

Signal transducer and activator of transcription 6 (STAT-6) expression and function in asthmatic bronchial epithelium

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Background: Asthma is associated with increased production of IL-4 and IL-13.

Objective: Because many of the effects of these cytokines are mediated by activation of signal transducer and activator of transcription 6 (STAT-6), we investigated expression and function of this transcription factor in the airways.

Methods: STAT-6 expression was investigated through use of immunohistochemistry or RT-PCR applied to bronchial biopsy specimens or brushings from normal control or asthmatic subjects. STAT-6 function was investigated by means of Western blotting and ELISA applied to primary epithelial cell cultures. **Results:** Immunohistochemistry revealed that the bronchial epithelium was the major site of STAT-6 expression, both cytoplasmic and nuclear staining being observed. The level of STAT-6 expression in subjects with mild asthma (median [range] percent epithelial staining, 3.4% [0% to 16.0%]; n = 14) did not differ significantly from that in normal controls (4.7% [0.0% to 20.0%]; n = 11); however, in subjects with severe asthma, epithelial STAT-6 expression (13.7% [4.8% to 25.7%]; n = 9) was increased in comparison with subjects with mild asthma and normal controls ($P < .05$). RT-PCR analysis showed that epithelial STAT-6 expression was heterogeneous and comprised both full-length STAT-6 and the dominant-negative variant that lacks the SH2 domain. Treatment of primary cultures of bronchial epithelial cells with IL-4 resulted in STAT-6 phosphorylation and stimulation of IL-8 secretion; however, no difference in the responses of epithelial cells was observed between normal (n = 12) and asthmatic (n = 14) donors.

Conclusion: These data demonstrate expression and activation of STAT-6 in normal and asthmatic bronchial epithelium. The activity of this transcription factor is likely to play a key role in mediating the responses of the bronchial epithelium to T_H2 cytokines that are characteristic of the asthmatic phenotype. (J Allergy Clin Immunol 2001;108:832-8.)

Key words: Asthma, epithelium, STAT-6, IL-4, IL-13, T_H2 , phosphorylation, IL-8

Asthma is a chronic inflammatory disorder superimposed on remodeled airways; the consequence is bronchial hyperresponsiveness and variable airflow obstruction and symptoms. The inflammatory component is characterized by mast cell and eosinophil infiltration resulting from T-lymphocyte polarization toward a T_H2 phenotype, leading to coordinate secretion of IL-3, -4, -5, -9, -13, and GM-CSF encoded in a cluster on chromosome 5q31-33.¹ IL-4 and IL-13 have long been recognized as key components in the pathogenesis of asthma because of their fundamental role in the commitment of T-helper cells to the T_H2 phenotype and the production of IgE.^{2,3} More recently, in vivo studies using animal models have identified the airways' structural cells as additional important targets for these cytokines. These effects include (1) goblet cell hyperplasia,^{2,3} (2) subepithelial fibrosis, a characteristic feature of remodeled asthmatic airways, and (3) enhanced epithelial expression of the eosinophil chemoattractant eotaxin.⁴ Consistent with these in vivo observations, direct effects of IL-4 and IL-13 on mucin⁵ and GM-CSF⁶ expression by airway epithelial cells have been reported.

IL-4 and IL-13 exhibit overlapping, but not identical, effector profiles, which is partly due to the shared use of the IL-4 receptor α chain (IL-4R α) in the multimeric IL-4 and IL-13 receptor complexes.⁷ However, the sphere of influence of IL-4 can be extended by an interaction of IL-4R α with the common γ chain of the IL-2 receptor, whereas the IL-13R $\alpha1$ chain can also interact with the high-affinity IL-13R $\alpha2$ chain. Because both IL-4 and IL-13 use the IL-4R α as a receptor component, these cytokines activate many common signaling pathways through activation of the Janus-kinase (JAK) family of tyrosine kinases.⁸ These initiate a series of phosphorylation events that ultimately lead to activation of the transcription factor signal transducer and activator of transcription 6 (STAT-6), as well as of insulin receptor substrates 1 and 2,⁹ which have been linked to cell survival and proliferation.

Phosphorylation on tyrosine residues provides a mechanism by which 2 STAT-6 molecules can form homo-

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Abbreviations used

STAT-6: Signal transducer and activator of transcription-6
R α : Receptor α chain
JAK: Janus-associated kinase

dimers through reciprocal SH2 domain interactions.¹⁰ This enables nuclear translocation and binding to specific TTN6AA recognition sequences within the promoter region of target genes to initiate transcription.¹⁰ The close relationship between STAT-6 and IL-4 and IL-13 has been revealed by studies of STAT-6-deficient mice, which demonstrate characteristics that contrast strikingly with those of IL-4 and IL-13 transgenic mice.^{11,12}

A number of functional and promoter polymorphisms of the IL-4/IL-13 cytokine and receptor systems have been detected that affect receptor signaling and cytokine expression levels, and these are associated with asthma susceptibility and severity.⁷ Although STAT-6 function might be affected by these genetic polymorphisms, its activity also appears to be regulated by differential splicing, leading to production of 3 naturally occurring variants: STAT-6a (referred to as *STAT-6*), STAT-6b, and STAT-6c.¹³ STAT-6b has an NH₂ terminal truncation, is poorly phosphorylated, and demonstrates reduced transcriptional activity. STAT-6c exhibits an SH2 domain deletion and is not phosphorylated in response to IL-4 or IL-13; this isoform is unable to form dimers and cannot induce transcription, but it appears to act as a dominant-negative inhibitor of STAT-6 function. Of these isoforms, STAT-6 appears to be the most abundant in human tissues, the dominant-negative isoform being found in greater amounts in whole lung extracts than in other human tissues.¹³

Expression of IL-4R α ¹⁴ and IL-13R α ¹⁵ has recently been reported in human bronchial epithelial cells; however, there is little information about expression of STAT-6 or any of its kinase regulators. A recent study has reported STAT-6 expression in bronchial biopsy specimens, an increase in STAT-6 positive cells being detected in subjects with atopic asthma.¹⁶ However, that study focused on submucosal cells rather than the bronchial epithelium.

In view of the effects of IL-4 and IL-13 expression on epithelial function in transgenic animal models and the opposing effects of STAT-6 deletion on these responses, we postulated that STAT-6 was involved in these processes in human airways in asthma. Therefore, we investigated the expression of STAT-6, the STAT-6c isoform, and JAK1 in bronchial biopsy specimens from normal and asthmatic subjects. We further investigated the role of STAT-6 in functional responses of bronchial epithelial cells to IL-4 and IL-13 using normal and asthmatic bronchial epithelial cells cultured in vitro.

MATERIALS and METHODS

All materials were purchased from Sigma (Poole, United Kingdom), unless otherwise indicated.

Subjects

Archival glycol methacrylate-embedded bronchial biopsy specimens obtained from 11 control subjects without asthma (5 female; mean [$\pm$ SD] age, 23.8 $\pm$ 7.5 years; age range, 18 to 46 years) with a mean ($\pm$ SD) FEV₁ of 102% $\pm$ 9.9% predicted (range, 86% to 116%), 9 subjects with severe asthma (8 female; mean [$\pm$ SD] age, 29.2 $\pm$ 15.6 years; age range, 13 to 50 years) with a mean ($\pm$ SD) FEV₁ of 75.0% $\pm$ 15.2% predicted (range, 53% to 91%), and 14 subjects with mild asthma (9 female; mean [$\pm$ SD] age, 28.2 $\pm$ 10.7 years; age range, 18 to 54 years) with a mean ($\pm$ SD) FEV₁ of 93.9% $\pm$ 12.5% predicted (range, 71% to 114%). The subjects with mild asthma were using inhaled bronchodilators for symptom relief, whereas the subjects with severe asthma were using between 2000 and 4000 pg/day inhaled corticosteroids and 5 to 100 mg/day oral corticosteroids. All subjects were nonsmokers, and none had experienced recent symptoms of upper respiratory tract infection within at least 6 weeks before the study. Definition of disease severity was based on symptoms, treatment, and lung function and was in accordance with the Global Initiative for Asthma guidelines.¹⁷ The study was approved by the Southampton Joint University and Hospitals Ethics Committee, and each subject gave written consent after being fully informed about the nature and purpose of the study.

Sample processing and immunohistochemistry

Processing of the tissue into glycol methacrylate and the immunohistochemical method applied to this material have been described in detail elsewhere.¹⁸ Briefly, 2- μ m sections were cut with an ultramicrotome (Leica, Wetzlar, Germany) and stained by immunohistochemistry through use of the streptavidin-biotin peroxidase detection system with diaminobenzidine as chromogen. The sections were stained through use of a primary rabbit anti-STAT-6 polyclonal antibody (Santa Cruz Biotechnologies, Santa Cruz, Calif) at 6.6 μ g/mL for 1 hour at room temperature. After washing, swine antirabbit biotinylated IgG Fab fragment at 1:300 (Dako Ltd, Wycombe, United Kingdom) containing 20% v/v normal swine serum was applied to the sections for 1 hour. Sections were counterstained with Mayer's hematoxylin and mounted in DPX (BDH Laboratory Supplies, Lutterworth, United Kingdom). Control incubations were performed through use of primary antibody to which a 10-fold molar excess of immunizing peptide was added.

Quantitative analysis

Airway epithelial expression of STAT-6 was quantified by computer-assisted image analysis (Colourvision 1.7.6, Improvision, Coventry, United Kingdom). For each biopsy specimen, the entire intact epithelium, defined as that having a brush border, was assessed. The mean area of intact epithelium measured was 0.08 mm² for the normal, 0.09 mm² for the mild asthmatic, and 0.07 mm² for the severe asthmatic biopsy specimens. At the beginning of each session, the image analysis system was standardized through use of the same section of bronchial mucosa stained for STAT-6 to ensure reproducibility of analysis. Each tissue section was analyzed on 2 separate occasions by the same observer, who was unaware of the patient group from which the specimen was obtained; data were analyzed through use of the Mann Whitney *U* test. A *P* value of less than .05 was considered to be significant.

Epithelial responses to IL-4 and IL-13

Primary bronchial epithelial cell cultures from 12 normal subjects (median age, 21 years; age range, 19-44 years; FEV₁ [range], 100% predicted [78% to 104%]) and 14 subjects with asthma (median age, 25 years; age range, 19-55 years; FEV₁ [range], 90% predicted [68% to 103%]) were grown from bronchial brushings in

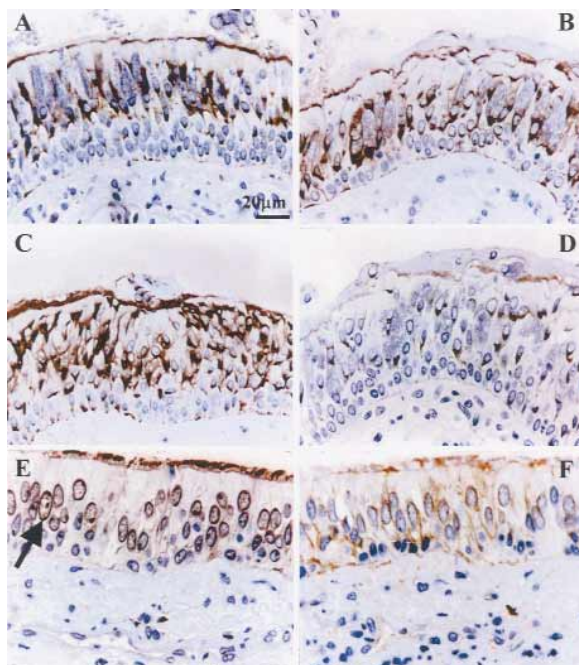


FIG 1. Immunohistochemical analysis of STAT-6 and JAK1 expression in bronchial epithelium: representative staining of biopsy specimens from subjects with normal (A), mild (B and D), and severe (C) asthma through use of anti-STAT-6 (A-C) or a preadsorbed antibody control (D). E, An asthmatic biopsy specimen displays nuclear staining for STAT-6. F, A typical section stained for JAK1. Scale bar, 20 μ m.

bronchial epithelial growth medium (Clonetics, San Diego, Calif), as previously described.¹⁹ Cells were used at passage 2 and were seeded into 24-well trays at 5×10^4 cells per well. When the cells were 80% to 90% confluent, they were rendered quiescent by transfer into bronchial epithelial basal medium (Clonetics) containing insulin, transferrin, and sodium selenite. The medium was then replaced with fresh basal medium in the absence or presence of IL-4 or IL-13 (20 ng/mL) and the cells were incubated for 24 hours. Cells were then fixed for analysis of cell number through use of the methylene blue assay.²⁰ Culture supernatants were harvested by centrifugation and stored for measurement of IL-8 release by ELISA through use of a commercial kit, according to the manufacturer's instructions. Data were normalized for cell number and analyzed through use of the Wilcoxon rank sum test.

Analysis of STAT-6 phosphorylation

Phosphorylation of STAT-6 was measured in primary bronchial epithelial cell cultures prepared exactly as described above, except that cells were exposed to IL-4 or other cytokines for the time indicated in the Results section. On termination of the incubation, the cells were solubilized into SDS lysis buffer and analyzed (15 μ g protein per lane) by SDS polyacrylamide gel electrophoresis and Western blotting, as previously described.²¹ The Western blot was probed through use of the pan-STAT-6 antibody used for immunohistochemistry or a rabbit anti-phospho-STAT-6 antibody (New England BioLabs, Hitchin, United Kingdom) at a 1:800 dilution and an antirabbit immunoglobulin horseradish peroxidase conjugate at a 1:1000 dilution through use of enhanced chemiluminescent detection (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom).

Analysis of STAT-6 variant expression by RT-PCR

Bronchial brushings or primary cultures of bronchial epithelial cells were lysed directly into Trizol (Life Technologies GIBCO BRL, Paisley, United Kingdom) and processed according to the manufacturer's instructions. To optimize RNA extraction, glycogen (Life Technologies GIBCO BRL) was used as a carrier for the RNA. One to 2 μ g of RNA was reverse transcribed through use of random hexamers in the SuperScript first-strand synthesis system (Life Technologies GIBCO BRL) according to the manufacturer's instructions. Analysis of the STAT-6 isoforms was achieved by RT-PCR through use of a forward primer (5'TTT TGG CAG TGG TTT GAT GGT3') common to all of the variants and reverse primers that specifically targeted transcripts containing (1) the SH2 domain (5'GTT TGC TGA TGA AGC CAA TGA TC3'), which is absent in STAT-6c, (2) the sequence spanning the splice site (5'CCA ATC TCT GAG TCG CGG TC3') formed by the deletion of 84 base pairs in the SH2 domain to form the dominant-negative variant, STAT-6c, and (c) a sequence (5'TGA CAT GGG CAA TGG TGA TG3') common to both STAT-6 and STAT-6c molecules.¹³ Control reactions were also performed through use of β -actin primers to assess RNA quality.

RESULTS

STAT-6 expression in human airways

To investigate whether STAT-6 was a likely mediator of IL-4 and IL-13 responses in human airway mucosa, immunohistochemistry was used to determine the presence of STAT-6 in bronchial biopsy specimens of normal and asthmatic subjects. Immunohistochemical analysis revealed the ciliated columnar cells of the bronchial epithelium to be the major site of STAT-6 expression, mucus secreting goblet cells also showing prominent positive immunoreactivity around the nuclear region (Fig 1, A-C). In contrast, the cells in the basal layer of the epithelium were only weakly positive. STAT-6 was mainly detectable in the cytoplasm, but in some sections a nuclear ring pattern of staining was apparent in both normal and asthmatic subjects (Fig 1, E), suggesting a degree of transcription factor activity irrespective of disease status; however, this remains to be confirmed. The specificity of STAT-6 immunoreactivity was confirmed by preadsorbing the antibody with an excess of its immunizing peptide, which abolished all staining apart from a small amount on the brush border (Fig 1, D). To avoid quantitation of this nonspecific staining, the brush border was excluded from the area of epithelium selected for quantitation. Computerized image analysis revealed that the level of STAT-6 expression in the bronchial epithelium in subjects with mild asthma did not differ significantly from that in normal controls (median [range] percent epithelial staining, (3.4% [0% to 16.0%]) vs 4.7% [0% to 20.0%]). In subjects with severe asthma, epithelial STAT-6 expression (13.7% [4.8% to 25.7%]) was significantly increased in comparison with subjects with mild asthma ($P = .001$) and normal controls ($P = .037$; Fig 2).

Positive immunoreactivity for JAK1 was much weaker and more diffuse than that observed for STAT-6 throughout the epithelium. This low level of staining might be due to the catalytic function of JAK1. The principal site of expression was the cytoplasm of columnar

epithelial cells; much lower levels were seen in the basal layer. In some sections, staining was evident around the plasma membranes, as would be expected for a surface receptor-associated kinase (Fig 1, *F*). In view of the low intensity of staining for JAK1 in the bronchial epithelium, quantification of staining was not undertaken.

To investigate the molecular forms of STAT-6 expressed in bronchial epithelium, RNA from bronchial epithelial brushings of 2 normal and 2 asthmatic subjects were analyzed by means of RT-PCR. In each case, mRNA transcripts for full-length STAT-6 was detected. However, with a primer specific for the splice site formed by deletion of the SH2 domain, the presence of the dominant-negative variant, STAT-6c, was also revealed (Fig 3). The splice variant was also detected in primary cultures of bronchial epithelial cells and the H292 cell line (data not shown).

STAT-6 function in bronchial epithelial cells

Treatment of primary bronchial epithelial cells with IL-4 caused a sustained increase in STAT-6 phosphorylation from 15 to 120 minutes (Fig 4, *A*); in contrast, in the absence of IL-4 or in the presence of EGF, transforming growth factor α , IFN- γ , and TNF- α had no effect on STAT-6 phosphorylation after 1 hour of incubation (Fig 4, *B*). STAT-6 activation was much more difficult to detect in H292 and 16HBE 14o- bronchial epithelial cell lines, in which low and variable responses were obtained (data not shown). The poor response of H292 cells was surprising, because IL-4 has been shown to enhance mucus gene expression in this cell line⁵; this might reflect an important difference between normal and transformed cells. The functional response of primary bronchial epithelial cells to IL-4 and IL-13 was measured as release of IL-8 24 hours after exposure to the cytokines. Through use of primary cultures from either normal ($n = 12$) or asthmatic ($n = 14$) donors, treatment with IL-4 or IL-13 caused an increase in release of IL-8 into the culture supernatant (Fig 5). Stimulation of IL-8 release above baseline was observed in 23 of the 26 cases studied; however, the magnitude of the response varied (median [range] fold stimulation, 3.5 [0.9-28.1] or 1.9 [0.6-19.8] and 3.1 [0.6-25.0] or 2.8 [0.6-18.8] for normal and asthmatic cultures treated with IL-4 or IL-13, respectively), suggesting that the individual cultures possessed a differential ability to respond to the 2 T_H2 cytokines. Furthermore, through use of the H292 bronchial epithelial cell line, IL-4 treatment for 24 hours failed to cause a significant increase in IL-8 release (867 ± 257 vs 707 ± 206 pg per 4×10^5 cells; $n = 6$; $P > .05$).

DISCUSSION

These studies have demonstrated for the first time that the expression of STAT-6 in the bronchial epithelium is higher in subjects with severe asthma than in subjects with mild asthma or normal controls. STAT-6 thus joins a variety of other transcription factors, including STAT-1,²² NF κ B,²³ and AP-1,²⁴ whose levels and activity are

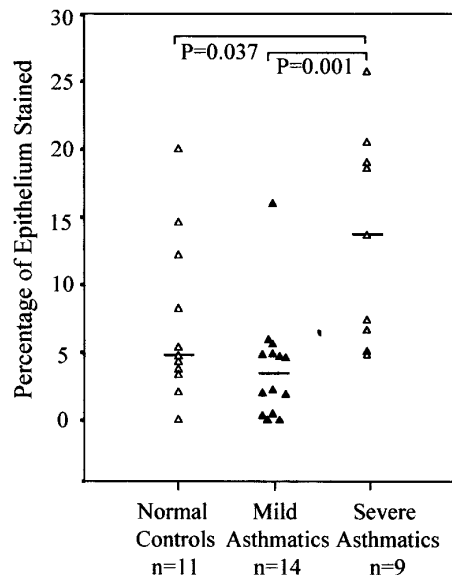


FIG 2. Quantitative analysis of epithelial STAT-6 staining in bronchial biopsy specimens. Immunostaining for STAT-6 was quantified in all intact epithelium, after delineation and exclusion of the non-specifically stained brush border region in each bronchial biopsy specimen, by means of computerized image analysis. Data were analyzed through use of the Mann Whitney *U* test.

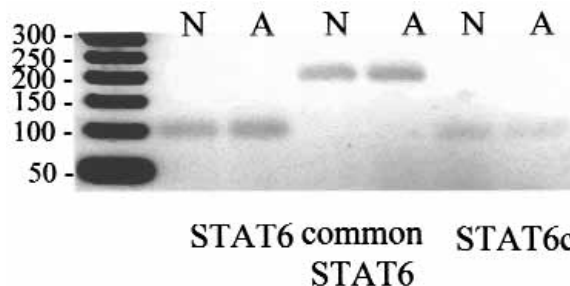


FIG 3. Detection of STAT-6 isoforms in normal and asthmatic bronchial epithelial cells. STAT-6 transcripts containing or lacking the SH2 domain required for STAT-6 dimerization were detected through use of a common forward primer and reverse primers specific for (a) the SH2 domain (114 bp product), (b) the common sequence 3' to the splice site to detect both isoforms (190 bp product), and (c) the splice site created after deletion of the SH2 domain form STATc (84 bp product). The figure shows the presence of each transcript in normal (*N*) and asthmatic (*A*) bronchial epithelial cells. In each case, a strong positive signal was also obtained through use of β -actin primers.

upregulated in the asthmatic bronchial epithelium. The activity of each of these transcription factors is likely to make a substantial contribution to the proinflammatory environment in the airways through induction of cytokine and adhesion molecule expression. Synergistic interactions, such as those that occur between STAT-6 and NF κ B to enhance eotaxin expression,²⁵ might make a further important contribution in the context of a T_H2 environment that occurs in all forms of asthma.

In addition to a proinflammatory role, *in vivo* studies have demonstrated that STAT-6 plays an important role in promoting mucus production,¹² and our finding that

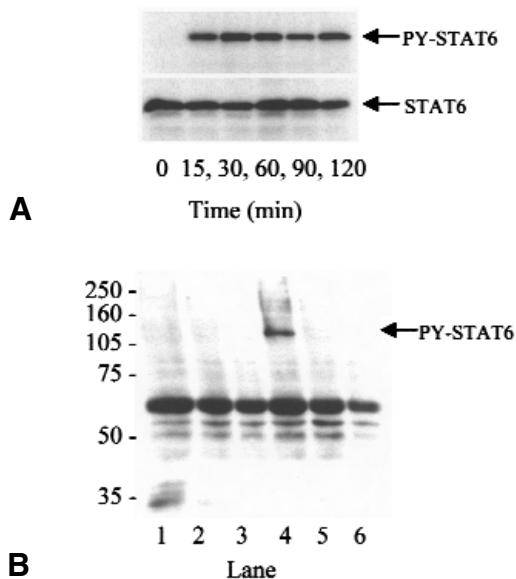


FIG 4. STAT-6 phosphorylation in vitro. **A**, Primary bronchial epithelial cell cultures ($n = 3$) were incubated in the presence of 75 pmol/L IL-4 for 0 to 120 minutes, and STAT-6 phosphorylation was determined through use of an anti-phospho-STAT-6 antibody or a pan-STAT-6 antibody (to confirm equivalent loading). **B**, Primary bronchial epithelial cell cultures ($n = 3$) were incubated in medium alone (*lane 1*) or with 1 nmol/L EGF (*lane 2*), 1 nmol/L TGF α (*lane 3*), 70 pmol/L IL-4 (*lane 4*), 60 pmol/L TNF- α (*lane 5*), or 60 pmol/L IFN γ (*lane 6*) for 1 hour and then analyzed by SDS-PAGE and Western blotting for the presence of phosphorylated STAT-6, seen at 100 kDa.

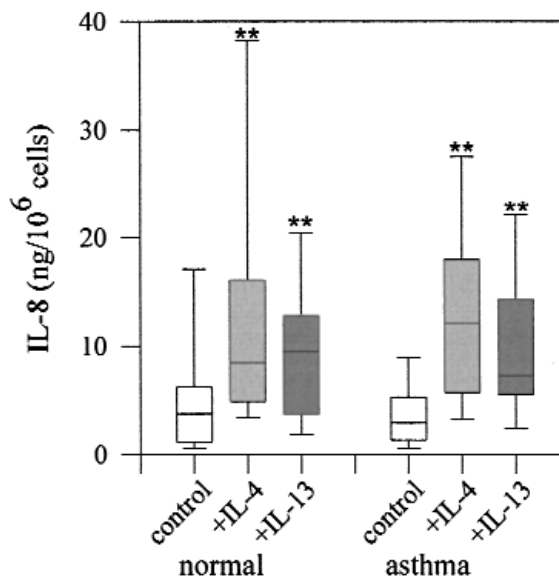


FIG 5. Stimulation of IL-8 release by IL-4 and IL-13. Primary cultures of normal (n = 12) or asthmatic (n = 14) bronchial epithelial cells were exposed to IL-4 (20 ng/mL) or IL-13 (20 ng/mL) for 24 hours, and IL-8 release was measured by ELISA. Data are corrected for cell number and analyzed through use of Wilcoxon rank-sum testing. The box plot shows the 25th and 75th percentiles, the 50th percentile value being indicated in the *box*. The *error bars* show the 10th and 90th percentiles. Data were tested through use of Wilcoxon rank sum testing. $^{**}P < .01$ vs untreated controls.

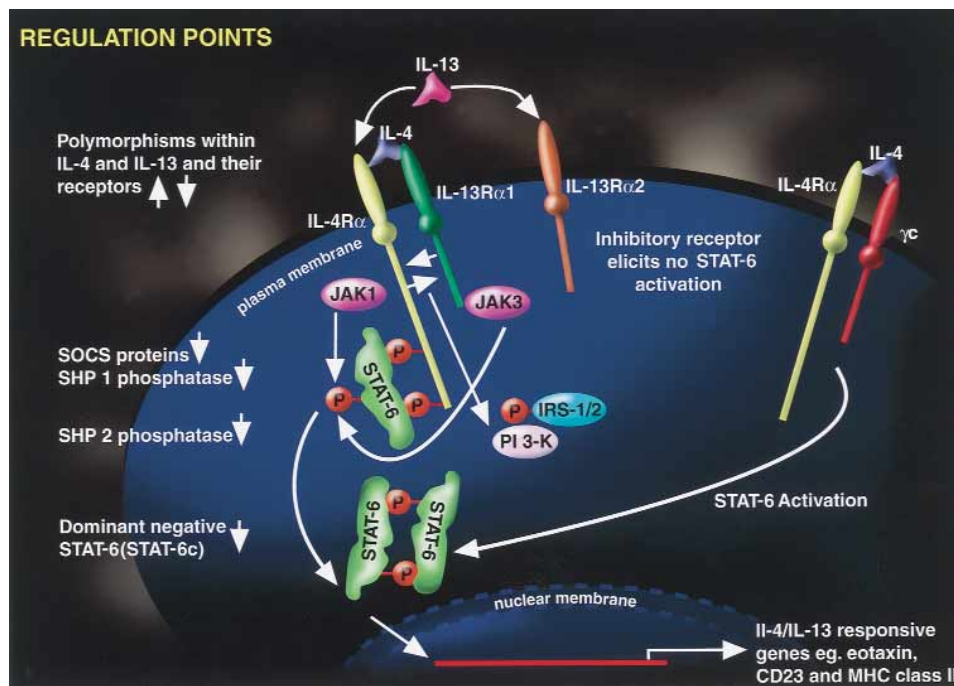


FIG 6. Schematic representation of regulatory points in the IL-4 and IL-13 signaling pathways. Binding of IL-4 or IL-13 to their heterodimeric receptor complexes induces activation by phosphorylation of the associated tyrosine kinases, the JAKs. The phosphorylated receptor serves as a docking site for STAT-6, which also becomes phosphorylated. This enables it to form a homodimer and to translocate to the nucleus to initiate transcription of IL-4/IL-13 responsive genes. The regulatory points in the signaling pathways are indicated on the left; *small arrows* (up or down) indicate their effects on signal transduction.

STAT-6 is strongly expressed in goblet cells is consistent with such a function. Although the *in vivo* studies implicate IL-4 and/or IL-13 receptors in the process of goblet cell differentiation, we observed relatively weak staining for STAT-6 in the basal cell layer. This suggests that low levels of STAT-6 are sufficient for induction of differentiation toward the goblet cell phenotype or that other signaling molecules that activated by IL-4 or IL-13 (eg, phosphatidyl inositol 3-kinase) might play an early role in the differentiation process, STAT-6 expression and activation occurring as the cells stratify to form the goblet cells within the columnar layer. Responses might also be influenced by the types and numbers of IL-4 and IL-13 receptors in the different epithelial cell types. However, though it is known that these receptors are expressed,^{14,15} there is no detailed description of their distribution and relative level of expression in basal cells, ciliated cells, and goblet cells, and there is no detailed information as to how their levels change during the differentiation process.

Although STAT-6 expression in the submucosa was not quantified in the present study, a previous study has provided evidence that STAT-6 is more strongly expressed in infiltrating cells in the submucosa of atopic asthmatic subjects than in that of nonatopic asthmatic subjects.¹⁶ Furthermore, in allergic rhinitic subjects, STAT-6 immunoreactive cells are increased after allergen challenge and correlate with levels of infiltrating CD3⁺ T cells and the numbers of IL-4 mRNA⁺ cells.²⁶ Thus, as indicated in animal models, STAT-6 appears to play an important role in regulating IL-4-mediated responses in several different cell types within the airways.

Although we were able to demonstrate increased levels of STAT-6 in severe asthma, we were unable to determine whether STAT-6 activation was increased in asthma; further immunohistochemical studies with the anti-phospho-STAT-6 antibody used for Western blotting were inconclusive because of low levels of immunostaining (data not shown). According to the results of our *in vitro* studies, activation of epithelial STAT-6 does occur in the presence of IL-4, though we found no evidence of any disease-related difference in STAT-6 activation or functional response as determined by IL-8 release. Thus our results suggest that it is the availability of IL-4 and IL-13 in the asthmatic state that makes the most significant contribution to the difference between the normal individual and the individual with asthma. However, inasmuch as our studies were performed with maximally stimulating doses of IL-4 and IL-13, differences in sensitivity that might arise from functional polymorphisms (see the following paragraph) might not have been apparent. Furthermore, it was evident that the magnitude of IL-8 release in response to IL-4 or IL-13 varied markedly between individual donors. Because we detected the dominant-negative STAT-6c isoform in bronchial epithelial cells, it is possible that this was acting as a natural suppressor of STAT-6 phosphorylation as a result of the absence of an SH2 domain. Future work will be needed to relate the phosphorylation state and transcriptional activity of STAT-6 to the level of STATc expression and functional outcome.

Although a part of the variation in responsiveness might reflect differences in the levels of the dominant-negative STAT-6 isoform, which we found in both normal and asthmatic subjects, there are also several functional polymorphisms of the IL-4/IL-13 receptor systems⁷—as well as of intracellular regulators, including SHP phosphatases and suppressors of cytokine signaling proteins²⁷—that might also contribute to responsiveness (Fig 6). For example, the IL-4R α gene on chromosome 16p12.1 has 8 polymorphisms in the coding region that lead to amino acid changes.²⁸ These include an extracellular variant, I50V, that upregulates receptor responses to IL-4, leading to increased STAT-6 activation and IgE synthesis,²⁹ and Q576R, a polymorphism in the intracellular domain located in the STAT-6 binding region that enhances signaling which is strongly associated with asthma severity. A polymorphism within the STAT-6 gene on chr12q1 5-24 has been associated with mild atopy in a Japanese population.³⁰ We have also identified linkage between asthma phenotypes and microsatellite markers on chromosome 12q13-24,³¹ indicating that disease-related genes are located in this region. Because it is difficult to identify which polymorphisms and their combinations contribute to the associations seen with asthma, it will be important to study their interrelationships by identifying disease-associated haplotypes and their functional effects on signaling in different cell types. With the availability of primary epithelial cell cultures, it will be possible to link phenotypic responses to genotype.

In conclusion, we have demonstrated for the first time the presence of STAT-6 in bronchial epithelium of normal and asthmatic subjects and have found it be raised in severe disease. Although the exact contribution of this transcription factor to altered epithelial function remains to be fully elucidated, our finding of IL-8 release by normal and asthmatic epithelial cell cultures is consistent with the proinflammatory role of these cytokines in asthma. The presence of a naturally occurring dominant-negative isoform suggests a distinct mechanism whereby STAT-6 activity can be modulated, in addition to the diverse influences of genetic polymorphism associated with the IL-4/IL-13 cytokine and receptor systems.

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