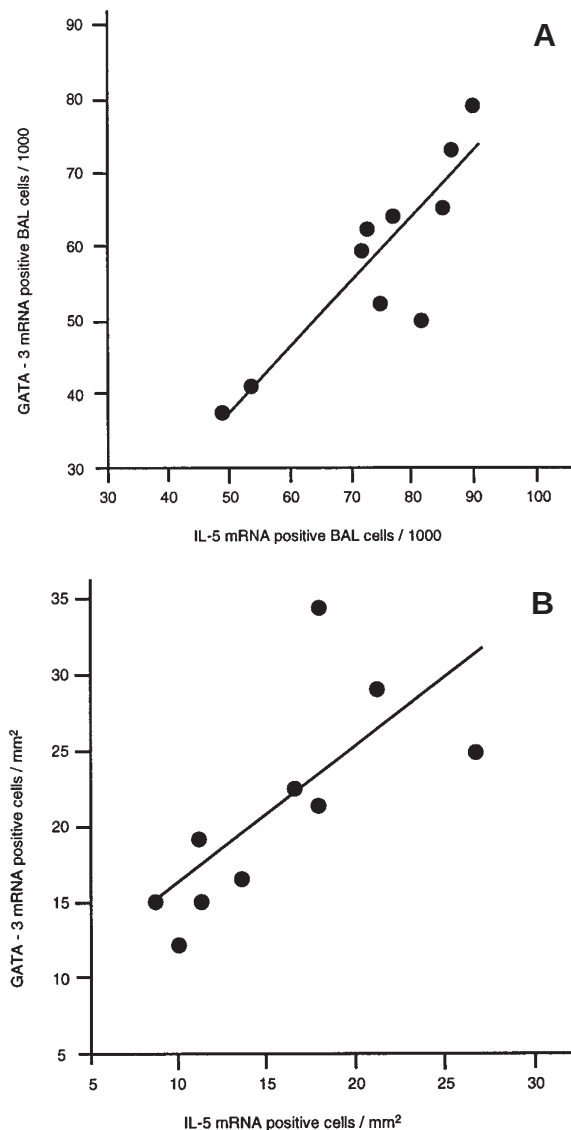


FIG 4. Correlation of GATA-3 mRNA⁺ cells and IL-5 mRNA⁺ cells in BAL fluid (**A**) and bronchial biopsy specimens (**B**) from atopic asthmatic subjects. Correlations were determined by linear regression analysis. Numbers of cells expressing GATA-3 mRNA correlated significantly with IL-5 mRNA⁺ cell numbers in BAL fluid and biopsy specimens from asthmatic subjects ($P < .001$, $r = 0.879$ for BAL fluid [**A**]; $P < .05$, $r = 0.721$ for bronchial biopsy specimens [**B**]).

GATA-3 in vitro²⁸ indicates that its effect on pulmonary function is likely a consequence of IL-5-mediated upregulation of eosinophil activity. In agreement with prior studies, IL-5 gene expression was found to be significantly increased in asthmatic airways compared with normal airways.¹³ Numbers of IL-5 mRNA⁺ cells both in BAL fluid and bronchial biopsy specimens correlated significantly with the numbers of cells expressing GATA-3 mRNA. To more precisely examine the nature of the association of GATA-3 with IL-5, double in situ hybridization was carried out to colocalize transcription factor and

cytokine gene transcripts. Seventy-six percent of GATA-3 mRNA⁺ cells were found to express IL-5 mRNA, and the remainder (GATA-3 mRNA⁺/IL-5 mRNA⁻ cells, 24%) might be indicative of cells that express GATA-3 but lack other transcription factors that are also necessary to induce IL-5 gene expression. IL-5 promoter activation also requires binding of proteins to the AP-1 site, which is achieved once the cells are activated.²⁸ A number of factors could be related to the presence of small numbers (<10%) of IL-5 mRNA⁺/GATA-3 mRNA⁻ cells. GATA-3 mRNA may be present, albeit at an undetectable copy number, in IL-5 mRNA⁺/GATA-3 mRNA⁻ cells. This hypothesis is attractive in light of a recent study demonstrating that IL-5 mRNA is highly stable compared with mRNA for other cytokine genes,⁴¹ although the relative stability of IL-5 versus GATA-3 mRNA is not known. It is also possible that certain stimuli in asthmatic airways could increase IL-5 mRNA expression in these minority of cells by a mechanism that bypasses the requirement for GATA-3. However, such a mechanism would likely not be dependent on direct stimulation with antigen or agents that induce an increase in intracellular levels of cAMP.²⁸

In addition to transactivating the IL-5 promoter, GATA-3 was also implicated in the expression of other T_{H2}-type cytokines, including IL-4, IL-6, and IL-10, although the mechanism or mechanisms by which this occurred has yet to be defined.³² Other transcription factors have also been suggested to play a role in asthma. For example, nuclear factor- κ B (NF- κ B) may regulate a large number of genes encoding products that are believed to be involved in inflammatory responses, including cytokines, chemokines, and adhesion molecules. However, there have been few measurements of NF- κ B activation in inflammatory cells or inflamed tissues. Nonetheless, one mechanism that has been proposed to account for many of the anti-inflammatory actions of glucocorticoids involves direct interaction of the glucocorticoid receptor with NF- κ B.^{42,45} A second mechanism involving upregulation of I κ B α synthesis has also been demonstrated in some cell types.^{46,47} The absence of significant levels of GATA-3 mRNA in BAL cells recovered from patients with tuberculosis (T_{H1}-mediated disease with low expression of IL-5) support the concept that GATA-3 is mainly involved in the regulation of T_{H2}-type cytokines. We have not included in this study specimens from atopic nonasthmatic subjects, and thus it is not clear whether GATA-3 is upregulated in association with atopy or asthma or both.

In conclusion, the results of this study provide the first evidence of increased GATA-3 gene expression in association with IL-5 mRNA⁺ cells in asthmatic airways. These findings support a causal association between augmented GATA-3 expression and dysregulated IL-5 expression in atopic asthma. In this study only GATA-3 mRNA was examined. Further studies to confirm the presence of GATA-3 protein in T cells of asthmatic subjects are necessary.

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