

Increased density of intraepithelial mast cells in patients with exercise-induced bronchoconstriction regulated through epithelially derived thymic stromal lymphopoietin and IL-33

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Background: Exercise-induced bronchoconstriction (EIB) is a prototypical feature of indirect airway hyperresponsiveness. Mast cells are implicated in EIB, but the characteristics, regulation, and function of mast cells in patients with EIB are poorly understood.

Objectives: We sought to examine mast cell infiltration of the airway epithelium in patients with EIB and the regulation of mast cell phenotype and function by epithelially derived cytokines.

Methods: Endobronchial biopsy specimens, epithelial brushings, and induced sputum were obtained from asthmatic patients with and without EIB and healthy control subjects. Mast cell proteases were quantified by using quantitative PCR, and mast cell density was quantified by using design-based stereology. Airway epithelial responses to wounding and osmotic stress were assessed in primary airway epithelial cells and *ex vivo* murine lung tissue. Mast cell granule development and function were examined in cord blood–derived mast cells.

Results: Tryptase and carboxypeptidase A3 expression in epithelial brushings and epithelial mast cell density were

selectively increased in the asthma group with EIB. An *in vitro* scratch wound initiated the release of thymic stromal lymphopoietin, which was greater in epithelial cells derived from asthmatic patients. Osmotic stress induced the release of IL-33 from explanted murine lungs, which was increased in allergen-treated mice. Thymic stromal lymphopoietin combined with IL-33 increased tryptase and carboxypeptidase A3 immunostaining in mast cell precursors and selectively increased cysteinyl leukotriene formation by mast cells in a manner that was independent of *in vitro* sensitization. **Conclusions:** Mast cell infiltration of the epithelium is a critical determinant of indirect airway hyperresponsiveness, and the airway epithelium might serve as an important regulator of the development and function of this mast cell population. (*J Allergy Clin Immunol* 2014;133:1448-55.)

Key words: Asthma, airway hyperresponsiveness, eicosanoid, epithelial cell, leukotriene

Mast cells are present in the conducting airways of the lung and have important roles in host defense, repair, and allergen-induced inflammation^{1,2}; however, the types of mast cells infiltrating the airways in asthmatic patients and their function remains incompletely understood. Mast cells are phenotypically divided into MC_T and MC_{TC} types based on the composition of their secretory granules, which contain tryptase in both types of cells but with the addition of carboxypeptidase A3 (CPA3) and chymase in the MC_{TC} phenotype.³ Initial studies found that mast cells in mucosal surfaces of the lung are predominantly MC_T cells.^{4,5} A recent genomic study identified an increase in tryptase and CPA3 levels in the airway epithelium of asthmatic patients, suggesting a novel mast cell phenotype in asthmatic patients,⁶ particularly in patients with “T_H2-high” asthma,⁷ but the full physiologic significance of this mast cell population is not fully established. In a genome-wide expression study among asthmatic patients, we found that tryptase and CPA3 levels are specifically increased in induced sputum cells of patients with exercise-induced bronchoconstriction (EIB), a prototypical feature of indirect airway hyperresponsiveness (AHR) in asthmatic patients.⁸ We have previously demonstrated mast cell degranulation after exercise challenge in patients with EIB.⁹ Thus we hypothesized that this novel tryptase- and CPA3-expressing mast cell population might play a key role in EIB. Because recent genome-wide association studies have highlighted the potential importance of the genes encoding IL-33 and thymic stromal lymphopoietin (TSLP), which are avidly expressed in the airway epithelium,¹⁰⁻¹² we tested a

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Abbreviations used

AHR: Airway hyperresponsiveness
CBMC: Cord blood–derived mast cell
CPA3: Carboxypeptidase A3
CysLT: Cysteinyl leukotriene
EIB: Exercise-induced bronchoconstriction
FVC: Forced vital capacity
PG: Prostaglandin
TSLP: Thymic stromal lymphopoietin

potential contribution from epithelially derived cytokines toward the accumulation and activation of these EIB-associated mast cells.

We conducted endobronchial biopsies, epithelial brushings, and sputum induction in patients with asthma and EIB and made comparisons with asthmatic patients who have AHR to methacholine (ie, direct AHR) but do not have EIB and healthy control subjects. To appreciate the origin of this mast cell population, we examined the response to wounding *in vitro* by using organotypic cultures of primary epithelial cells from patients with and without asthma and an *ex vivo* model of osmotic stress in lung tissue derived from mice with and without allergen-induced inflammation. Because these model systems led to the release of TSLP and IL-33, we examined the effects of these epithelially derived cytokines on mast cell granule development and mast cell production of eicosanoids. The results support a potential role of this novel mast cell population in patients with indirect AHR and that the airway epithelium might regulate the development and function of this mast cell population through TSLP and IL-33.

METHODS

Full experimental details are provided in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Study subjects and study protocol

We used endobronchial biopsy specimens, epithelial brushings, and induced sputum from a repository of samples collected at the University of Washington designed to examine differences between asthmatic patients with and without EIB and nonasthmatic control subjects.¹³ Induced sputum and research bronchoscopy were conducted 2 to 10 days apart. Written informed consent was obtained from all participants, and the University of Washington Institutional Review Board approved the study protocol. Patients with asthma, based on a positive methacholine challenge result, were characterized as EIB(+) or EIB(–) based on the response to exercise challenge.¹⁴

Either epithelial brushings or endobronchial biopsy samples were available from 10 control subjects, 12 EIB(–) asthmatic patients, and 19 EIB(+) asthmatic patients. Endobronchial biopsy tissue was inadequate for stereology assessment in 1 control subject, 2 EIB(–) asthmatic patients, and 1 EIB(+) asthmatic patient. Insufficient RNA was available from the epithelial brushings for PCR analysis in 1 control subject, 2 EIB(–) asthmatic patients, and 2 EIB(+) asthmatic patients.

Copy number quantitative PCR

Real-time PCR analysis was conducted by using TaqMan primer probe sets with FAM probes for *TPSAB1* (Hs02576518_gH), *CPA3* (Hs00157019_m1), *CMA1* (Hs01095979_g1), *IL33* (Hs00369211_m1), *TSLP* (Hs00263639_m1) and, when applicable, a primer-limited VIC probe

for *HPRT1* (4326321E) as an endogenous control.¹⁵ In some samples the PCR amplification of *HPRT1* was low, and these samples were excluded. The number of samples with accurate PCR data for each group is noted in the figures.

Immunohistochemistry and design-based stereology

We used the physical disector method to enumerate the density of mast cells in the airway epithelium relative to the volume of the epithelium.⁷ The surface area of the basal lamina relative to the volume of the epithelium was quantified to calculate the number of mast cells relative to the surface area of the basal lamina.

Primary airway epithelial cell culture and scratch wound model

Primary bronchial epithelial cells isolated during bronchoscopy were expanded in culture and cryopreserved. Cryopreserved primary epithelial cells were differentiated in air-liquid interface organotypic cultures.⁸ A series of scratch wounds were created with a pipette tip on the apical surface of the air-liquid interface culture. TSLP and IL-33 levels were assayed in basolateral medium by using ELISA.

Murine osmotic stress model and allergen-induced airway inflammation model

Cells of the murine pre-B-cell line Ba/F3 were stably transfected with full-length ST2L and a nuclear factor κ B–luciferase reporter. Conditioned supernatants from C57Bl/6 mouse lung explants were evaluated for murine IL-33 and for ST2-activating biological activity by using the Ba/F3 cell assay. Osmotic stress was applied to mouse lung tissue by adding sorbitol or mannitol at concentrations ranging from 0.06 to 0.5 mol/L for 48 hours. Cockroach extract (100 μ g) was administered by means of intranasal delivery every 3 days for 2 weeks before removal of lung explants to examine the effects of allergen-induced airway inflammation. All animal studies were reviewed and approved by the Amgen Animal Care and Use Committee.

Human cord blood–derived mast cell culture

Human umbilical cord blood was obtained from anonymous donors to the Puget Sound Blood Center (Seattle, Wash). CD34⁺ cells were isolated and treated with IL-3 (30 ng/mL), IL-6 (100 ng/mL), and stem cell factor (100 ng/mL) during the first week and transferred to a new flask containing IL-6 (100 ng/mL) and stem cell factor (100 ng/mL) in subsequent weeks.¹⁶

Flow cytometry and immunocytochemistry of granule development

Granule development in CD34-selected cord blood cells was assessed at 1 and 3 weeks in culture medium alone or with IL-33 (10 ng/mL), TSLP (10 ng/mL), or both IL-33 and TSLP (both at 10 ng/mL).¹⁷ Intracytoplasmic staining was evaluated by means of flow cytometry with primary antibodies directed against CPA3, tryptase, and chymase. Immunocytochemistry for CPA3 was conducted on cytospin preparations by using the 3,3'-diaminobenzidine technique.

Assessment of eicosanoid formation by mature cord blood–derived mast cells

Mature cord blood–derived mast cells (CBMCs) were passively sensitized with human IgE for 7 days and activated with the murine mAb CRA1 (clone AER-37) that activates the high-affinity IgE receptor (Fc ϵ RI) for 1 hour.¹⁸ Before activation, CBMCs were treated with or without IL-13, IL-25, IL-33, TSLP, or both IL-33 and TSLP (all at 10 ng/mL) for 7 days.¹⁶ ELISAs were used to measure leukotriene C₄, cysteinyl leukotriene (CysLT), and prostaglandin (PG) D₂ levels.

Statistical analysis

Differences in the characteristics of the groups were assessed with a χ^2 , ANOVA, or Kruskal-Wallis test. Differences in mast cell gene expression and density were tested with a Kruskal-Wallis test. Associations between mast cell markers and the severity of EIB were assessed by using linear regression. Differences across multiple cell-culture conditions were assessed with a 1-way ANOVA. Differences in cell phenotype, as well as treatment conditions (ie, wounding), were assessed with a 2-way ANOVA.

RESULTS

Subjects' characteristics

The groups of subjects were similar with respect to age, sex, ethnicity, race, and baseline lung function measured based on FEV₁ and forced vital capacity (FVC) values (Table I). There were notable differences between the groups for baseline airflow obstruction reflected in the FEV₁/FVC ratio and direct AHR to methacholine challenge, with overall differences among the 3 groups, as well as differences between the 2 asthma groups, regarding the FEV₁/FVC ratio ($P = .03$) and methacholine PC₂₀ ($P = .03$). The severity of EIB was markedly greater in the EIB(+) group than in either of the other groups, with marked differences between the 2 asthma groups with respect to the maximum decrease in FEV₁ after exercise challenge ($P < .001$) and area under the FEV₁ time curve ($P < .001$).

Epithelial mast cell protease gene expression is increased in asthmatic patients with EIB

The expression of the tryptase (*TPSAB1*; Fig 1, A) and *CPA3* (Fig 1, B) genes in epithelial brushings were increased in the EIB(+) asthma group relative to the EIB(−) asthma group and the control group, but there was no difference between the control subjects and the EIB(−) group. Immunohistochemistry of airway biopsy specimens revealed leukocytes in the epithelium that contain tryptase and CPA3, which is consistent with intraepithelial mast cells as the origin of these findings in epithelial brushings (see Fig E1 in this article's Online Repository at www.jacionline.org). The expression of chymase (*CMA1*) was more than 1000 times lower than the expression of either *TPSAB1* or *CPA3* in the epithelium. The expression of *CMA1* was increased in the EIB(+) asthma group relative to that seen in the control group but not relative to that seen in the EIB(−) group (Fig 1, C). Gene expression analysis of induced sputum cells confirmed our prior genomic findings in a separate cohort of subjects.⁸ The expression of *TPSAB1* in induced sputum cells was increased in the EIB(+) asthma group relative to that seen in the control group, whereas the expression of *CPA3* was increased in the EIB(+) group relative to that seen in the EIB(−) asthma group and the control group (Fig 1, D and E). There was no difference in *CMA1* expression in induced sputum cells between the groups (Fig 1, F). The severity of EIB measured based on the maximum decrease in FEV₁ after exercise was associated with the number of copies of *TPSAB1* ($r^2 = 0.31$, $P = .0006$) and *CPA3* in the airway epithelium ($r^2 = 0.34$, $P = .0004$) but less associated with the number of copies of chymase ($r^2 = 0.11$, $P = .08$, see Fig E2 in this article's Online Repository at www.jacionline.org).

Intraepithelial mast cell density is specifically increased in the EIB(+) phenotype of asthma

Differences in epithelial mast cell density were quantified by using design-based stereology, a technique that avoids the usual

TABLE I. Study population

Characteristic	Control subjects, n = 10	Asthmatic patients		P value
		EIB(−), n = 12	EIB(+), n = 19	
Age (y)	30.4 ± 12.7	24.8 ± 5.0	26.8 ± 8.6	.35
Sex, male (%)	20.0	25.0	31.6	.79
Ethnicity, white (%)	70.0	91.7	84.2	.46
FEV ₁ (% predicted)	96.5 ± 11.3	90.7 ± 9.7	88.8 ± 10.9	.19
FVC (% predicted)	95.7 ± 13.5	96.2 ± 8.9	103.3 ± 9.6	.10
FEV ₁ /FVC ratio	0.87 ± 0.06	0.80 ± 0.09	0.73 ± 0.09	<.001*
Methacholine PC ₂₀	>8 ± 0	1.8 ± 1.3	0.6 ± 1.5	<.001*
Exercise challenge				
Maximum decrease in FEV ₁ (%)	1.7 ± 2.1	2.3 ± 2.6	27.7 ± 9.5	<.001†
AUC30 FEV ₁	−7.4 ± 58.1	−13.4 ± 69.5	624.7 ± 295.7	<.001†

P values represent the overall comparison between the 3 groups. Comparisons between the asthma groups were as follows: * $P = .03$ and † $P < .001$.

AUC30, Area under the FEV₁ time curve.

sources of bias encountered in 2-dimensional sections.^{7,19} The density of mast cells per volume of the epithelium was greater in the EIB(+) group relative to that seen in the control group and the EIB(−) asthma group (Fig 2, A). The surface area of the basal lamina relative to the volume of the epithelium was not altered in asthmatic patients ($P = .74$, see Fig E3 in this article's Online Repository at www.jacionline.org). The number of mast cells relative to the surface area of the basal lamina was greater in the EIB(+) group relative to that seen in the control group (Fig 2, B). The severity of EIB measured by the maximum decrease in FEV₁ after exercise was associated with the density of mast cells relative to the epithelial volume ($r^2 = 0.24$, $P = .002$) and epithelial mast cells relative to the area of the basal lamina ($r^2 = 0.12$, $P = .03$, see Fig E4 in this article's Online Repository at www.jacionline.org).

TSLP and IL-33 are generated in response to physiologically relevant stressors implicated in asthmatic patients

To further understand the origin of the intraepithelial mast cell population we identified, we conducted studies examining the release of IL-33 and TSLP by epithelial cells. We postulated that IL-33 and TSLP might influence mast cell development because both mature mast cells and early CD34⁺ progenitor cells express the IL-33 receptor (ST2L) and the TSLP receptor.¹⁷ Although gene expression for *IL33* and *TSLP* in epithelial brushings was similar among the 3 different groups (see Fig E5 in this article's Online Repository at www.jacionline.org), we further examined the release of TSLP and IL-33 in response to mechanical wounding and osmotic stress. These stimuli are relevant to the pathogenesis of indirect AHR because epithelial shedding is increased in patients with EIB.²⁰ Also, because the minute ventilation increases during exercise challenge, osmotic stress occurs, leading to acute bronchoconstriction and an injury syndrome linked to asthma in athletes training at high levels.²¹ A scratch wound in fully differentiated primary airway epithelial cells in organotypic culture caused a time-dependent release of TSLP that was greater in epithelial cells derived from EIB(+) asthmatic donors (n = 3) relative to that seen in cells from healthy control subjects (n = 2, Fig 3). Although IL-33 release presumably occurs in response to injury and mechanical stress, extracellular IL-33 was less than the ELISA detection limit in this model.

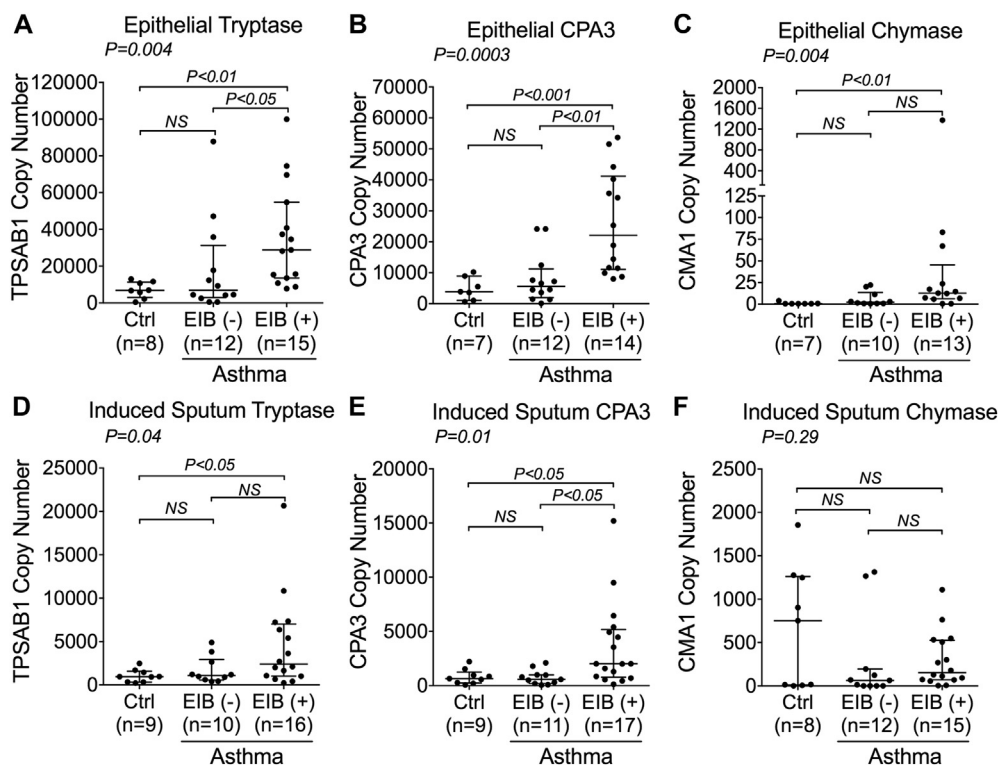


FIG 1. Airway gene expressions of specific mast cell proteases are increased in a precise phenotype of asthma. Quantitative PCR of airway epithelial brushings (**A-C**) and induced sputum cells (**D-F**) for *TPSAB1*, *CPA3*, and *CMA1* expression is shown. The overall *P* value for the Kruskal-Wallis test is shown in the upper left of each pane, and the *P* values for *post hoc* tests are shown above the horizontal bars. NS, Not significant.

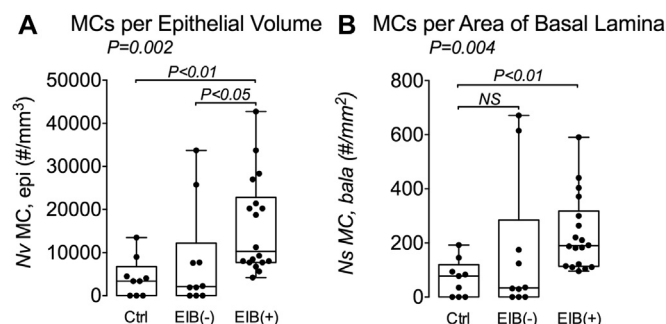


FIG 2. Quantification of the density of intraepithelial mast cells (*MCs*) by using design-based stereology. The density of intraepithelial mast cells was quantified based on the numeric density of intraepithelial mast cells relative to the volume of the airway epithelium (*Nv MC, epi*; **A**) and the numeric density of mast cells relative to the surface area of the basal lamina (*Ns MC, bala*; **B**). NS, Not significant.

We used an *ex vivo* murine model to examine the release of IL-33 in response to epithelial stress initiated by osmotic agents. Ba/F3 cells stably transfected with murine ST2L and an nuclear factor κ B-luciferase reporter were used to detect IL-33 activity (see the Results section in this article's Online Repository at www.jacionline.org). Lung explants exposed to increasing concentrations of sorbitol from 0.06 to 0.5 mol/L for 48 hours caused a dose-dependent increase in ST2 activity in the culture medium (**Fig 4, A**). Lung explants exposed to mannitol had a similar concentration-dependent increase in ST2 activity in the supernatant that reached a maximum at approximately

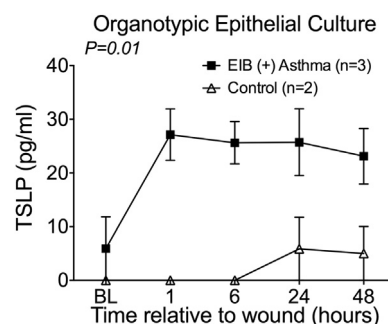


FIG 3. A mechanical scratch wound initiates TSLP release from primary airway epithelial cells in organotypic culture. Airway epithelial cells at passage 2 from asthmatic and control subjects differentiated in organotypic culture release TSLP protein into the basolateral media after a series of mechanical scratch wounds. The release of TSLP was greater from epithelial cells derived from asthmatic patients. BL, Baseline.

0.3 mol/L, possibly because of the limited solubility of mannitol (**Fig 4, B**). A comparison of ST2 activity in response to 0.5 mol/L concentrations of sorbitol or mannitol from the same lung explants revealed a similar increase in ST2 activity (**Fig 4, C**). Immunoprecipitation of the lung supernatant revealed IL-33 bands from the lungs treated with osmotic agents but not in the supernatant from lung explants exposed to medium alone (see **Fig E6, C**, in this article's Online Repository at www.jacionline.org).

Lung tissue from allergen-challenged mice had increased ST2 activity compared with that seen in explants from naive or

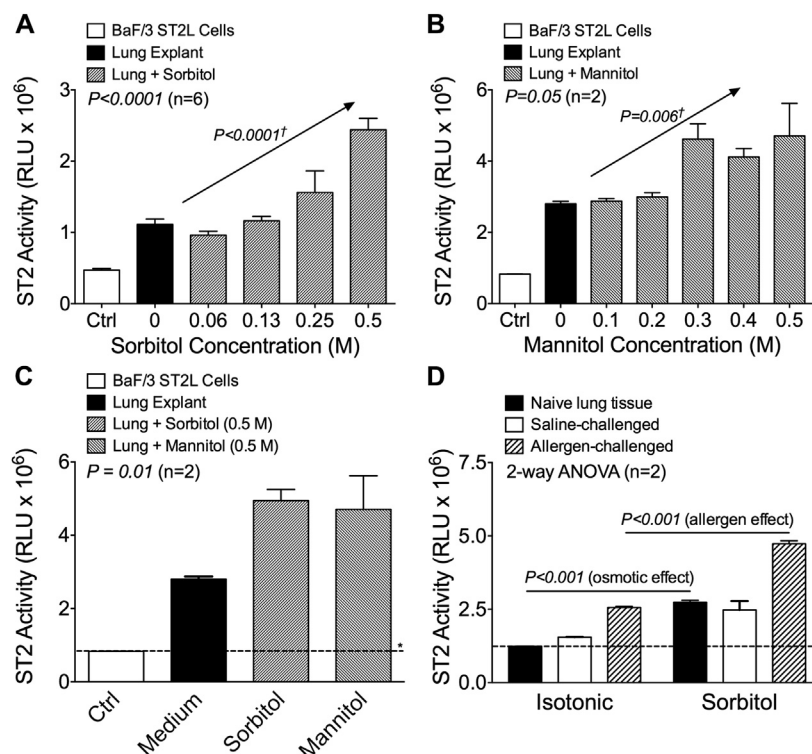


FIG 4. Osmotic stress in murine lung tissue initiates the release of biologically active IL-33. Conditioned medium from explanted lung tissue exposed to osmotic stress in the form of sorbitol (**A**) or mannitol (**B**) increased ST2 reporter activity in Ba/F3 cells that was related to the amount of osmotic stress. †Test for linear trend. Release of ST2 activity was similar for equal molar concentrations of sorbitol or mannitol (**C**). Allergen challenge increased the basal release of ST2-activating activity and increased the release of this activity in response to osmotic stress (**D**).

saline-challenged mice ($P < .001$). Osmotic stress applied to allergen-challenged lung tissue caused a substantially greater release of ST2 activity than the same osmotic stress applied to explants from either naive or saline-challenged mice (**Fig 4, D**).

Development of the mast cell granule phenotype is influenced by TSLP and IL-33

Because TSLP and IL-33 were released from the epithelium, we examined the effects of these cytokines on the development of mast cell proteases in CD34⁺ cord blood cells after 1 and 3 weeks of *in vitro* mast cell differentiation. After the first week, there was no discernible effect of TSLP, IL-33, or both cytokines combined on intracytoplasmic staining for tryptase (see **Fig E7, A**, in this article's Online Repository at www.jacionline.org) or CPA3 (see **Fig E7, B**), as determined by using flow cytometry. After 3 weeks of culture with the same cytokines, there was an overall increase in the mean intensity of immunostaining for tryptase ($P = .02$, see **Fig E7, C**) and CPA3 ($P = .05$, see **Fig E7, D**) in the cells treated with TSLP, IL-33, or both. Because the intracellular immunostaining for chymase could not be identified in fully mature CBMCs, we did not assess chymase by using flow cytometry. We further quantified CPA3 immunostaining by means of immunocytochemistry on the same cord blood–derived cells after 3 weeks of culture, demonstrating that there was an increase in the percentage of CPA3⁺ cells after treatment with TSLP, IL-33, or both ($P = .05$, see **Fig E7, E and F**).

TSLP and IL-33 alters mast cell formation of CysLTs and PGD₂

We further examined the effects of IL-33, TSLP, or both on the function of mast cells. Treatment of CBMCs with IL-33, TSLP, or both for 7 days before activation through FcεRI increased CysLT formation when both IL-33 and TSLP were administered together but not with IL-33 or TSLP alone (**Fig 5, A**). When CBMCs were passively sensitized with human polyclonal IgE, there was an increase in CysLT formation in response to the FcεRI-activating antibody ($P = .06$). In passively sensitized CBMCs, treatment with IL-33 and TSLP together increased CysLT formation, whereas treatment with either IL-33 or TSLP alone did not (**Fig 5, B**). Although activation by the FcεRI-activating antibody initiated PGD₂ synthesis by unsensitized and passively sensitized CBMCs, there was no increase in PGD₂ formation mediated by IL-33, TSLP, or both (**Fig 5, C and D**).

We also examined the effects of IL-25 on CBMCs because epithelial cells are a prominent source of IL-25 and the IL-25 receptor is expressed on airway mast cells.²² Treatment of CBMCs with IL-25 did not alter CysLT or PGD₂ formation with or without passive sensitization (**Fig 6, A and B**). We also found that IL-13 did not alter CysLT or PGD₂ formation in unsensitized CBMCs; however, after passive sensitization, there was an increase in CysLT but not PGD₂ formation in IL-13–treated CBMCs after FcεRI-mediated activation (**Fig 6, C and D**). Collectively, these results indicate that IL-33 and TSLP together have a selective influence on the 5-lipoxygenase pathway that is independent of sensitization,

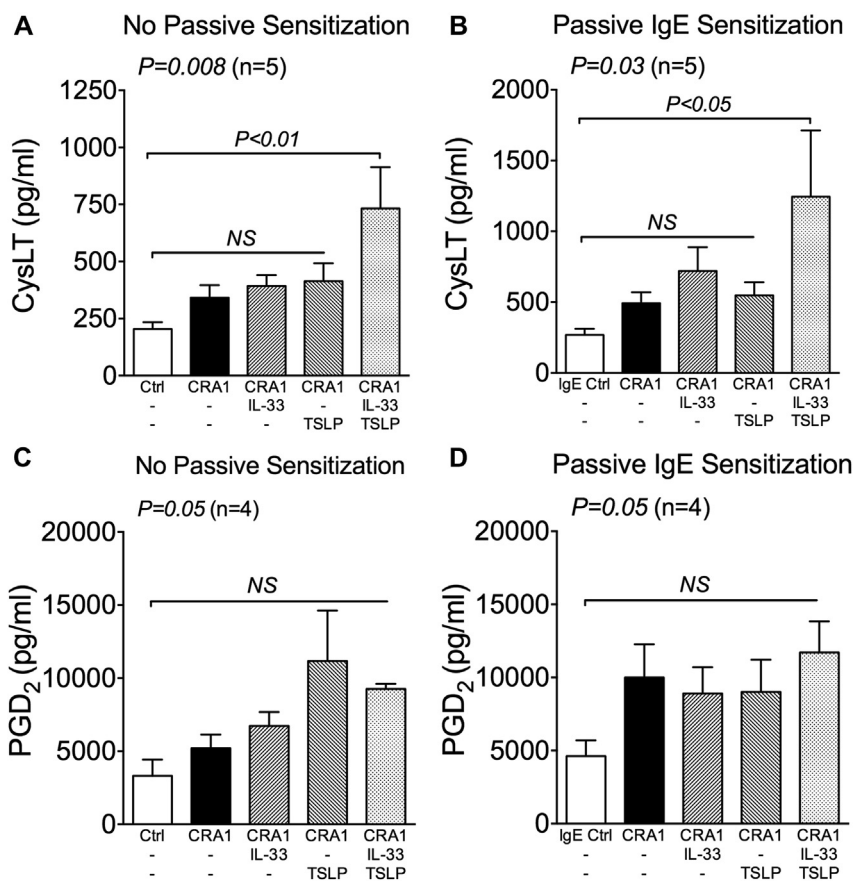


FIG 5. Influence of IL-33 and TSLP on IgE-dependent mast cell production of CysLTs and PGD₂. Treatment of CBMCs for 7 days with IL-33 in combination with TSLP increased CysLT formation in response to the FcεRI-activating antibody CRA1 both without passive sensitization (**A**) and after passive sensitization with polyclonal IgE for 7 days (**B**). There was no increase in PGD₂ formation in response to IL-33, TSLP, or both without passive sensitization (**C**) or after passive sensitization (**D**). NS, Not significant.

whereas IL-13 selectively alters the 5-lipoxygenase pathway only during *in vitro* sensitization.

DISCUSSION

Our findings demonstrate that the intraepithelial mast cells with high CPA3 and tryptase expression but low chymase expression recently identified in asthmatic patients^{6,7,23} are restricted to patients with indirect AHR in the form of EIB. We found that airway epithelial cells release TSLP and IL-33 in response to wounding and osmotic stress, respectively, and that these 2 cytokines are generated in increased quantities by epithelial cells from asthmatic patients and from allergen-sensitized airways of mice, respectively. TSLP and IL-33 enhanced the EIB-associated granule phenotype and increased IgE receptor-mediated CysLT formation by mast cells with or without passive sensitization. These results highlight an important function of the airway epithelium to regulate mast cell phenotype and function, particularly in the setting of indirect AHR.

Among subjects with asthma, approximately 30% to 50% of subjects have EIB, a feature of asthma that is closely tied to the severity of indirect AHR.²⁴ Asthmatic patients with EIB have epithelial shedding into the airway lumen and increased production of CysLTs.²⁰ Mast cell degranulation and sustained generation of both CysLTs and PGD₂ occur after exercise

challenge in patients with EIB.^{9,25} Recent work demonstrates that the density of intraepithelial mast cells is increased in asthmatic patients,⁷ especially in patients with a “T_H2-high” genomic signature.^{6,7} Another study found the epithelial expression of both tryptase and CPA3 was increased in asthmatic patients but was similar across differing levels of asthma severity.²³ We found that tryptase and CPA3 expression in epithelial brushings and mast cell infiltration of the epithelium in endobronchial tissue are selectively increased in the group of asthmatic patients with EIB, revealing an important physiologic implication of this mast cell population. The group of asthmatic patients without EIB in our study all had positive methacholine challenge test results but had mast cell density and gene expression that were no different than in the control group, suggesting that differences in direct and indirect hyperresponsiveness might be mast cell mediated.

Recent studies demonstrate that the epithelium can regulate AHR in a murine model through mast cells²⁶ and that epithelial cells alter the activation of mast cells.²⁷ Cross-talk between mast cells and airway epithelial cells in culture has also been described.²⁸ We focused on the response to epithelial injury and osmotic stress because these factors have been implicated in the “injury syndrome” that is associated with the development of asthma and EIB in athletes who train at high levels in cold and dry environments.²⁹ We found that epithelial cells isolated from

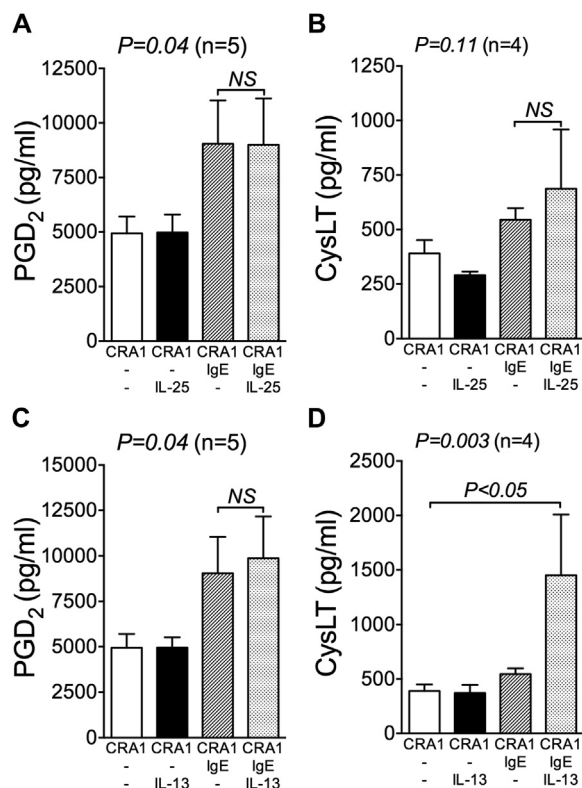


FIG 6. Effects of IL-25 and IL-13 on IgE-dependent mast cell CysLT and PGD₂ formation. Treatment of mature CBMCs with IL-25 did not alter the formation of IgE receptor-mediated (CRA1) formation of PGD₂ (A) or CysLTs (B) with or without passive sensitization. Treatment of CBMCs with IL-13 did not alter the formation of PGD₂ either with or without passive sensitization (C). The formation of CysLTs was increased by IL-13 after passive sensitization but not without passive sensitization of the CBMCs (D). NS, Not significant.

asthmatic patients released more TSLP after a scratch wound, suggesting that disruption of susceptible epithelium might bias immunity toward allergen sensitization.³⁰ Because prior work revealed that TSLP expression is increased in the skin after trauma³¹ and plays an important role in wound repair,³² these data further indicate that airway epithelial cells of asthmatic patients exhibit features of a chronic wound.

Osmotic stress during exercise or through inhalation of a solution with high osmolarity, such as mannitol, causes acute bronchoconstriction in asthmatic patients with indirect AHR.²⁴ We demonstrate here that osmotic stress applied to murine lung tissue generates IL-33 activity *in vitro* and that allergen-induced airway inflammation primes the lung tissue for greater osmotic stress-induced IL-33 release. Although we do not demonstrate that the IL-33 was of epithelial origin *per se*, small-airway epithelial immunostaining for IL-33 was lost rapidly after osmotic stress (not shown). The form of IL-33 released from lung explants was approximately 24 kDa, indicating that proteolytic processing occurred, which is possibly coincident with recently described processed and more active forms of IL-33 (ie, IL-33₉₅₋₂₇₀, IL-33₉₉₋₂₇₀, or IL-33₁₀₉₋₂₇₀).³³ Because IL-33 can be both activated and inactivated by posttranslational processing, our assay based on ST2 activity suggests that osmotic stress leads to the release of an active form of IL-33.^{34,35} Because osmotic stress does not lead directly to cell death, IL-33 might be released by an active but still incompletely defined pathway.³⁶

The release of IL-33 in murine airways is provoked by ATP release,³⁷ and the nuclear to cytoplasmic export of IL-33 is also ATP dependent,³⁶ suggesting that ATP release in response to water loss or osmotic stress might initiate IL-33 release.

The effects of IL-33 and TSLP on granule development have not been studied in detail. A prior study indicated that conditioned media from IL-13-treated epithelial cells reduces the expression of chymase without changes in tryptase and CPA3 expression in CBMCs.⁷ In CD34⁺ progenitor cells IL-33 increased tryptase immunostaining at 3 weeks of culture during *in vitro* mast cell differentiation.³¹ Our results suggest that IL-33 in combination with TSLP increases both tryptase and CPA3 expression early during *in vitro* CBMC differentiation, but the effects were modest in magnitude.

The formation of CysLTs is important during the sensitization phase of allergen-induced inflammation,³⁸ and CysLTs and PGD₂ are implicated in the development of bronchoconstriction after exercise or osmotic challenges to the airways.²¹ Although IL-33 induces the generation of PGD₂ in murine bone marrow-derived mast cells,³⁹ IL-33 did not generate PGD₂^{31,40} or the CysLT LTC₄ in human CD34⁺ progenitor-derived mast cells.³¹ However, IL-33 in combination with TSLP had prominent effects on IL-5 and IL-13 release in human mast cells,³¹ and TSLP-mediated effects were enhanced in the presence of an IL-1 family member, including IL-33.⁴¹ Supernatant from necrotic murine structural cells induces CysLT generation by bone marrow-derived mast cells at least in part through IL-33.⁴² Our work demonstrates that IL-33 in combination with TSLP augmented IgE receptor-mediated CysLT generation in CBMCs, and this “priming” effect occurred with or without passive sensitization. In contrast, IL-13 augmented the production of CysLTs only in the context of passive sensitization.

We conclude that mast cell infiltration of the airway epithelium is a key feature of indirect AHR and that the epithelium might play a important role in the retention and activation of mast cells through the generation of TSLP and IL-33 in response to epithelial stress.

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Key messages

- Infiltration of the airway epithelium with tryptase- and CPA3-positive mast cells is selectively increased in patients susceptible to EIB.
- Epithelial cells release TSLP and IL-33 in response to mechanical wounding and osmotic stress.
- TSLP in combination with IL-33 increases mast cell formation of eicosanoids, which are important in patients with EIB.

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