Thymic stromal lymphopoietin activation of basophils in patients with allergic asthma is IL-3 dependent

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Background: Thymic stromal lymphopoietin (TSLP) released after antigenic stimulation of allergic asthmatic airways is a key initiator of type 2 inflammation. Basophils are important effectors of allergic inflammation in the airways. Murine basophils have been shown to respond to TSLP independently of IL-3 by increasing functional thymic stromal lymphopoietin receptor (TSLPR) expression.
Objective: The purpose of this study was to investigate the effect of TSLP stimulation on human basophil function.
Methods: Ten patients with mild allergic asthma underwent diluent and allergen inhalation challenges. Peripheral blood and sputum samples were collected at baseline and 7 and 24 hours after challenge, and bone marrow samples were collected at baseline and 24 hours after challenge to measure basophil TSLPR expression. *In vitro* experiments were conducted on purified human basophils to measure the effect of TSLP on degranulation, expression of activation markers and T_H2 cytokines, and eotaxin-induced shape change.
Results: Allergen inhalation increased basophil numbers in the airways and significantly upregulated the expression of activation markers, T_H2 intracellular cytokines, and receptors for TSLP, IL-3, and eotaxin in blood, bone marrow, and sputum basophils. *In vitro* stimulation with TSLP primed basophil migration to eotaxin and induced rapid and sustained basophil activation mediated directly through TSLPR and indirectly through an IL-3–mediated basophil autocrine loop. Basophils responded to TSLP at a similar magnitude and potency as the well-described basophil-activating stimuli IL-3 and anti-IgE.
Conclusion: Our findings indicate that basophil activation during early- and late-phase responses to inhaled allergen might be driven at least in part by TSLP. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *Basophils, allergic asthma, thymic stromal lymphopoietin, epithelium-derived cytokines, CD203c, T_H2 cytokines*

Abbreviations used
EAR: Early asthmatic response
GM-CSFR: GM-CSF receptor
IL-3R: IL-3 receptor
IL-5R: IL-5 receptor
LAR: Late asthmatic response
TSLP: Thymic stromal lymphopoietin
TSLPR: Thymic stromal lymphopoietin receptor

Asthma is a chronic respiratory disease characterized by reversible airway obstruction, airway hyperresponsiveness, and heterogeneous inflammation of the airways of which eosinophils and basophils are prominent components. The cause of this disorder is multifaceted, and recent evidence indicates an array of genetic and environmental mechanisms at play.

The epithelium-derived cytokine thymic stromal lymphopoietin (TSLP) has been identified as a master switch for allergic inflammation in several mouse models.[1] TSLP is produced in response to proinflammatory stimuli, which in turn promotes primarily type 2 inflammatory responses. In both animal and *in vitro* human models, this mediator has been shown to exert its effects on innate and adaptive immune cells, including dendritic cells, macrophages, lymphocytes, granulocytes, mast cells, innate lymphoid type 2 cells, and $CD34^+$ progenitor cells.[2]

In studies of human asthma, TSLP mRNA expression in the airways was greater in asthmatic patients compared with that seen in healthy control subjects and correlated with asthma severity.[3-5] Gauvreau et al[6] demonstrated that treatment with an mAb to TSLP in patients with mild allergic asthma significantly attenuated allergen-induced early asthmatic responses (EARs) and late asthmatic responses (LARs) in parallel with a reduction in blood and sputum eosinophil numbers. Given that allergen-induced EARs and LARs are mediated by mast cell and basophil activation, the above findings suggest that TSLP can activate these cells in addition to other inflammatory cells, such as eosinophils.

Emerging evidence demonstrates that epithelium-derived factors can regulate basophil function. TSLP can promote murine and human bone marrow progenitor cells to differentiate into basophils.[7] Human peripheral $CD34^+$ cells preincubated with IL-3 and TNF-α have enhanced sensitivity to TSLP-mediated basophil lineage commitment,[8] and mast cell–activated bone marrow mesenchymal stromal cells produce TSLP, which can enhance the differentiation of $CD34^+$ progenitors into eosinophil/basophil colonies.[9] Furthermore, in the presence or absence of IL-3, TSLP can induce murine basophil receptor changes, including increased CD69, CD62 ligand, CD11b, CD123, IL-33 receptor, and IL-18 receptor expression.[7] Basophils are a significant source of TSLP, enabling the possibility of a positive feedback loop.[10] It has recently been confirmed that human peripheral basophils express thymic stromal lymphopoietin receptor (TSLPR), which can be upregulated after incubation with IL-3.[11] Additionally, allergen stimulation leads to increased TSLPR expression on circulating basophils from patients with atopic asthma compared with nonatopic control subjects.[12] Lastly,

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Disclosure of potential conflict of interest: G. M. Gauvreau has received research support from Genentech, Sanofi, and CSL Pharma. The rest of the authors declare that they have no relevant conflicts of interest.
Received for publication November 12, 2014; revised March 19, 2015; accepted for publication March 25, 2015.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2015.03.039

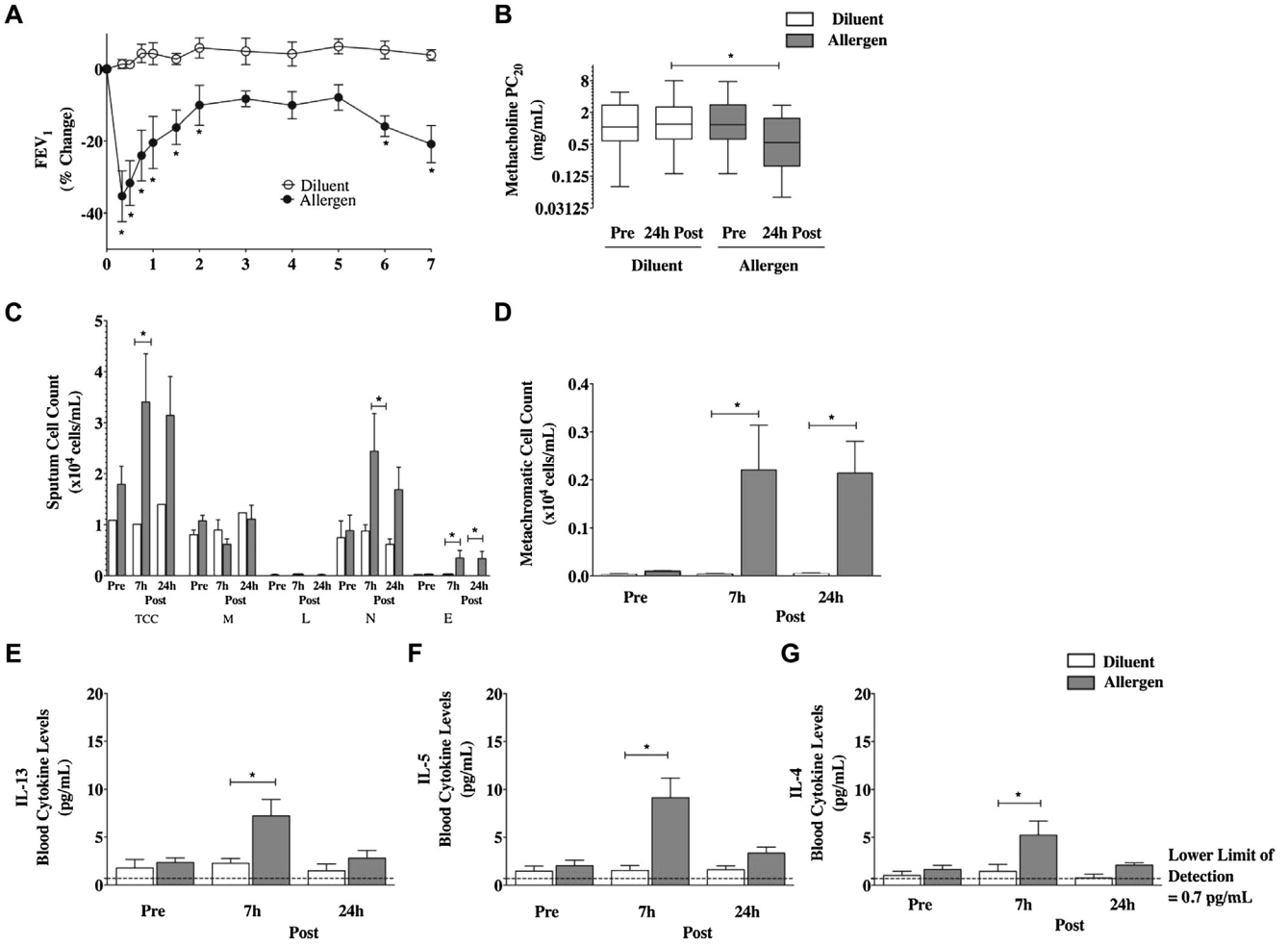

FIG 1. Allergen inhalation induces airway changes in patients with mild allergic asthma. **A** and **B**, Percentage change in FEV_1 (Fig 1, *A*) and methacholine PC_{20} (in milligrams per milliliter; Fig 1, *B*) for allergen *(gray bars)* versus diluent *(open bars)*. **C** and **D**, Sputum cell counts after diluent *(open bars)* and allergen *(gray bars)*. *E*, Eosinophils; *L*, lymphocytes; *M*, macrophages; *N*, neutrophils; *TCC*, total cell count. **E-G,** T_H2 cytokines in serum after allergen *(gray bars)* versus diluent *(open bars)*. Data are presented as means ± SEMs, with the exception of methacholine PC_{20}, which was presented as geometric means ± geometric SEMs. *Significantly different from diluent control ($P < .05$).

a correlation has been found between increased airway TSLP expression and exaggerated basophil responses in patients with eosinophilic esophagitis, and TSLP neutralization, basophil depletion, or both ameliorates disease-like symptoms, suggesting that the interaction between TSLP and basophils might be an important component of airway inflammation.[13]

Although TSLP has been identified as an important initiator of type 2 inflammatory responses in both animal and human models of allergic asthma, the basophil-TSLP axis in human allergic inflammatory responses has not been well characterized. The purpose of this study was to further elucidate the relationship between basophils and TSLP in human allergic asthma.

METHODS

Subjects

Ten patients with mild allergic asthma were enrolled in a diluent-controlled allergen bronchoprovocation study (see Table E1 in this article's Online Repository at www.jacionline.org). Subjects were required to have a positive skin test response to common aeroallergens, a methacholine PC_{20} value of 16 mg/mL or less, FEV_1 of 70% of predicted value or greater, and a dual-phase response to allergen (≥20% decrease in FEV_1 within 2 hours after allergen and ≥15% decrease in FEV_1 between 3 and 7 hours after allergen).

Blood for *in vitro* experiments was collected from 8 donors with positive skin prick test responses to common aeroallergens. Approval was granted from the Hamilton Integrated Research Ethics Board, and all subjects provided signed informed consent.

Study design

Baseline measurements of methacholine PC_{20}, peripheral blood, bone marrow aspirate, and sputum were conducted on day 1. Subjects were randomized to inhale diluent or allergen on day 2. Blood and sputum samples were obtained 7 hours after challenge, and blood, bone marrow, and sputum samples were collected 24 hours after challenge on day 3. This triad was repeated after a 2-week recovery period. Allergen and diluent challenges were conducted, as previously described (see the Methods section in this article's Online Repository at www.jacionline.org).[14]

Blood, bone marrow, and sputum samples

Approximately 10 mL of blood was collected into sodium heparin vacutainers. The same volume of bone marrow was aspirated from the iliac