Allergen-induced IL-9 directly stimulates mucin transcription in respiratory epithelial cells

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A hallmark of asthma is mucin overproduction, a condition that contributes to airway obstruction. The events responsible for mucin overproduction are not known but are thought to be associated with mediators of chronic inflammation. Others have shown that T-helper 2 (Th2) lymphocytes are required for mucous cell metaplasia, which then leads to mucin overproduction in animal models of allergy. We hypothesized that Th2 cell mediators are present in asthmatic airway fluid and directly stimulate mucin synthesis in airway epithelial cells. Results in cultured airway epithelial cells showed that samples of asthmatic fluid stimulated mucin (MUC5AC) synthesis severalfold more potently than non-asthmatic fluid. Consistent with this, lavage fluid from the airways of allergen-challenged dogs stimulated mucin synthesis severalfold more potently than that from non–allergen-challenged dogs. Fractionation of dog samples revealed 2 active fractions at <10 kDa and 30–100 kDa. Th2 cytokines in these molecular weight ranges are IL-9 (36 kDa), IL-5 (56 kDa), and IL-13 (10 kDa). Antibody blockade of ligand-receptor interaction for IL-9 (but not IL-5 or IL-13) inhibited mucin stimulation by dog airway fluid. Furthermore, recombinant IL-9, but not IL-5 or IL-13, stimulated mucin synthesis. These results indicate that IL-9 may account for as much as 50–60% of the mucin-stimulating activity of lung fluids in allergic airway disease.


Introduction

Mucous cell metaplasia is often observed in airway inflammation and is the leading cause of airway obstruction in asthma, chronic bronchitis, and cystic fibrosis (1–4). A key question in the pathogenesis of these illnesses is the extent to which inflammation itself contributes to mucous cell metaplasia, which then leads to mucin overproduction in animal models of allergy. We hypothesized that Th2 cell mediators are present in asthmatic airway fluid and directly stimulate mucin synthesis in airway epithelial cells. Results in cultured airway epithelial cells showed that samples of asthmatic fluid stimulated mucin (MUC5AC) synthesis severalfold more potently than non-asthmatic fluid. Consistent with this, lavage fluid from the airways of allergen-challenged dogs stimulated mucin synthesis severalfold more potently than that from non–allergen-challenged dogs. Fractionation of dog samples revealed 2 active fractions at <10 kDa and 30–100 kDa. Th2 cytokines in these molecular weight ranges are IL-9 (36 kDa), IL-5 (56 kDa), and IL-13 (10 kDa). Antibody blockade of ligand-receptor interaction for IL-9 (but not IL-5 or IL-13) inhibited mucin stimulation by dog airway fluid. Furthermore, recombinant IL-9, but not IL-5 or IL-13, stimulated mucin synthesis. These results indicate that IL-9 may account for as much as 50–60% of the mucin-stimulating activity of lung fluids in allergic airway disease.
The experiments described in this article indicate that the Th2 cytokine IL-9 acts directly on epithelial cells to stimulate mucin transcription, and they suggest that analysis of the IL-9–triggered mucin response may reveal new antihypersecretion drug targets.

**Methods**

**Cell culture.** All tissue culture media and supplies were obtained from Gibco BRL (Grand Island, New York, USA) or Clonetech (San Diego, California, USA). A human pulmonary mucoepidermoid carcinoma cell line, NCI-H292, was purchased from the American Type Culture Collection (Rockville, Maryland, USA) and maintained in RPMI-1640 with 2 g/L glucose, 0.3 g/L L-glutamine, 2 g/L NaHCO₃, 10% fetal calf serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C in 5% CO₂. Human primary bronchial epithelial cells (Clonetics) were maintained in bronchial epithelial basal medium (BEBM) with added growth factors according to the recommendations of the supplier. Both cell types express receptors for IL-5, IL-9, and IL-13 as determined by RT-PCR of whole-cell RNA (see below).

For function-blocking antibody experiments, antibodies to cytokine receptors were purchased from R&D Systems Inc. (Minneapolis, Minnesota, USA) or Chemicon International (Temecula, California, USA) and used at concentrations recommended by the manufacturers (1–3 μg/mL). We immunoprecipitated cytokines from experimental medium using standard methods and cytokine-or receptor-specific antibodies (R&D Systems Inc.).

**Human tracheal aspirates.** Tracheal aspirates were obtained from subjects recruited from Moffitt-Long and San Francisco General Hospital (University of California–San Francisco, San Francisco, California, USA). Medical consent was obtained, and a medical questionnaire was filled out for each patient. Subjects who had smoked cigarettes or used any other recreational inhalant in the past 10 years were excluded from the study. Also excluded were subjects who had evidence of congestive heart failure or who had a history of lung disease other than asthma. Five patients (4 women, 1 man; mean age 39.8 ± 6.9 SEM) diagnosed as having acute severe asthma by intensive care physicians, and who had been intubated for the management of their asthma, and 5 patients (2 women, 3 men; mean age 32.8 ± 5.4 SEM) undergoing delivery of general anesthesia for nonpulmonary surgical procedures all had tracheal aspirates collected 0–12 hours after intubation. Standard clinical methods were used for collecting tracheal aspirates from healthy and asthmatic subjects in this study. A 30-cm thin plastic catheter (Suction Catheter Kit; Kendall Health Care Products Company, Mansfield, Massachusetts, USA) was passed through the endotracheal tube and into the airway. Mucus was aspirated into a clean plastic container. Aspirate volume was determined and mixed with an equal volume of sterile saline. This was then diluted with saline containing DTT (10% sputolysin [0.01% final concentration]; Behring Diagnostics Inc., Somerville, New Jersey, USA). The aspirated sample (now diluted 1:4 from the original sample) was incubated in a shaking water bath at 37°C for 15 minutes. The samples were then centrifuged at 1,037 g for 5 minutes, and the supernatants were stored at −70°C. We exposed NCI-H292 cells to human tracheal aspirates diluted 1:6 with culture medium for 8 hours. We harvested cultured cell RNA and monitored it for the presence of MUC5AC mRNA by RT-PCR. MUC5AC transcriptional activity was evaluated after 16 hours by measuring luciferase activity (see below).

**Allergic dog lavage.** Dogs were sensitized as pups with ragweed antigen and 1 lung in each of 5 adult dogs was challenged with ragweed. See Collie et al. (21) for detailed sensitization, challenge, and lavage procedures. Challenges were followed by timed bronchoalveolar lavages. For the present study, we focused on the “3-day pre-challenge” and the “4-day post-challenge” samples as these showed the greatest difference in in vitro mucin-inducing activity in preliminary screens. NCI-H292 cells were transfected with MUC5AC luciferase (see below) and treated with dog lavage samples diluted 1:3 with serum-free RPMI. Macrosep centrifugal concentrators (Pall Filtron Corp., Northborough, Massachusetts, USA) were used for fractionation of lavage fluids by molecular weight (100kDa, 30kDa, 10kDa, and 3kDa) according to manufacturer’s recommendations. The Macrosep device was rinsed with buffer, loaded with biological fluid, and then centrifuged at 3,000–5,000 g for 60–90 minutes. All retentates were then volume corrected with PBS to maintain original concentration of the retained molecules. Some lavage samples were incubated with proteinase K (1:20, proteinase/protein ratio) at 60°C for 1 hour followed by 15 minutes of boiling to determine whether the...
active factor was a protein. Negative controls consisting of PBS with proteinase K alone were used to determine possible effects of proteinase K on mucin transcription.

RT-PCR. Reverse transcription of RNA to cDNA was carried out using the following reaction mixture: 5 μg total RNA from each sample in 8 μL diethyl phosphate–treated (DEPC) water, 4 μL 5X first strand buffer ( Gibco BRL), 2 μL 10 mM deoxynucleotide triphosphate (dNTP) ( Gibco BRL), 1 μL RNase inhibitor (40 U; Roche Molecular Biochemicals, Indianapolis, Indiana, USA), 2 μL 10X hexonucleotides (Roche Molecular Biochemicals), and 1 μL SuperScript II (Gibco BRL). The reaction mixture was incubated at 37 °C for 1 hour, then at 75 °C for 15 minutes, and stored at 4°C. PCR was carried out using Ready-To-Go PCR Beads (Pharmacia Biotech Inc., Piscataway, New Jersey, USA) in a 25-μL reaction mixture containing: 1.5 U Taq polymerase, 10 mM Tris-HCl (pH 9.0 at room temperature), 50 mM KCl, 1.5 mM MgCl2, 200 μM of each dNTP and stabilizers, including BSA, 0.2 μM of each primer, and 1 μL of RT-PCR reaction product. Custom primers were obtained from Life Technologies (Gaithersburg, Maryland, USA). Human GAPDH primers were 3′: GGG GTC TAC ATG GCA ACT GTG AGG AGG GGA and 5′: AAA CCT GCC AAA TAT GAT GAC ATC AAG AAG; human MUC5AC primers 3′: TCA CAG CCG GGT ACG CTG TGG CAC AAG TGG and 5′: TGC TAT TAT GCC CTG TGT AGC CAG GAC TGC; human IL-9 receptor (IL-9R) α primers were 3′: TGC CTG ACA AAC TCA GTG CCA CAC TGG and 5′: CCT GTG TGA ACC TGT CGT GCA AAG CTC; human IL-13Rα primers were 3′: GCC CTT GTT AGG ATC AAA CCC ATC and 5′: TGA GTC TAA CGG TCT TCC GGA TGA; human IL-5Rα primers were 3′: CAA CAA GCC AGG TGC AGT GAAG and 5′: CCA CTG AGA TAC TGC AAG CTG AC. We used a standard PCR protocol: 5 minutes at 95 °C followed by 35 cycles of 30 seconds at 95 °C, 30 seconds at either 60 °C or 55 °C (depending on the annealing temperature of the primers), and 60 seconds at 72 °C. After cycling, the samples were kept at 72 °C for 20 minutes and stored at 4 °C. Samples were run on a 1.2% agarose gel containing ethidium bromide for visualization under an ultraviolet lamp.

Luciferase assay. Approximately 3.7 kb of the 5′ flanking region of the human MUC5AC gene (22) was cloned into pGL3-Basic luciferase vector (Promega Corp., Madison, Wisconsin, USA). NCI-H292 cells were seeded in 6-well tissue culture plates (10^5 cells/well) and left for 18 hours in complete growth medium. Lipofectamine (Gibco BRL) transfection of cells was done in accordance with the instructions of the manufacturer. Briefly, 1 μg DNA, 0.8 μg histone, and 2 μg Lipofectamine in 1 mL OPTIMEM (Gibco BRL) reduced serum medium was added to each well. After 4 hours, the OPTIMEM medium was replaced with complete growth medium and the cells allowed to recover overnight. Luciferase activity was measured according to manufacturer’s recommendations (Promega Corp.). Briefly, 250 μL of lysis buffer (Promega Corp.) was added to each well and plates were agitated for 1 hour at room temperature. The wells were then scraped with a rubber cell scraper, and the contents of the wells were transferred into 1.5-mL tubes and briefly centrifuged to remove cell debris. Twenty microliters of supernatant was added to 100 μL luciferase assay reagent and light units measured for 30 seconds on a Monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, California, USA). All transfections were carried out in triplicate. Luciferase activity was normalized with respect to relative light units emitted from serum-free, medium-treated controls.

ELISA. Serial dilutions of each dog lavage or human aspirate sample were plated in duplicate on 96-well microtiter plates (Immulon; PGC Scientific, Gaithersburg, Maryland, USA). Wells containing no sample served as negative controls, and wells containing recombinant human IL-9, IL-5, or IL-13 (R&D Systems Inc.) served as positive controls and concentration standards on each plate. Antigen was dried onto the plates

**Figure 2**
Ragweed-challenged dog lavage stimulates MUC5AC reporter in airway epithelial cells in culture. NCI-H292 cells were transfected with the 3.7-kb MUC5AC luciferase construct and challenged with BAL for 16 hours. Bars represent means ± SEM (n = 4 dogs in triplicate). * Significantly different from SFM control; P < 0.05.

**Figure 3**
Fractionated dog lavage stimulates MUC5AC reporter in airway epithelial cells in culture. NCI-H292 cells were transfected with the 3.7-kb MUC5AC luciferase construct and challenged with BAL for 16 hours. Bars represent means (n = 4 dogs in duplicate).
Figure 4
Demonstration of receptor RNA for IL-9, IL-5, and IL-13 in NCI-H292 airway epithelial cell line (H292) and primary cultures of human primary bronchial epithelial cells (HBE) by RT-PCR of unique receptor α subunits.

Figure 5
Demonstration of cytokines in dog airway lavage and human airway aspirate.
(a) ELISA showing IL-5, IL-9, and IL-13 in post-RW lavage compared with pre-RW lavage. (b) ELISA showing IL-5, IL-9, and IL-13 in normal and asthmatic aspirates. Bars represent means ± SEM (n = 4 in duplicate). *Significantly different from pre-RW (a) or normal (b) control.
in either anti–IL-9R (R&D Systems Inc.) or isotype control (anti-trinitrophenol; PharMingen, San Diego, California, USA) for 2 hours. After washing in PBS/Tween 20, the tissue was treated with a secondary biotinylated antimouse IgG (Vector Laboratories) for 1 hour and washed thoroughly. The slides were then developed with diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories) for 4 minutes at room temperature and counterstained with hematoxylin (Sigma Chemical Co.).

Intratracheal instillation of IL-9 in mice. Female (age 8 weeks, weighing 20–23 g) C57BL6/J mice were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA). Mice were anesthetized with Avertin (2.5% solution, 0.15 mL/10 g intraperitoneally). The trachea was surgically exposed by making a 1.5-cm incision in the skin and carefully separating the thyroid gland and overlying strap muscles. A 100-μL volume of rmIL-9 (10 μg/mL in PBS; R&D Systems Inc.), or PBS was instilled into the trachea using a 27-gauge needle. The incision was closed with surgical silk, and the mice were observed until they recovered from anesthesia (usually 1 hour). The mice were returned to filter-topped, autoclaved cages for 1 week with free access to food and water.

The mice were sacrificed by cervical dislocation, and their lungs were excised and fixed by perfusion through the trachea with 4% paraformaldehyde at a constant pressure of 20 cm H2O. After 2 hours perfusion-fixation, the lungs were immersed in a large volume of the same fixative overnight. Tissues were embedded in paraffin, sectioned (5 μm), and then stained with periodic acid/Schiff reagent (PAS) and hematoxylin.

The trachea and main stem bronchi were the focus of study as these airways have a quantifiable population of mucin-producing goblet cells, and these airway structures are easily identifiable in section. We obtained measurements from 17–19 cross sections of the main stem bronchi from each mouse and 14–16 longitudinal sections of each trachea taken at 20-μm intervals. Using ×400 magnification, all PAS-positive cells lining the trachea and main stem bronchi were counted. The length of the basal lamina for each sectioned airway was determined using a computerized stereology system that used software capable of making calibrated linear measurements from contour tracings (Stereoinvestigator; Microbrightfield Inc., Colchester, Vermont, USA). The data for each group were expressed as the mean number of PAS-positive cells per millimeter of basal lamina.

Results

Upregulation of MUC5AC by asthmatic airway fluid. Exposure to tracheal aspirates from asthmatic and non-asthmatic patients caused MUC5AC mRNA (Figure 1a) and transcriptional activity (Figure 1b) to increase in NCI-H292 cells. The increase in transcriptional activity was severalfold greater in response to asthmatic aspirates when compared with non-asthmatic aspirates.

Because of difficulties inherent in obtaining and working with human samples, we evaluated the possibility of using allergic airway fluids from animals. Results showed that dog bronchoalveolar lavage, like human airway fluid, stimulated MUC5AC transcription; post-challenge (ragweed) lavage was severalfold more potent than pre-challenge lavage (Figure 2). Stimulation by both human and dog lavage was inhibited by proteinase K treatment of the lavage samples (not shown), thus indicating that the active factors in both human and dog samples were proteins.

Fractionation of dog lavage samples revealed activities in fractions containing molecules between 30 and 100 kDa and less than 10 kDa (Figure 3). The stimulatory effect of full medium could be essentially reconstituted by combining the effects of the 30–100 and less than 10 kDa fractions, which contributed 62% and 32%, respectively, to the overall response. Based on data published previously implicating Th2 mediators in allergy-associated mucin production (17), we selected those Th2 cytokines between 30-100 kDa and <10 kDa (i.e. IL-5, 9, or 13) as candidate pro-mucin cytokines (Table 1).

Table 1

| Known cytokines categorized by molecular weights (based on review by Curfs et al. [17]). Categories reflect the molecular weight cutoffs used for fractionation. Th2-type cytokines are boldfaced. |
|-------------------------------|-----------------|-----------------|-----------------|
| TNFβ = 20-180 kDa | ≥100 | ≤100 | ≤30 | ≤10 |
| IL-5 = 50 kDa | IL-9 = 39 kDa | IL-12 = 76 kDa | IL-14 = 60 kDa | IL-16 = 56 kDa | IL-17 = 38 kDa |
| IL-1 = 18 kDa | IL-1ra = 18 kDa | IL-2 = 20 kDa | IL-3 = 15-40 kDa | IL-4 = 14 kDa | IL-6 = 26 kDa | IL-7 = 22 kDa |
| IL-IL-10 = 18 kDa | IL-11 = 24 kDa | IL-15 = 24 kDa | TGFB = 25 kDa | TGFβ = 25 kDa |
| IL-1 = 18 kDa | IL-1ra = 18 kDa | IL-2 = 20 kDa | IL-3 = 15-40 kDa | IL-4 = 14 kDa | IL-6 = 26 kDa | IL-7 = 22 kDa |
A prerequisite for testing the pro-mucin effects of these cytokines was evidence showing that receptors for all 3 cytokines were present on airway epithelial cells. We found that NCI-H292, the epithelial cell line used in our transcription assays, synthesizes RNA encoding receptors for IL-9, IL-5, and IL-13 at approximately the same levels as those seen in primary bronchial epithelial cells (Figure 4). ELISA analysis in dog tracheal lavage and showed that all 3 cytokines were increased in lavage fluid in response to ragweed challenge (Figure 5a). In addition, the levels of IL-9 and IL-13 were elevated in asthmatic compared with normal human tracheal aspirates (Figure 5b).

To examine the role of cytokines IL-9, IL-5, and IL-13 in mucin stimulation, we exposed epithelial cells to dog BAL in the presence of function-perturbing antibodies directed against 2 IL receptors (hIL-5Rα or hIL-9R), or against the cytokine IL-13. Neither anti-IL5Rα nor anti-IL13 significantly inhibited the effect of dog BAL on MUC5AC transcription (P > 0.05 for both, Figure 6). In contrast, anti-IL-9R attenuated the dog BAL effect in a concentration-dependent manner by up to 60% (P < 0.05; Figure 6). Isotype controls, mouse IgG and goat IgG, had no effect (P > 0.05; Figure 6). Consistent with the results obtained using anti-receptor antibodies, human recombinant IL-9, but not IL-5 or IL-13, increased MUC5AC transcription (Figure 7a). The effect was dose-dependent (Luciferase = (0.003)(dose) + 1.321; r² = 0.976). Recombinant human IL-9 increased MUC5AC message in NCI-H292 cells as measured by RPA (Figure 7b). A similar increase in MUC5AC steady state was observed when human primary bronchial epithelial cells were treated with rhIL-9 (Figure 7c).

Demonstration of IL-9R in human airway epithelium and NCI-H292 cells. Bronchial biopsies were immunohistochemically stained for the presence of IL-9R (Figure 8, a and b). Pseudostratified epithelial cells had the greatest degree of immunoreactivity, but staining was also associated with the endothelium of small blood vessels and smooth muscle fibers in the lamina propria. There were no obvious differences in the degree of IL-9R epithelial staining between the normal and asthmatic biopsies. To confirm that our cell line also expressed IL-9R protein, Western blots were performed using cellular extracts from NCI-H292 cells and K562 human lymphoblasts (positive control) (Figure 8c).

Upregulation of goblet cell concentration by IL-9 in mouse tracheas. Tracheal instillation of rmIL-9, but not the PBS vehicle, significantly increased the number of PAS-positive cells in both the trachea and main stem bronchi of mice (Figure 8) (n = 3 animals per group; P < 0.05).

Discussion
Mucin overproduction is a common feature of asthma and an important contributor to airway obstruction. The strong correlation between mucus overproduction and inflammation in asthma suggests that inflammation might directly stimulate mucin synthesis. Recently, this was supported by animal studies that went on to identify Th2 cells as being instrumental in the generation of mucin-containing epithelial cells in allergic airways (17, 24). That study did not, however, indicate whether Th2 mediators acted directly to stimulate mucin production in epithelial cells or acted through an intermediate cell type.

Here we analyzed the airway fluid of asthmatic subjects to identify factors that can directly stimulate mucin transcription. Using a human MUCSAC mucin transcription assay and differential filtration we found major activities between 30 and 100 kDa and below 10 kDa. Based on results from Cohn et al. (24), we focused on Th2 cytokines in these size ranges (IL-5, IL-13, and IL-9). In a series of experiments using cytokine- and cytokine receptor function-perturbing antibodies, we observed inhibition of the mucin-stimulating effect of dog airway fluid by an antibody inhibiting the effects of IL-9, but not IL-5 and IL-13. Support for the hypothesis that IL-9...
IL-9 does directly stimulate mucin transcription, whereas IL-5 and IL-13 do not, was provided by results from the converse experiment in our reporter assay showing that recombinant human IL-9 did stimulate mucin transcription, whereas IL-5 and IL-13 did not.

However, IL-9 may not be the only substance in asthmatic airway fluid contributing to mucin production. Although we could stimulate MUC5AC transcription with physiologic doses of rhIL-9, our receptor blocking data showed that anti-IL-9R inhibited only approximately 50%–60% of the effect of post-challenge dog BAL treatment. Our data therefore suggest the presence of additional stimulatory factors and possibly inhibitors of IL-9/IL-9R in the lavage fluid.

The identity of accessory factors remains unknown. Obvious sources are proteins other than Th2 cytokines present in the active fractions of airway fluid. For example, IL-8 (8 kDa) was abundant in our asthmatic samples and could contribute to mucin stimulation. However, this is unlikely based on our data showing that rhIL-8 fails to stimulate mucin transcription (data not shown). Thus, there are pro-mucin factors yet to be defined in allergic BAL. Isolating and characterizing these accessory pro-mucin factors will be the object of future studies.

The Th2 cytokines IL-4, IL-5, and IL-9 are regarded as pro-mucin agents because airway overexpression of these 3 cytokines is correlated with the appearance of an increased number of mouse airway goblet cells (18–20). Recently, however, it was shown that sensitized Th2 cells and ovalbumin could induce mucus hyperplasia in mouse airways even when Th2 cells failed to express IL-4 or IL-5 (17). Our results are consistent with this because they show that antibodies directed against IL-4 receptor (not shown) or IL-5 receptor failed to inhibit the response to dog BAL.

Our studies are performed on a simplified in vitro model of the mammalian airway. To confirm that the stimulation of mucin production by IL-9 occurs in vivo as well as in vitro, we administered rmIL-9 to mice intratracheally. Consistent with the in vitro data, we found that mice receiving IL-9 intratracheally showed an increased number of mucus cells in their tracheas and main stem bronchi 1 week later. This indicates that the ability of IL-9 to stimulate mucin in vitro is reflective of a biological phenomenon that also occurs in vivo.

How does IL-9 exert its effect on mucin synthesis? It is clear from the work of Kay et al. (13) that Th2 lymphocytes are present in asthmatic airways in numbers exceeding those found in normal airways. Moreover, the adoptive transfer experiments of Cohn et al. (24) indicate
that TH2 cells, which release IL-9, contribute to mucin overproduction in an animal model of the disease. Shimbara et al. (25) have recently shown that the IL-9 message is dramatically increased in the human asthmatic airway, and the primary source of this cytokine is activated TH2 lymphocytes. Although IL-9 effects had been described previously for only hematopoietic cells, our data reveal both IL-9 receptors and IL-9 responsiveness in epithelial cells. We have shown that IL-9 receptors are present on airway epithelial cells, but a preliminary comparison of asthmatic and normal bronchial biopsies suggests that the number of IL-9 receptors is not increased in asthma. This is supported by observations by Shimbara et al. (25) of asthmatic bronchial biopsy tissue. This strongly suggests that TH2 cell–released IL-9 activates epithelial IL-9 receptors that are normally present on airway epithelium, this in turn triggers a signaling cascade that activates MUC5AC transcription. Notably, the studies here consider only 1 of the 9 known human mucin genes. It is possible that other mucin genes are upregulated in asthma by other cytokines.

The effects of IL-9 on hematopoietic cells (e.g., mast cells and lymphocytes) are mediated by JAK/STAT signaling pathways (26). Because there is no previous evidence for IL-9 effects on epithelial cells, the nature of the relevant signaling cascades is unknown. Analysis of the IL-9 signaling pathway in epithelial cells may suggest novel therapeutic approaches to the treatment of mucus hypersecretion.

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