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Accumulation of intraepithelial mast cells with a unique protease phenotype in T_H2-high asthma

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Abstract

Background—Previously, we found that mast cell tryptases and carboxypeptidase A3 (CPA3) are differentially expressed in the airway epithelium in asthmatic subjects. We also found that asthmatic subjects can be divided into 2 subgroups (“T_H2 high” and “T_H2 low” asthma) based on epithelial cell gene signatures for the activity of T_H2 cytokines.

Objectives—We sought to characterize intraepithelial mast cells (IEMCs) in asthma.

Methods—We performed gene expression profiling in epithelial brushings and stereology-based quantification of mast cell numbers in endobronchial biopsy specimens from healthy control and asthmatic subjects before and after treatment with inhaled corticosteroids (ICSs). We also performed gene expression and protein quantification studies in cultured airway epithelial cells and mast cells.

Results—By means of unsupervised clustering, mast cell gene expression in the airway epithelium related closely to the expression of IL-13 signature genes. The levels of expression of mast cell genes correlate positively with lung function improvements with ICSs. IEMC density was 2-fold higher than normal in subjects with T_H2-high asthma compared with that seen in subjects with T_H2-low asthma or healthy control subjects ($P = .015$ for both comparisons), and these cells were characterized by expression of tryptases and CPA3 but not chymase. IL-13 induced expression of stem cell factor in cultured airway epithelial cells, and mast cells exposed to conditioned media from IL-13-activated epithelial cells showed downregulation of chymase but no change in tryptase or CPA3 expression.

Conclusion—IEMC numbers are increased in subjects with T_H2-high asthma, have an unusual protease phenotype (tryptase and CPA3 high and chymase low), and predict responsiveness to ICSs. IL-13-stimulated production of stem cell factor by epithelial cells potentially explains mast cell accumulation in T_H2-high asthmatic epithelium.

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Keywords

Asthma; mast cells; tryptase; chymase; carboxypeptidase A3; stem cell factor

Mast cells play key roles in host defense, homeostasis, tissue repair, and mechanisms of allergic inflammation.¹ Two major human mast cell phenotypes have been described²: (1) mast cell–tryptase (MC-T), or mucosal mast cells found in the lung and gastrointestinal tract, and (2) mast cell–tryptase/chymase (MC-TC), or connective tissue mast cells found predominantly in the skin, peritoneum, and joints. MC-T cells predominantly express high levels of tryptases but not chymase or carboxypeptidases, whereas MC-TC cells express all 3 proteases.³ In lung tissues MC-T cells usually predominate, but smooth muscle, submucosal glands, blood vessels, and pleura are enriched in MC-TC cells.^{4,5}

In recent genome-wide expression studies of cells obtained by means of epithelial brushing during research bronchoscopy in asthmatic and control subjects, we found that 2 mast cell genes (*tryptase* and *carboxypeptidase A3* [CPA3]) are among the top 10 most differentially expressed genes in asthmatic subjects. We also found that 3 other genes (*periostin*, *chloride channel accessory 1* [CLCA1], and *serpin B2*) among the top 10 most differentially expressed genes are regulated by IL-13.⁶ We subsequently proposed these 3 genes as a signature for IL-13–induced activation of airway epithelial cells.⁷ Surprisingly, when we classified asthmatic subjects based on expression of IL-13–inducible genes in epithelial brushings, we identified 2 evenly sized and distinct subgroups: one that had an IL-13 signature and another that did not.⁷ Our approach was unsupervised hierarchical clustering based on the microarray expression levels of *periostin*, *CLCA1*, and *serpin B2*. These 2 subgroups differed significantly in gene expression for IL-5 and IL-13 in homogenates of bronchial biopsy specimens, and we therefore named the 2 groups “T_H2 high” and “T_H2 low.”

Our findings for increases in expression of mast cell proteases in airway epithelial brushings in an unbiased gene-profiling study in asthmatic subjects suggest that mast cells infiltrate the airway epithelium in asthmatic subjects. Increases in intraepithelial mast cell (IEMC) numbers in asthmatic subjects have been shown in some prior studies^{8,9} but not in others,^{10,11} and all of these studies have been limited by relatively small numbers of subjects, imprecise methods of quantification, and confounding by inhaled corticosteroid (ICS) use. Therefore uncertainty remains about whether IEMC numbers are increased in asthmatic subjects, and little is known about the phenotype of this mast cell subset *in vivo*. In addition, it is unknown whether T_H2 status influences mast cell accumulation in the airway epithelium. Here we set out to use rigorous stereology-based methods in a relatively large number of steroid-naïve asthmatic subjects to determine whether the numbers of IEMCs are increased in asthmatic subjects and to determine the influence of T_H2 status on these numbers. We also used PCR-based gene profiling and immunohistology to determine the expression of tryptases, CPA3, and chymase in IEMCs in asthmatic subjects.

METHODS

Airway Tissue Bank at the University of California, San Francisco

We studied biological samples from the Airway Tissue Bank at the University of California, San Francisco (UCSF). These samples had been collected during research bronchoscopy in healthy and asthmatic volunteers. Bronchoscopy included collection of epithelial brushings and bronchial biopsy specimens by using methods previously described.^{6,12–14} For this study, we reviewed mast cell gene expression data from our previously generated epithelial

microarray and quantitative RT-PCR (qPCR) data⁶ and then generated new data by using samples from the tissue bank.

Study population

We studied 42 steroid-naïve subjects with mild-to-moderate asthma and 36 healthy nonasthmatic control subjects (Table I). Thirty-two of the asthmatic subjects were also enrolled in a double-blind randomized controlled clinical trial of inhaled fluticasone (500 µg twice daily) or matched placebo (ClinicalTrials.gov Identifier: NCT00187499).

Gene expression

Microarray analyses on epithelial brushings had been performed as described previously.⁷ These data are available in MIAME-compliant format at Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo/>, accession no. GSE4302). Real-time RT-PCR (qPCR) analyses for *tryptases*, *CPA3*, and *chymase* were performed with RNA from epithelial brushings by using methods previously described⁶ and with primers and probes listed in Table E1 (available in this article's Online Repository at www.jacionline.org). Summary data for the *tryptase* and *CPA3* PCR results presented here were published previously as validation of gene expression data obtained by using microarrays.⁶

Design-based stereology

Four to 6 bronchial biopsy specimens had been embedded in paraffin with isector molds and regular paraffin molds, as previously described.¹³ Three serial sections, 3 µm thick, were cut, and tryptase immunostaining was used to identify mast cells (see below). We used the Computer Assisted Stereology Toolbox (C.A.S.T.) grid system (Olympus, Albertslund, Denmark) to measure the numeric density of mast cells using the physical dissector method (full details are available in the Methods section of this article's Online Repository at www.jacionline.org).^{14,15}

Immunohistochemistry

The antibody reagents used were as follows: (1) tryptase, AA1, mouse monoclonal anti-human mast cell tryptase (Thermo Scientific, Fremont, Calif); (2) chymase, CC1 mouse monoclonal anti-human mast cell chymase (ABD Serotec, Oxford, United Kingdom); (3) CPA3, rabbit anti-human mast cell CPA3 (Sigma, St Louis, Mo); and (4) basophils, BB1 mouse mAb against basogranulin (a generous gift from Dr Andrew Walls). Three-micrometer sections of formalin-fixed and paraffin-embedded lung sections from a patient who died from asthma were used to test and optimize the mast cell protease antibodies.

Studies in cultured airway epithelial cells

Normal human bronchial epithelial cells (Clonetics, San Diego, Calif) were cultured in Grey media and seeded onto 12-well transwell inserts, as described in the Methods section of this article's Online Repository. Cells were then grown at the air-liquid interface with the addition of cytokines to the basal media for 4 days: control (Grey media alone), IL-13 (10 ng/mL), TNF-α (10 ng/mL), or IL-1β (10 ng/mL). On day 4, cells and media were collected for analysis (full details are available in the Methods section of this article's Online Repository).

Studies in cord blood–derived cultured mast cells

Mast cells were derived from umbilical cord blood mononuclear cells (as described in the Methods section of this article's Online Repository). The cells were exposed to conditioned media (CM) from human bronchial epithelial cells that had been activated with IL-13 (10

ng/mL on days 0 and 2) or control (no cytokine). Mast cells were cultured with the epithelial cell CM for 2 days and processed for immunostaining and qPCR.¹⁶

Statistics

Values are presented as means $\pm$ SDs or medians (interquartile ranges) unless otherwise specified. Correlation was performed with the Pearson correlation. Nonparametric 2-group comparisons were made with the Wilcoxon signed-rank test, and multiple group comparisons were made with Kruskal-Wallis 1-way ANOVA. A 2-tailed *P* value of less than .05 was taken as statistically significant. All statistical analyses were performed with STATA SE 10.1 software (StataCorp, College Station, Tex). Unsupervised hierarchical clustering of gene expression data with Pearson correlation as a distance metric was performed with the R package hclust (see the Methods section of this article's Online Repository).

RESULTS

Gene expression for mast cell proteases is increased in asthmatic epithelium, especially in the T_H2-high subgroup of asthma, and predicts responsiveness to ICSs

We previously provided summary data for gene expression for *tryptase* and *CPA3* in epithelial brushings from 42 steroid-naïve subjects with asthma and 28 healthy control subjects.⁶ These summary data showed that tryptase and CPA3 expression was higher than normal in asthmatic subjects. Here we show that each of these 2 genes is overexpressed in some, but not all, of the 42 asthmatic subjects (Fig 1). Interestingly, using microarray- or qPCR-based gene expression profiling, we found no difference in chymase expression in airway epithelial brushings in asthmatic subjects (Fig 1). Mast cells expressing both *tryptase* and *CPA3* traditionally are of the MC-TC phenotype and also coexpress chymase. This was not the case here, suggesting a unique pattern of protease gene expression by IEMCs in asthmatic subjects. Notably, the ratio of *CPA3* to *tryptase* was similar in the asthmatic and healthy subgroups (0.46 vs 0.39, *P* = .13), indicating that the IEMC phenotype of increased *CPA3* expression is not asthma specific but rather indicative of an intraepithelial cell phenotype, of which there is an accumulation in asthmatic subjects. We did observe a trend for an increased *CPA3/tryptase* ratio among subjects with T_H2-high asthma, suggesting that *CPA3* expression is especially increased in IEMCs from subjects with T_H2-high asthma (see Fig E1 in this article's Online Repository at www.jacionline.org).

To gain insight into potential mechanisms of mast cell accumulation within the airway epithelium, we examined the relationships between expression levels of the 2 mast cell genes and other genes in the microarray expression data from epithelial brushings among the 42 asthmatic and 28 healthy subjects. Specifically, we performed unsupervised hierarchical clustering analysis to classify genes based on overall gene expression without regard to subject category labels. The hierarchical tree yielded 40 gene clusters, the largest of which contained 40 genes. This cluster includes several mast cell protease genes: tryptases *TPSAB1* and *TPSB2*, *CPA3*, and the mast cell surface receptor c-kit (*KIT*), which is a transmembrane receptor for the mast cell growth stimulant and attractant stem cell factor (*SCF*; otherwise known as kit ligand; Fig 2). Notably, in the same "mast cell cluster" we found *periostin*, *serpin B2*, and *CLCA1*, the 3 genes we have previously proposed as an epithelial gene signature for T_H2-high asthma. *Tryptase* gene expression was found to strongly correlate with each of these T_H2 signature genes (see Fig E2 in this article's Online Repository at www.jacionline.org). Although *SCF* was not specifically found in this cluster, we did find significantly higher epithelial *SCF* gene expression among subjects with T_H2-high asthma compared with that seen in subjects with T_H2-low asthma and healthy control subjects (see Fig E3 in this article's Online Repository at www.jacionline.org). These findings suggest

that upregulation of mast cell gene expression in the airway epithelium in asthmatic subjects occurs specifically in the T_H2-high subgroup.

A subset of the 42 asthmatic subjects (n = 32) had been enrolled in a clinical trial in which they had been randomized to either inhaled fluticasone, 500 µg twice daily, or inhaled placebo for 8 weeks after bronchoscopy. Here we examined the relationship between baseline gene expression levels for *tryptase* and *CPA3* in epithelial brushings and fluticasone-induced improvement in lung function. In fluticasone-treated asthmatic subjects we found significant positive correlations between baseline expression of tryptase in epithelial brushings and the change in FEV₁ at 4 weeks ($r = 0.53$, $P = .03$) and between baseline expression of *CPA3* and the change in FEV₁ at 4 weeks ($r = 0.48$, $P < .05$; Fig 3). This analysis shows that asthmatic subjects with higher baseline expression levels for mast cell genes in their epithelium have greater improvements in lung function, suggesting that some of the clinical improvement from ICSs could be a result of decreased activation of IEMCs.

Number of IEMCs is increased in asthmatic subjects, especially in those with T_H2-high asthma

We examined a subset of samples from the UCSF Airway Tissue Bank with adequate intact epithelium (26 asthmatic 23 healthy control subjects) and quantified IEMC density using design-based stereology. We found that the number of IEMCs was higher than normal in asthmatic subjects (Fig 4, A); when stratified by T_H2 subgroup, the number of IEMCs was significantly higher in the subjects with T_H2-high asthma than the subjects with T_H2-low asthma, and the T_H2-low subgroup did not differ significantly from normal values (Fig 4, B).

IEMCs and not basophils express tryptase

Basophils express tryptase, albeit at lower expression levels than mast cells, and basophils are increased in sputum from asthmatic subjects¹⁷ and increase after allergen challenge.¹⁸ To confirm that the tryptase gene expression in epithelial brushings in asthmatic subjects derives from mast cells and not basophils, we immunostained serial sections of airway biopsy specimens for basophils by using a basophil-specific antibody (BB1).¹⁹ We found that basophil staining was sparse or absent in the airway epithelium and that tryptase-positive cells within the epithelium did not costain with the basophil antibody (Fig 5, A and B). The positive control slides (fatal asthma) showed that the BB1 antibody worked to show basophil staining (see Fig E4 in this article's Online Repository at www.jacionline.org). We conclude that tryptase-positive cells in the airway epithelium in asthmatic subjects are mast cells and not basophils.

IEMCs express tryptase and CPA3 but not chymase

We immunostained serial sections of airway mucosal tissue biopsy specimens from asthmatic subjects for tryptase, CPA3, and chymase. We consistently found that IEMCs expressed tryptase and CPA3 but not chymase (Fig 5). We observed chymase-positive staining in submucosal mast cells and mast cells localized to smooth muscle cells, but we were unable to detect chymase staining in IEMCs. These data confirm the results of gene expression by means of microarray and qPCR from epithelial brushings and suggest a unique pattern of protease expression among IEMCs.

Expression of SCF, an important mast cell growth factor and chemokine, is induced in airway epithelial cells by IL-13

As noted above, we identified a mast cell gene cluster that included several mast cell protease genes and c-kit, an important cell-surface receptor on mast cells. The major ligand for c-kit is SCF, which is an important growth factor for mast cells and is expressed by airway epithelial cells.^{20,21} Given our finding for an increase in IEMCs in subjects with T_H2-high asthma, we hypothesized that IL-13 stimulates SCF production by epithelial cells and therefore promotes intraepithelial accumulation of mast cells. Using an *in vitro* model of primary bronchial epithelial cells grown at an air-liquid interface and stimulated with IL-13, we found that IL-13 induced a significant increase in *SCF* gene expression (Fig 6, A). In addition, we found that IL-13 induced secretion of SCF protein into the basal medium underneath the epithelial cells (Fig 6, B), a finding that provides a mechanism for how epithelial secretion of SCF could promote migration of mast cells from the submucosa to the epithelium.

CM from IL-13–treated epithelial cells promote the IEMC protease phenotype

Epithelial cells can promote and support the growth of mast cells *in vitro*.²¹ Given the unique protease phenotype of IEMCs (chymase low and CPA3/tryptase high) and their accumulation in subjects with T_H2-high asthma, we hypothesized that CM from epithelial cells treated with IL-13 would promote changes in IEMC gene expression consistent with this phenotype. We cultured cord blood–derived mast cells in the presence of CM from epithelial cells treated with IL-13–supplemented or control media and analyzed gene and protein expression (Fig 7). We found that CM from IL-13–treated epithelial cells significantly downregulated *chymase* gene expression by cord blood–derived cultured mast cells, whereas there was no significant change in expression of *tryptase* or *CPA3*. (Of note, there were no significant changes in gene expression with IL-13 alone [data not shown].) Mast cell chymase is packaged within granules, and therefore the effect of IL-13 we observe here might be to cause degradation of granules with chymase and formation of new granules lacking chymase. This is consistent with our immunostaining of IEMCs *in vivo* and suggests a direct role for the epithelium under the influence of T_H2 cytokines to directly modify the phenotype of IEMCs after their recruitment into the epithelium.

DISCUSSION

We found that the numbers of mast cells in the airway epithelium were increased in some, but not all, of the 26 asthmatic subjects we studied. By classifying the asthmatic subjects as having T_H2-high or T_H2-low asthma based on expression of IL-13–responsive genes in their epithelial brushings, we discovered that IEMC numbers in the T_H2-high subgroup were significantly higher than those in the T_H2-low subgroup. In fact, the numbers of IEMCs in the T_H2-low subgroup were similar to those in the healthy nonasthmatic subjects. We also found that mast cell genes and T_H2 signature genes clustered together in unsupervised hierarchical clustering analyses of the microarray data from epithelial brushings from asthmatic and healthy subjects, lending further support to a link between IEMCs and T_H2 inflammation. Thus our findings here add increases in IEMC numbers to the list of pathologies characteristic of T_H2-high asthma. Other characteristics we have reported include airway and systemic eosinophilia, sub-epithelial fibrosis, and abnormal expression of gel-forming mucins.⁷

The levels of expression of *tryptase* and *carboxypeptidase* in epithelial brushings in the asthma subgroup correlated positively with lung function improvement after treatment with ICSSs. This finding is consistent with the T_H2 subtype of asthma being corticosteroid

sensitive,⁷ and it suggests that some of the clinical improvement from ICSs could be a result of decreased activation of IEMCs.

c-kit, a cell-surface receptor on mast cells, was also in this mast cell cluster. SCF is a key ligand for *c-kit*, and levels of SCF correlate with asthma disease severity.²² We found *SCF* expression to be increased among subjects with T_H2-high asthma and also found *SCF* expression to be increased at both the gene transcript and protein levels in cultured airway epithelial cells stimulated with IL-13. Interestingly, SCF protein was secreted basally by the epithelial cells, raising the possibility that basal secretion of this mast cell chemokine and growth factor by epithelial cells in response to IL-13 could be a mechanism for mast cell migration from the subepithelial matrix into the intraepithelial space.

We found IEMCs to have upregulation of tryptase and CPA3 but no upregulation of chymase. This pattern of protease expression is not consistent with the patterns observed for MC-T or MC-TC cells. MC-T cells predominantly express high levels of tryptase, but not chymase or carboxypeptidases, whereas MC-TC cells express all 3 proteases.³ To our knowledge, our data for a tryptase-high and CPA3-high subset of mast cells is the first evidence that IEMCs in subjects with mild-to-moderate asthma have a unique phenotype with a different program of gene expression from mast cells in other lung microenvironments. It has previously been shown that mast cells grown in coculture with epithelial cells downregulate chymase.²¹ We have shown here that CM from IL-13-stimulated epithelial cells downregulate chymase while maintaining CPA3 and tryptase expression in cord blood-derived cultured mast cells. These findings raise the possibility that the T_H2-activated epithelium alters the phenotype of mast cells once they accumulate in this location. Our data contrast with those for IEMCs in mouse intestine, which produce chymase that is important for worm expulsion.²³ In addition, increased numbers of chymase-producing mast cells have been reported in the small airways of patients with severe asthma,²⁴ suggesting that mast cell phenotypes in the airway vary by disease severity.

The expression of CPA3 in IEMCs suggests a potentially important role for this protease in asthma. Mast cell carboxypeptidase cleaves C-terminal aromatic and some aliphatic amino acids, and several potential natural substrates have been identified, including angiotensins, apolipoproteins, endothelin, and venoms.^{25–28} Murine mast cell CPA3 can convert angiotensin I to angiotensin II by removing C-terminal amino acids.²⁹ Angiotensin II is proposed to play a major role in airway bronchoconstriction and enhanced airway hyperresponsiveness in asthmatic subjects.³⁰ It is therefore possible that CPA3 plays a role in asthma through production of angiotensin II. Our finding of CPA3 as a major product of IEMCs should prompt further investigation into the potential biologic role of carboxypeptidases in asthma.

The mechanism for how mast cells accumulate in the epithelium is incompletely understood, although there are clues. For example, mast cells adhere avidly to tracheal epithelium through interactions between epithelial cells and cell-surface proteins on mast cells.³¹ Human lung mast cells express several integrins and immunoglobulin superfamily adhesion receptors, including CD18, very late antigen 4, and $\alpha\text{E}\beta 7$.^{32–34} Normal epithelial cells express important counterligands for these receptors, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1, and E-cadherin, respectively.^{35,36}

Mast cell products have several important paracrine effects on airway epithelial cell function. First, tryptases can stimulate production of IL-8 and ICAM-1 by epithelial cells.³⁷ ICAM-1 is the primary receptor for the major group rhinoviruses, an important cause of asthma exacerbations. Second, numerous mast cell products can act as mucin secretagogues, including prostaglandin D₂, chymase, and leukotriene C₄.^{38–40} Finally, mast cells can

produce T_H2 cytokines, including IL-4 and IL-13,⁴¹ which activate specific programs of gene expression by epithelial cells, leading to mucus hypersecretion, eosinophilic inflammation, and airway hyperresponsiveness.^{42,43}

In summary, we conclude that increases in IEMC numbers occurs in subjects with T_H2-high asthma but not in subjects with T_H2-low asthma and that IEMCs have an unusual phenotype, showing upregulation of tryptases and CPA3 but no upregulation of chymase. These findings highlight an important pathologic abnormality in asthma that has implications for mechanisms of epithelial cell activation.

Key messages

- IEMCs are a pathologic characteristic of T_H2-high asthma, a distinct molecular phenotype of asthma.
- The levels of expression of mast cell genes in the airway epithelium correlate positively with lung function improvements with corticosteroid treatment.
- The mechanism of accumulation of mast cells in the airway epithelium in asthmatic subjects might involve IL-13–stimulated secretion of SCF by airway epithelial cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CLCA1	Chloride channel accessory 1
CM	Conditioned media
CPA3	Carboxypeptidase A3
ICAM-1	Intercellular adhesion molecule 1
ICS	Inhaled corticosteroid
IEMC	Intraepithelial mast cell
MC-T	Mast cell–tryptase (tryptase-positive, chymase-negative)
MC-TC	Mast cell–tryptase/chymase (tryptase-positive, chymase-positive)
qPCR	Quantitative RT-PCR
SCF	Stem cell factor
UCSF	University of California, San Francisco

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FIG 1. Epithelial gene expression of mast cell proteases, as determined by means of gene array (*top row*) and qPCR (*bottom row*). Lines represent median values. A, Asthmatic; H, healthy. * $P < .0001$.

FIG 2.

Heat map showing genes in cluster number 24 from unsupervised hierarchical clustering of gene expression data from epithelial brushings of asthmatic subjects (T_H2 high, $n = 22$; T_H2 low, $n = 20$) and healthy control subjects ($n = 28$). The color bar along the top indicates subject status: *green*, healthy; *blue*, T_H2 -low asthma; *red*, T_H2 -high asthma.

FIG 3.

Baseline mast cell protease gene expression is positively correlated with improvement in lung function from ICSs. Baseline (pretreatment) *tryptase* (**A**) and *CPA3* (**B**) mRNA expression levels (as measured by means of qPCR) in subjects subsequently randomized to inhaled fluticasone correlated with treatment-related improvements in lung function at 4 weeks.

FIG 4.

Density of mast cells within airway epithelium among subjects stratified by disease status (A) and T_H2 subgroup (B). Lines represent median values, and boxes represent interquartile ranges. A, Asthmatic; H, healthy. * $P = .011$, Kruskal-Wallis test.

FIG 5.

Immunohistochemistry in endobronchial biopsy specimens for mast cells and basophils. **A** and **B**, Serial sections show tryptase-positive cells within the epithelium (*arrows*; Fig 5, **A**) and absence of basophils (Fig 5, **B**). **C** and **D**, Serial sections show tryptase-positive cells within the epithelium (*arrow*; Fig 5, **C**) and chymase-positive cells in the submucosa (*arrow*; Fig 5, **D**). **E**, Anti-CPA3 shows CPA3-positive cells (*arrows*). Additional immunostaining is shown in Fig E5.

FIG 6. IL-13 induces epithelial cell production of SCF. qPCR (**A**) and protein (**B**) levels in basal media after 4 days of treatment with control (Grey media), IL-13, TNF- α , and IL-1 β . Results are shown as the fold induction over control averaged across 4 different donor cell lines. * $P = .02$ and ** $P = .004$ versus control.

FIG 7.

CM from IL-13-treated epithelial cells suppresses mast cell chymase but not tryptase or CPA3. Cord blood-derived mast cells were grown in CM from human epithelial cells treated with control media or IL-13 (10 ng/mL; *CM/IL-13*). **A** and **B**, qPCR data (means $\pm$ SDs). **C**, Immunostaining of mast cells for tryptase, CPA3, and chymase. * $P = .002$.

TABLE I

Subjects' characteristics

	Healthy control subjects	Asthmatic subjects
No.	36	42
Sex	18 F/18 M	25 F/17 M
Age (y)	35 ± 9	36 ± 12
FEV ₁ (% predicted)	105 ± 14	87 ± 12 [*]
FEV ₁ /FVC ratio	0.81 ± 0.07	0.72 ± 0.08 [*]
PC ₂₀ (mg/dL)	>16	0.43 (0.14–1.11) [*]

Data are presented as means ± SDs or medians (interquartile ranges). No subjects with asthma were using corticosteroids or long-acting β-agonists before enrollment. *F*, Female; *FVC*, forced vital capacity (volume of air exhaled in an entire forced exhalation); *M*, male.

^{*} $P < .05$ compared with healthy control subjects based on *t* tests (PC₂₀ log transformed before analysis).