

Letter to the Editor

Increased IL-33 expression by epithelial cells in bronchial asthma

To the Editor:

Allergic asthma is a chronic inflammatory disease of the lungs characterized by T_H2 -type immune bias. Apart from the infiltrating immune cells, the epithelium is increasingly considered as an active immune player producing numerous inflammatory mediators.

IL-33, also called IL-1F11 or nuclear factor from high endothelial venules (NF-HEV), is a member of the IL-1 cytokine family. IL-33 is an inducer of the T_H2 branch of adaptive immunity and signals through a complex including the membrane-bound ST2 protein.¹ Aside from T_H2 cells, ST2 is present on mast cells, basophils, eosinophils, natural killer cells, and natural killer T cells. The IL-33/ST2 axis triggers the release of several proinflammatory mediators, such as chemokines and cytokines, and induces systemic T_H2 -type inflammation *in vivo*.¹ The IL-33/ST2 pathway also contributes to allergen-induced airway inflammation and hyperresponsiveness,² both important features of asthma. IL-33 effects were antagonized by administration of soluble ST2 decoy receptor or blocking antibodies *in vivo* and *in vitro*.^{2,3} Elevated IL-33 mRNA levels were measured in the lung tissue from subjects with asthma compared with healthy controls.⁴ Together, these data support a role for IL-33 in the pathogenesis of allergic asthma. Although studies reported that structural cells, including endothelial cells,^{4,5} fibroblasts, smooth muscle cells, and epithelial cells,⁶ express IL-33 in various organs,¹ this study is the first to investigate expression of IL-33 by the airway epithelium in bronchial asthma and compared that with controls.

Adult subjects with and without asthma were recruited from the Montreal Chest Institute and the Sacré-Coeur Hospital based on the criteria of the American Thoracic Society Workshop on Refractory Asthma, and patients' characteristics were reported previously.⁴ Study protocols were approved by ethics committee boards, and all subjects gave written informed consent. Endobronchial tissue specimens from healthy controls without asthma ($n = 5$) and subjects with asthma ($n = 13$: 4 with mild, 4 with moderate, and 5 with severe asthma) were fixed in formaldehyde, and paraffin sections were used for immunocytochemistry staining by using mAb against human IL-33 (0.01 mg/mL, mouse IgG1 κ [Nessy-1]; Alexis Biochemicals, Axxora LCC, San Diego, Calif) and a peroxidase-based detection method.⁵ As reported in other organs,⁶ bronchial epithelial cells express IL-33 protein (Fig 1, A-C). Specificity of IL-33 immunostaining was confirmed by preabsorbing the antihuman IL-33 antibody with recombinant human IL-33 for 1 hour at 4°C (0.05 mg/mL; Axxora LLC), which resulted in a complete abolishment of the IL-33 staining across the tissue sections (Fig 1, D). IL-33 expression in controls without asthma appeared mostly localized in both the cell nuclei and cytoplasm of basal cells (Fig 1, C), which contrasted with widespread distribution of the signal throughout the epithelium in sections from asthmatic subjects (Fig 1, A and B, respectively). Semiquantitative assessment of epithelial cell IL-33 immunoreactivity was achieved through blind grading of both nuclear and cytoplasmic staining intensity based on a 0 to 8 scale where 0 corresponds to no staining (similar to preabsorbed antibody control) and 8 represents the darkest staining in which all epithelial cells

were positive, both in the cytoplasm and cell nuclei. We observed an increased IL-33 staining intensity in subjects with severe asthma compared with healthy controls ($P < .05$), but not other subjects with asthma (Fig 1, E).

IL-33 mRNA expression by epithelial cells was confirmed by using bronchial epithelial cells isolated from bronchial biopsies of subjects with asthma ($n = 5$; mean age, 24 years; FEV₁, 96.3 $\pm$ 6.3%; PC₂₀, 3.6 $\pm$ 5.3 mg/mL) and healthy subjects without asthma ($n = 4$; mean age, 23.2 years; FEV₁, 105.6 $\pm$ 4.3%; PC₂₀, 115.2 $\pm$ 28.6 mg/mL) (mean $\pm$ SD). Subjects were recruited from the Institut Universitaire de Cardiologie et de Pneumologie de Québec (Québec City, Quebec, Canada). All subjects with asthma were atopic (positive skin test for house dust) and were nonsmokers, and none used corticosteroids. The isolated epithelial cells (99% purity) were subcultured *in vitro* between P3 and P5 and characterized by immunofluorescence staining using panepithelial type II cytokeratins (clone AE3) and antifibroblast vimentin antibodies.⁷ Epithelial cells were processed for gene expression profiling by RT-PCR and quantitative real-time PCR.⁴ We detected significantly increased IL-33/glyceraldehyde-3-phosphate transcript ratios in cells from subjects with asthma compared with those from controls without asthma (Fig 2, A). Detection of IL-33 mRNA was also confirmed in epithelial cells isolated from frozen bronchial tissue sections from normal subjects and subjects with asthma by laser-capture microdissection and RT-PCR (data not shown). These data confirm that bronchial epithelium of subjects with asthma expresses elevated levels of IL-33 compared with controls, which supports the concept that bronchial epithelial cells in asthma exhibit altered and proinflammatory phenotype compared with cells from healthy controls.

We then sought to determine whether IL-33 is secreted in the lumen of asthmatic airways. Our research proposal was approved by the Tissue Bank of the Respiratory Health Network of the Fonds de la Recherche en Santé du Québec, which enabled the use of bronchoalveolar lavage fluid (BALF) samples from age-matched subjects with mild and moderate asthma on the basis of American Thoracic Society criteria, and from healthy nonatopic controls without asthma ($n = 10$ per group). Subjects with asthma were all atopic, and displayed BAL eosinophilia: subjects with mild asthma had an FEV₁ of 77.3 $\pm$ 2.9 (3/10 on bronchodilators; none on steroids), whereas subjects with moderate asthma had an FEV₁ of 86.0 $\pm$ 1.6 (all on bronchodilators; 8/10 on inhaled steroids). By using a human IL-33 ELISA kit (CytoSet; Invitrogen, Carlsbad, Calif), we detected increased IL-33 levels in the BALF supernatants from subjects with moderate asthma (average of 120.8 $\pm$ 22.4 pg/mL) compared with those with mild asthma (89.8 $\pm$ 26.4 pg/mL) or controls without asthma (89.4 $\pm$ 12.4 pg/mL; FEV₁, 110.0 $\pm$ 5.2), respectively (Fig 2, B). This suggests that bronchial epithelium and other airway resident cells express elevated levels of IL-33 in asthma^{1,4} and may also be subjected to conditions favoring its release. In asthma, several reports documented intense epithelial cell turnover caused by cell damage/death and aberrant repair leading to thickening of epithelium.⁸ Recent studies on IL-33-expressing endothelial cells reported significant release of IL-33 in supernatants of damaged and/or necrotic endothelial cells compared with supernatants from live cells.⁹ It is therefore tempting to speculate that increased epithelial damage caused by chronic inflammation of the

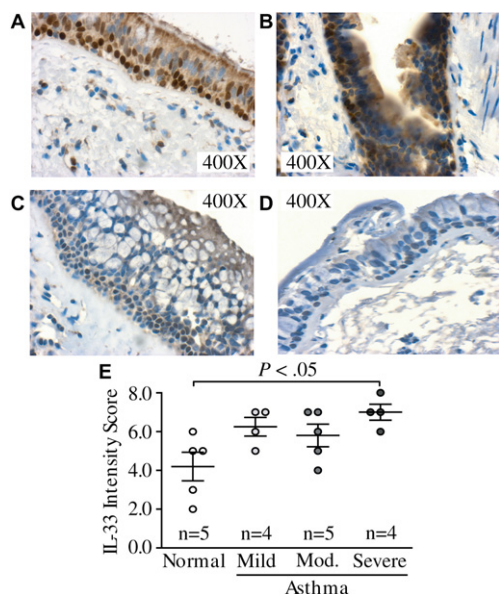


FIG 1. Epithelial IL-33 immunoreactivity in bronchial asthma compared with controls. Endobronchial tissue sections from subjects with mild asthma (**A**), subjects with severe asthma (**B**), and controls (**C**) were stained by immunocytochemistry. Preabsorption of primary antibody with recombinant IL-33 abolished staining on a severe asthma section (**D**). **E**, IL-33 staining intensity was blindly graded and revealed an increased immunoreactivity in subjects with severe asthma versus control subjects. *Mod.*, Moderate.

asthmatic bronchi contributes to the release of IL-33. However, discrepancies observed between the epithelial IL-33 immunoreactivity in subjects with moderate versus mild asthma (**Fig 1, E**) and the increased IL-33 levels in BALF from subjects with moderate versus mild asthma (**Fig 2**) suggest other cell types, like smooth muscle,⁴ may also be significant IL-33 sources in asthma.

Aside from the literature describing IL-33 as an “alarmin”-type cytokine with proallergic properties, there is a lack of studies regarding the sources of this cytokine and the regulation of its release in airway diseases. This study proposes that bronchial epithelium is an important IL-33 reservoir in the lung and that its expression is elevated in bronchial asthma. Histologic observations support the intracellular and nuclear localization of IL-33 in epithelial cells, whereas we also document the increasing release of this cytokine in the airway lumen along with asthma severity (moderate > mild asthma and controls). This study sheds light on the relevance of further using IL-33 BALF levels and studying epithelial cells’ IL-33 expression as potential biomarkers in moderate and severe asthma, respectively.

We thank Elsa Schotman and Dr Stéphane Lajoie-Kadoch for technical advice as well as Dr Ron Olivenstein, Dr Michel Laviolette, and Dr Catherine Lemièrre for their help with recruitment of the patients and collection of tissues.

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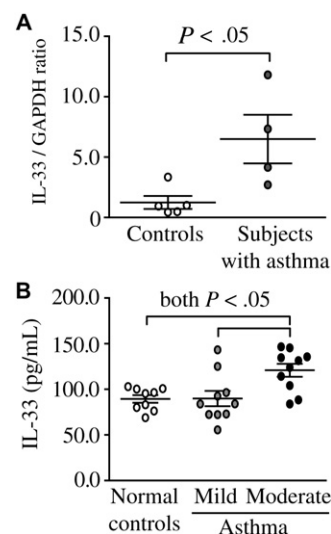


FIG 2. **A**, Cultured bronchial epithelial cells isolated from subjects with asthma exhibit elevated baseline IL-33/glyceraldehyde-3-phosphate (GAPDH) mRNA ratios versus cells from subjects without asthma ($P < .05$). **B**, Elevated IL-33 cytokine levels were detected in BALF from adults with moderate asthma compared with those from controls with mild asthma or without asthma ($n = 10$ per group; $P < .05$; Bonferroni test). ELISA experiments were run in duplicate.

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Supported by the Richard and Edith Strauss Canada Foundation (Montreal, Quebec, Canada), the Canadian Institutes of Health Research (Ottawa, Ontario, Canada), and the J. T. Costello Memorial Research Fund (Montreal, Quebec, Canada). The Meakins-Christie Laboratories and the McGill University Health Center—Research Institute are supported in part by a Center Grant from Fonds de la Recherche en Santé du Québec. D.P. is supported by a doctoral studentship from the Fonds de la Recherche en Santé du Québec and by an American Academy of Allergy, Asthma and Immunology’s Strategic Training in Allergy Research Award. A.S. is the recipient of a GSK/Canadian Institutes of Health Research fellowship.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict.

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doi:10.1016/j.jaci.2009.12.935